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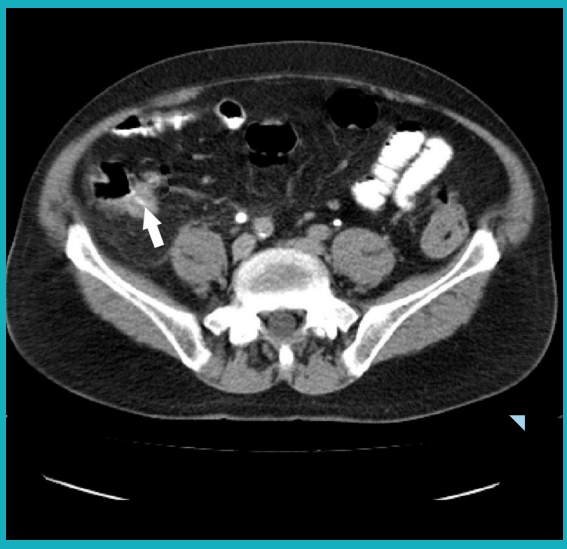

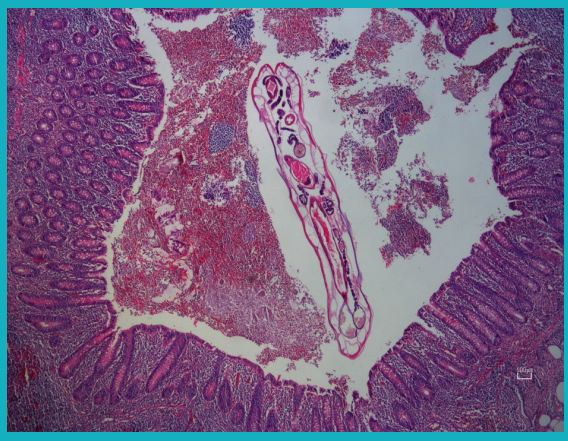

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E-mail: info@bezmialemscience.org

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Phone: +90 (212) 621 99 25

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Pharmaceuticals should be specified with their generic names, and medical products and devices should be identified with brand name and company name, city and country.

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Scientific or Technical Report: Smith P. Golladay K. Payment for durable medical equipment billed during skilled nursing facility stays. Final report. Dallas (TX) Dept. of Health and Human Services (US). Office of Evaluation and Inspections: 1994 Oct. Report No: HHSIGOE 169200860.

Thesis: Kaplan SI. Post-hospital home health care: the elderly access and utilization (dissertation). St. Louis (MO): Washington Univ. 1995.

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Epub ahead of print Articles: Aksu HU, Ertürk M, Gül M, Uslu N. Successful treatment of a patient with pulmonary embolism and biatrial thrombus. Anadolu Kardiyol Derg 2012 Dec 26. doi: 10.5152/akd.2013.062. [Epub ahead of print]

Manuscripts published in electronic format: Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis (serial online) l995 Jan-Mar (cited 1996 June 5): 1(1): (24 screens). Available from: URL: http:/ www.cdc.gov/ncidodlElD/cid.htm.

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EDITORIAL

Dear readers,

We are happy to be with you in a new issue. As the editorial board, we continue our work at a high tempo in the Covid-19 period, which we cannot escape. Our broadcast quality is increasing and your intense interest make us very happy. With the accepted articles, our issues are ready until the middle of 2022. Therefore, we will have two requests from you, our valuable readers. As it is known, we are a multi-disciplinary journal and we strive to make every issue balanced in terms of content. We have many pending surveys, reviews, reliability and validity studies. When you submit such articles, I would like you to think that they may be published later. Later publication of the articles we have accepted may not be suitable for our colleagues in academic endeavors. The second important issue is that there are still many reviews on Covid-19. As it is known, we made 3 special issues about Covid-19 and we will be able to publish a limited number of articles on this subject in our routine issues. This will cause us not to meet your early publication request.

In this issue, we present you many good articles including; Aba et al. Acute Appendicitis Caused by Enterobius Vermicularis: Case Report, Karakaya et al. Carbonic Anhydrase Isoenzymes I and II Inhibition Potentials of Leiotulus dasyanthus (K. Koch) Pimenov&Ostr. and Ferulago pauciradiata Boiss.&Heldr. (Apiaceae), Yıldız et al. Can the Nasal Schirmer Test be Used as a Topographic Test in Facial Paralysis Patients?, Uyarıcı et al. Kainic Acid and MPP+ Induce Upregulation of GLT-1 in Neuroblastoma and Glia Cells, Çeşme. Effectiveness of Diffusion Tensor Imaging in the Microstructural Evaluation of Corpus Callosum and Brain Parenchyma in Children with Neurofibromatosis Type I.

We have also chosen the cover art for this issue from ABA M. et al's study "Acute Appendicitis Caused by Enterobius Vermicularis: Case Report". Thank you for this beautiful picture.

Dear readers,

I hope that this pandemic period, in which we are experiencing the third wave in our country, will come to an end and we will have good days. See you in our next issue. All the best.

Best regards,

Prof. Dr. Adem AKÇAKAYA
Editor-in-Chief

Commentary

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Bezmialem Science 2021;9(2):125-6

Complementary Medicine and Phythotherapy

Tamamlayıcı Tıp ve Fitoterapi

Prof. Dr. Adem AKÇAKAYA*

Bezmialem Vakıf University Faculty of Medicine, Head of General Surgery Department, İstanbul, Turkey

Introduction

Traditional and Complementary Medicine (TCM), which has taken great steps recently in our country, continues to remain on the agenda with many controversial issues. This field, in which applications had been done previously without a scientific base, was discussed within the framework of the Ministry of Health 2013 - 2017 Strategic Plan and Tenth Development Plan of the Republic of Turkey and was reliably and effectively integrated into the health system with an evidence-based approach (1).

TCM is the whole of knowledge, skills and practices based on theories, beliefs and experiences specific to different cultures, used in the prevention of physical and mental illnesses, as well as diagnosis, healing and treatment, as well as protection of health and prevention of diseases (2). They are supportive and complementary methods of today's medicine. Complementary medicine is based on a holistic approach to the patient and disease. The most important sub-unit of complementary medicine is phytotherapy.

What is Phytotherapy?

Phytotherapy means treatment with herbs. The term was first used by Henri Lenclerc, a French physician and phytotherapist. Human beings have used herbal products since ancient times to treat diseases and have benefited. Phytotherapy products are used for treatment, prevention of diseases or to support other treatments. Often, phytotherapy is used for drug-free life. It is preferred because it is natural, considering that it is harmless. However, every food and food supplement can be a useful drug, as well as a harmful poison for the body.

In our country, the TCM regulation was published in 2014. In this regulation, the purpose, scope and legal basis of TCM are explained very clearly and the characteristics of the practitioners, trainers and the health institutions in which TCM is applied are specified (4). Although this regulation describes many areas, it has not eliminated the abuses made by people who have not received training in this field which is under the counter. The Ministry of Health has banned the application of TCM by persons other than physicians and dentists. The Regulation on the Clinical Researches of TCM Practices that entered into force in 2019 and the TCM Good Clinical Practice Guide prepared based on this determined the research and working conditions in this field (5). However, application of TCM by people from different branches have not received training in herbal products, phytotherapy and TCM could not be prevented.

There are many respected medical doctors dealing with phytotherapy in our country. Particularly, the academicians at the university were interested in this field and the academic studies in this field changed the negative opinions about TCM and the more widespread use of TCM treatments (1). More than 10 centers were given certified training authorization by the Ministry of Health, and more than 30 centers were licensed to practice (5). Unfortunately, specialization in this field has not been achieved in our country, which we think that it should be a branch of education. Although it is widely accepted by the public, some of the academicians, especially from the health community, are still skeptical about this area. Pharmacy faculties' pharmacognosy and phytotherapy departments, agricultural faculties and, medicinal and aromatic plants departments which were recently opened

Address for Correspondence: Adem AKÇAKAYA, Bezmialem Vakıf University Faculty of Medicine, Head of General Surgery Department, İstanbul, Turkey
E-mail: drakcakaya@yahoo.com **ORCID ID:** orcid.org/0000-0003-3116-7033

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at associate degree level increased the interest in this field and endemic vegetation in our country. Promising studies are carried out in the production, research and marketing of medicinal and aromatic plants and these departments are developing rapidly. Dealing with phytotherapy by people who have been educated and are from different branches, even outside the health field, can cause information pollution and wrong practices. When we look at who is interested in phytotherapy in our country, we see the following groups:

1- Those who have participated in the certification program with the trainings given by the Ministry of Health within the framework of TCM trainings and gained the competence to prescribe in this field. This group, which I am a part of, first entered the field of education with the Council of Higher Education approved Phytotherapy center opened at Bezmialem Vakıf University. Later, the TCM training center was opened in many universities and training and research hospitals for physicians and dentists.

2- Those who has become a doctor and completed phytotherapy master and doctorate programs opened by some universities' pharmacy faculties.

3- Pharmacists working in the pharmacognosy and phytotherapy departments of the Faculty of Pharmacy, who have gained experience in this field. Non-physician healthcare professionals holding master's and doctoral degrees in phytotherapy.

4- Those who study biology and chemistry at the faculties of Agriculture and Science and are interested in medicinal and aromatic plants.

5- Graduates of the Department of Medicinal and Aromatic Plants, who are studying at associate degree in some universities.

6- Those who turn to the field of phytotherapy with courses and other trainings after completing their education in a different field.

7- People who has grown up in a family tradition or herbalist shop in this field without any training or who claim to have discovered this area as a result of the illness of themselves or a relative.

As can be seen, only physicians and dentists in the first group of these groups are legal to use phytotherapeutic products in treatment. Any physician can recommend these products, provided that they are trained in this field. However, those who are commonly interested in this field and dominate the market consist of people in the 7th group who have never received any training. Unfortunately, this group has caused loss of trust in TCM applications due to the exploits made in the past. However, the President of the World Health Organization, Dr. As Chan said that abuse would be less in phytotherapy if it was applied by well-trained, experienced and licensed people (6).

As a result, herbal medicines are becoming popular and developing in our country day by day. The loss of reputation in this field due to the abuses committed by people who have not been trained is being regained day by day. The fact that some of our universities such as the Bezmialem Vakıf University, Yıldırım Beyazıt University, Yeditepe University, and Health Sciences University have also turned to this field contributes to the increase of this reputation. Phytotherapy will develop with the contributions of those who believe and trust academician-based phytotherapy. Phytotherapy will continue to be one of the promising fields of our country in terms of both health and economics by developing the cooperation of medicine, pharmacy, agriculture faculties, medical and aromatic plants departments, and putting their studies into a scientific framework.

*Chair of Phytotherapy Association and Trainer of Bezmialem Vakif University, Phytotherapy Training Center.

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Original Article

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Is a Single Rinse Effective on Evacuating the Residual Monomers After Orthodontic Bonding? An *In Vivo* Study

Braketleme Sonrası Tek Çalkalama Artık Monomerlerin Uzaklaştırılmasında Etkili Midir? Bir *İn Vivo* Çalışma

Erhan GÖRÜKMEZ[1], Berza ŞEN YILMAZ[2], Sabri İlhan RAMOĞLU[3]

[1]Nişantaşı University, Health Vocational School, Division of Oral and Dental Health, İstanbul, Turkey
[2]Bezmialem Vakıf University Faculty of Dentistry, Department of Orthodontics, İstanbul, Turkey
[3]Altınbaş University Faculty of Dentistry, Department of Orthodontics, İstanbul, Turkey

ABSTRACT

Objective: Orthodontic adhesives are known to release potentially harmful bio-chemicals such as Bisphenol A, a derivative from Bisphenol-A-Glycidyl-Methacrylate (Bis-GMA). The aim of this study was to evaluate the amount of Bis-GMA released after the use of orthodontic adhesives polymerizing chemically or with light. We also aimed to check whether a single rinse is effective on evacuating all the residual monomers.

Methods: Light curing (Transbond XT, Unitek, CA, USA and Opal Seal Ultradent, Utah, USA) and chemically curing adhesives (Rely-a bond, Reliance Orthodontic Products, Inc., USA and Unite, 3M/Unitek, CA, USA) were used to bond upper and lower braces of 48 patients. Patients gargled 25 mL drinking water for 1 minute; before bonding (T0), immediately after bonding (T1) and immediately after the first rinse (T2). The samples were placed in amber colored glass bottles, preserved in -20 °C and filtered through a 0.45-micron filter and analyzed with Liquid Chromatography Tandem Mass Spectrometry.

Results: Bis-GMA release was recorded with all the adhesives. Higher amount of residual monomer was recorded for the light curing composite adhesives; Transbond XT and Opal Seal, followed by chemically curing; Unite and Rely-a bond. There was no statistically significant difference in Bis-GMA concentration at T1 and T2 ($p>0.05$).

ÖZ

Amaç: Ortodontik adezivlerin kullanımı sonrası Bisfenol-A-Glisidil-Metrakrikattan (Bis-GMA) türeyen Bisfenol A gibi potansiyel zararlı biyokimyasal ajanların ortaya çıktığı bilinmektedir. Bu çalışmanın amacı; Kimyasal ya da ışıkla sertleşen adezivlerin kullanımıyla ortaya çıkan Bis-GMA miktarının değerlendirilmesinin yanı sıra braketleme sonrası tek çalkalamanın tüm artık monomerlerin tahliyesinde etkili olup olmadığının değerlendirilmesidir.

Yöntemler: Işıkla (Transbond XT, Unitek, CA, USA and Opal Seal Ultradent, Utah, USA) ve kimyasal (Rely-a bond, Reliance Orthodontic Products, Inc., USA and Unite, 3M/Unitek, CA, USA) polimerizasyon gösteren adezivlerle 48 hastanın alt ve üst braketleri yapıştırılmıştır. Hastalar braketleme öncesi (T0), braketlemeden hemen sonra (T1) ve ilk çalkalamayı takiben (T2) 25 mL'lik içme suyunu 1 dakika boyunca çalkalamıştır. Örnekler kehribar renkli şişelerde -20 °C'de korunmuş ve 0,45-mikron inceliğinde filtrelerden geçirilmiş ve likit kromatografi kütle spektrometresi ile ölçümler gerçekleştirilmiştir.

Bulgular: Tüm gruplarda braketleme sonrası Bis-GMA ortamda tespit edilmiştir. Işıkla sertleşen adezivlerde daha çok miktarda olmak üzere Transbond XT, Opal Seal, Unite ve Rely-a bond gruplarında sırasıyla azalacak miktarda Bis-GMA tespit edilmiştir. T1 ve T2 ölçümleri arasında istatistiksel olarak fark yoktur ($p>0,05$).

Address for Correspondence: Berza ŞEN YILMAZ, Bezmialem Vakıf University Faculty of Dentistry, Department of Orthodontics, İstanbul, Turkey
E-mail: berzasen@hotmail.com **ORCID ID:** orcid.org/0000-0002-7961-0535

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Conclusion: Significant release of Bis-GMA was observed following orthodontic bonding with either light or chemically curing adhesives. No significant difference was recorded between the first and the second rinses following bonding, meaning that a single rinse may not be effective in evacuating all the residual monomers.

Keywords: Bis-GMA, orthodontic adhesives, liquid chromatography

Sonuç: Kimyasal ve ışıkla sertleşen adeziv kullanımı sonrası ortamdaki Bis-GMA miktarı anlamlı derecede artmıştır. Braketleme sonrası ilk ve ikinci çalkalama örneklerindeki Bis-GMA miktarında anlamlı farklılık bulunmaması, tek çalkalamanın tüm artık monomerleri uzaklaştırmada etkili olamayacağı yönünde yorumlanabilir.

Anahtar Sözcükler: Bis-GMA, ortodontik adeziv, likit kromatografi

Introduction

Advancements in material science offered practitioners a variety of adhesive materials with different properties in clinical practice. Composite adhesives not only shorten treatment time but also provide easier handling.

The adhesive composites in orthodontics have similar chemical composition with dental restorative composite materials. They consist of inorganic fillers, a solvent base and an organic matrix. The organic matrix of composite materials changes into polymers with polymerization. However, some monomers not joining the polymer structure may remain (1).

The organic matrix part of composites most frequently includes glycidyl methacrylate (Bis-GMA), which decomposes into a potentially harmful bio-chemical called Bisphenol A. Bisphenol A is known to have undesirable effects on the reproduction capacity of rats and estrogenic activities (2,3). Its cytotoxicity and mutagenic properties have been confirmed in tissue cultures (4,5).

There are different studies in the literature to measure the residual monomer quantities (6-10). However, the concentration of the Bis-GMA is depending on factors such as the temperature, the pH, the solvent solution since it can dissolve to other components. The evacuation of the Bis-GMA from the oral medium in a short time prior to its conversion to BPA is important, however, its low concentration makes it difficult to detect in chromatography devices (11). However, there are no studies in the literature evaluating the short time release of Bis-GMA *in vivo* from different adhesives.

In the clinical practice, it is advised to ask patients to rinse their mouth with water following the bonding procedure to reduce the BPA presence to before bonding levels (12). However, there is no study in the literature evaluating the Bis-GMA concentration following orthodontic bonding cured by neither light nor chemically *in vivo*.

This study aims to investigate whether rinsing following bonding is effective on evacuating the residual Bis-GMA and to evaluate Bis-GMA concentration following bonding with light and chemically curing orthodontic adhesives *in vivo* with a sensitive method.

Methods

The study was approved by the Ethics Committee of Bezmialem Vakıf University (approval number: 71306642-050.01.04). The volunteers signed a consent form prior to participating in the study.

A power analysis was performed based on a former study (12). Accordingly, 12 patients in each of the groups would guarantee the power of 80% at the 5% confidence level allowing detection of differences.

Bonding Process

Forty-eight patients with no composite dental restoration and bonding the upper and lower jaws was possible at the same appointment were included (1st molar to 1st molar of the contralateral side). Patients with missing or extra tooth, with crowding requiring extraction, appliances containing acrylic parts such as a Nance appliance were excluded. The patients were randomly assigned into two groups (n=24) using a randomization web site (https://www.randomizer.org/) based on the adhesive type; light or chemically curing. As four brands were going to be tested, the groups were subdivided using the same software. Two light curing [Transbond XT, Unitek, CA, USA (n=12) and Opal Seal Ultradent, Utah, USA (n=12)] and two chemically curing adhesives [Rely-a bond, Reliance Orthodontic Products, Inc., USA (n=12) and Unite, 3M/Unitek, CA, USA (n=12)] were used to bond upper and lower braces. The chemical compositions of the adhesives are shown in Table 1.

The buccal surfaces of the teeth were cleaned with prophylaxis pumice and rinsed with a water-air spray. The teeth were etched with 37% orthophosphoric acid for 15 s, rinsed and dried for 4-5 s. Every patient received the same type of braces and molar tube set (18-inch slot, Mini Master Series; Roth, American Orthodontics, Sheboygan, USA).

The composite adhesives were applied in accordance with the manufacturer's instructions and finally light-cured by using a LED curing unit (VALO LED, Ultradent, South Jordan, USA) in power mode. The primer was cured for 3 s, the paste was cured for 3 s from the mesial and 3 s from the distal sides. For the chemically curing group, the primer was applied to both tooth and bracket surfaces, and the medium was isolated for 4 minutes following the last bracket placement.

The patients were given 25 mL of drinking water to rinse in a glass for 1 minute. The samples were collected at three instances: before bracket bonding (T0), immediately after bracket bonding (first rinse; T1) and immediately after the first rinse (second rinse; T2). 144 samples were collected and placed in amber colored glass flasks and stored at -20 °C until analysis.

The samples were filtered through a 0.45-micron filter. 1 μL from each sample was collected and analyzed in a liquid chromatographer (Agilent 1200, San Jose, CA, USA) and a mass spectrometer (Agilent 6460, San Jose, CA, USA). Analytical distinction was achieved by a Poroshell 120 EC-C18 3x50 mm with a particle size of 2.7 microns set at a flow speed of 0.2 mL per minute and column temperature of 35 °C. The gradient values are given in Table 2.

Electrospray ionization was carried out using N_2 in negative and positive ion mode sets at 300 °C and a flow rate of 11 mL per minute, while the nebulizer was set at a pressure of 45 psi. Capillary voltage was set at 3,500 V. The retention time of the peak value for Bis-GMA was 4.81 minutes.

Statistical Analysis

The Statistical Package for the Social Sciences for Windows 22.0 (Armonk, NY: IBM Corp, 2013) was used for the

Table 1. Chemical composition of the adhesives

	Chemical name	(C.A.S. No.)	wt% range)
Transbond XT adhesive paste	Silane treated quartz	100402-78-6	70 - 80
	Bisphenol a diglycidyl ether dimethacrylate	1565-94-2	10 - 20
	Bisphenol a Bis (2-hydroxyethyl ether) dimethacrylate	24448-20-2	5 - 10
	Silane treated silica	68611-44-9	< 2
	Diphennyliodonium hexafluorophosphate	58109-40-3	< 0.02
Transbond XT adhesive primer	bisphenol a diglycidyl ether dimethacrylate	1565-94-2	45 - 55
	triethylene glycol dimethacrylate	109-16-0	45 - 55
	Triphenylantimony	603-36-1	< 1
	4-(dimethylamino)-benzeneethanol	50438-75-0	< 0.5
	dl-camphorquinone	10373-78-1	< 0.3
	Hydroquinone	123-31-9	< 0.03
Opal bond adhesive	Bisphenol a diglycidyl ether dimethacrylate	1565-94-2	< 20
	Ethoxylatebisphenoldimethacrylate	41637-38-1	< 10
	Triethylene glycol dimethacrylate	109-16-0	< 5
	Aluminum oxide	1344-28-1	< 10
Opal seal adhesive	Bisphenol a diglycidyl ether dimethacrylate	1565-94-2	< 15
	Hydroxypropyl methacrylate	27813-02-1	45 - 55
	Ethyl alcohol	64-17-5	< 10
	Methacrylic acid	79-41-4	< 10
Rely bond adhesive paste	Bisphenol a diglycidyl ether dimethacrylate	1565-94-2	10-30
	Amorphous silica	60675-86-0	50-75
	Triethylene glycol dimethacrylate	109-16-0	5 - 10
	Aluminum oxide	1344-28-1	1 - 5
Rely bond adhesive primer	bisphenol a diglycidyl ether dimethacrylate	1565-94-2	10-30
	tetrahydrofurfuryl methacrylate	2455-24-5	30 - 50
	2-Hydroxyethyl methacrylate	868-77-9	10 - 30
	Triethylene glycol dimethacrylate	109-16-0	10 - 30
Unite adhesive paste	Silane treated quartz	100402-78-6	50 - 60
	Bisphenol a diglycidyl ether dimethacrylate	1565-94-2	10 - 20
	Benzoyl peroxide	94-36-0	< 2
	Silane treated silica	68611-44-9	5 - 10
	Dimethyl siloxane	67762-90-7	< 2
Unite adhesive primer	bisphenol a diglycidyl ether dimethacrylate	1565-94-2	5 - 15
	triethylene glycol dimethacrylate	109-16-0	70 - 80
	2,2'-(P-Tolylimino) diethanol	3077-12-1	5 - 15
	3- Methacryloxypropyltrimethoxysilane	2530-85-0	< 5
	Poly (Methyl methacrylate) (PMMA)	9011-14-7	< 5

statistical analysis. The statistical analysis was performed with Mann-Whitney U test for comparing the data between two independent groups and Kruskal-Wallis test for data in two or more independent groups. Data within a group were compared using Wilcoxon signed-rank test. All tests were performed at a significance level of α=0.05.

Results

The mean amounts of Bis-GMA detected at T0, T1 and T2, and comparison of the light and chemically curing adhesive groups are presented in Table 3. A statistically significant difference was observed between the light and chemically curing adhesive groups' samples at T1 and T2 (p=0.000). The light-cured group showed higher values than the chemically cured group.

Table 4 reports the results of the Mann-Whitney U test within the groups in terms of sample collection time. In all groups, the differences between T0 and T1 and between T0 and T2 were found to be statistically significant (p=0.000). The change between T1 and T2 was insignificant.

The mean Bis-GMA amounts recorded for the subgroups at T0, T1 and T2 and evaluation of the values for the same time period between the subgroups are given in Table 5. The differences between the means in the T0 period showed no significance (p=0.982), whereas significant differences were found for the T1 and T2 periods (p=0.000). Mann-Whitney U test was used to determine which group caused the difference (Table 6). For both the T1 and T2 periods, higher Bis-GMA concentrations were recorded in the Transbond group followed by Opal and Unite. The Bis-GMA levels for the Rely-a Bond group were significantly lower than the other groups.

Table 2. Gradient values related to analytical division

Time	Function	Parameters
1. min	Solvent composition change	Solvent change 95% A, 5% B
3. min	Solvent composition change	Solvent change 2% A, 98% B
7. min	Solvent composition change	Solvent change 2% A, 98% B
7.1 min	Solvent composition change	Solvent change 95% A, 5% B

A: 1 mM Ammonium formate, 0.1% formic acid in water
B: 0.1% formic acid In acetonitrile

Table 3. Bis-GMA amount comparison between light and chemically curing adhesive groups

	Groups	Mean (ppb)	SD	p
T0	Light cure	0.603	0.226	0.797
	Chemical cure	0.609	0.247	
T1	Light cure	29.683	5.324	**0.000****
	Chemical cure	13.693	4.888	
T2	Light cure	29.715	5.175	**0.000****
	Chemical cure	13.688	4.838	

Mann-Whitney U test, *p<0.05, **p<0.01, SD: Standard deviation

Table 4. Evaluation of the Bis-GMA amount within the groups according to sample collection time

Groups	p		
	T0-T1	T1-T2	T0-T2
Light cure	0.000**	0.732	**0.000****
Chemical cure	0.000**	0.943	**0.000****

Mann-Whitney U test, *p<0.05, **p<0.01

Table 5. Evaluation of the Bis-GMA amount within the subgroups according to sample collection time

	Groups	N	Mean (ppb)	SD	p
T0	Transbond	12	0.590	0.249	0.982
	Opal	12	0.615	0.212	
	Unite	12	0.601	0.252	
	Rely a bond	12	0.616	0.253	
T1	Transbond	12	34.892	0.214	**0.000****
	Opal	12	24.473	0.124	
	Unite	12	18.475	0.204	
	Rely a bond	12	8.910	0.133	
T2	Trans bond	12	34.772	0.229	**0.000****
	Opal	12	24.657	0.351	
	Unite	12	18.421	0.180	
	Rely a bond	12	8.955	0.141	

Kruskall-Wallis H test. *p<0.05. **p<0.01, SD: Standard deviation

Table 6. Evaluation of the group differences at T0. T1 and T2

	T0			T1			T2		
Groups	**Opal**	**Unite**	**Rely a bond**	**Opal**	**Unite**	**Rely a bond**	**Opal**	**Unite**	**Rely a bond**
Transbond	0.982	0.982	0.982	**0.00****	**0.00****	**0.00****	**0.00****	**0.00****	**0.00****
Opal		0.982	0.982		**0.00****	**0.00****		**0.00****	**0.00****
Unite			0.982			**0.00****			**0.00****

Mann-Whitney U test. *p<0.05. **p<0.01

Table 7. Within group comparison of the samples collected at T0. T1. and T2

Groups	p		
	T0-T1	T1-T2	T0-T2
Transbond	**0.002****	0.347	**0.002****
Opal	**0.002****	0.209	**0.002****
Unite	**0.002****	0.182	**0.002****
Rely-a Bond	**0.002****	0.170	**0.002****

Mann-Whitney U test. *p<0.05. **p<0.01.

The results for the intragroup comparison of the samples collected at T0, T1 and T2 are given in Table 7. The mean amount of Bis-GMA recorded at T1 and T2 for each group was found significantly higher in comparison to the T0 measurements, whereas the difference between the mean values at T1 and T2 was insignificant for all groups.

Discussion

There is a general belief that orthodontic adhesives are biologically safe, and this opinion is supported by the argument that adhesive resins are used in small quantities. However, the excess adhesive is not always completely removed prior to polymerization. Moreover, some unreacted material may remain since the composite located under the metallic components access light only by transillumination. Małkiewic et al. investigated the levels of BPA, BPA polymers and Bis-GMA resin in eluates of six commonly used orthodontic adhesives *in vitro* and demonstrated that most of the orthodontic adhesives released Bisphenol A or its derivatives. In the literature, it was reported that even the pellicle layer formation immediately after brushing may decrease monomer release proving that laboratory studies may not reflect precise clinical conditions (13), this is the reason why the present *in vivo* study was designed.

Studies analyzing residual monomers used various solvents such as distilled water, ethanol, saline, cell culture medium or artificial saliva (14). ISO recommends using distilled water to determine the chemicals that are released from resin-based materials, whereas US FDA recommends using a 75% water-ethanol solution. Accordingly, Moreira et al. (15) reported higher amounts of monomers with water-ethanol solution in comparison to using water only. In this study, drinking water presented in a glass was used as sample collection liquid since it is a routine to rinse with water following bonding.

Storage conditions are essential for specimens containing biological fluids, e.g. saliva. The saliva specimens were stored at -80 °C or -70 °C in the literature (16,17). Kloukos et al. (12) kept from rinsing specimens at 4 °C. In this study, the specimens were placed at -20 °C as described by Olea et al. (18).

Liquid chromatography combined with a tandem mass spectrometer was selected in our study for investigating monomers with high molecular weight since combining the mass spectrometer with chromatographic methods is proven to provide more accurate and sensitive results compared to other chromatographic methods (19). While only liquid or gas chromatography devices can measure up to 1 ppm (one millionth), the chromatography combined with mass spectrometry can measure up to 1 ppd (one billionth) of monomers (20), which means that the last one provides 1000 times more sensitive results.

It was shown that Bis-GMA released from composite resins hydrolyzes into Bis-HPPP after 25 hours (21). In another study, chemical and light cured composites were kept in 0.9% saline at 37 °C for 2 months. Following this period, Bis-GMA was not detected in the samples because of the fact that Bis-GMA hydrolyzes and transforms into Bis-HPP with time (22). Moreira et al. (15) found that 75% of the monomer release occurred within a few hours and 95% of the residual monomer was released within 48 hours, whereas Polydorou et al. (23) reported higher monomer release within the first 24 hours. Bis-GMA monomer levels may be affected from the water, esterase enzyme and the temperature of the oral cavity over time. Therefore, the amount of released Bis-GMA was measured in this study immediately after polymerization. Although the samples were collected at different times, similar results were obtained for both groups. This may be proving that Bis-GMA did not deteriorate under storage conditions in our study. On the other hand, in the study of Kloukos et al. (12), the amount of BPA, which is the product of Bis-GMA deterioration, was evaluated. In contradiction with our results, the authors reported that the increase in BPA concentration was reduced after the 2nd post-bonding rinse. Since the concentration of the Bis-GMA is known to be varying with the media temperature, these differences might be related to the fact that their samples were conserved at 4 °C till the measurements were performed while our samples were stored at -20 °C.

In some studies evaluating residual monomer release from chemical or light curing adhesives, a higher degree of monomer release was reported in the chemically-cured group (8,24). In the present study, higher Bis-GMA release was found in the light-cured groups, contradicting these studies. It was reported that increasing curing time leads to decreased residual monomer release (24). Depending on that, it may be hypothesized that using QTH light sources with longer exposure time would cause less residual monomer formation in comparison to high-power LED sources. This study demonstrates that reduced

exposure time with high intensity LEDs can result in composite restorations with inferior curing depth and increased leaching of monomers.

In our study, higher Bis-GMA concentrations were recorded with light-cured composites, especially in the Transbond XT followed by the Opal Seal, Unite and Rely a Bond groups. Considering the chemical contents of these composites, monomer concentrations appeared to be similar. The composites were used in the clinic with bonding agents from their own brands. Similarly, the concentration of Bis-GMA in the bonding agent content was in harmony with the adhesives. Therefore, the presence of unreacted monomers could be expected to be higher. Furthermore, the higher Bis-GMA concentration in the Transbond XT group may be explained by the high amount of Bis-GMA (wt. 45-55%) within the primer and the exposure of the primer to a relatively large surface.

In a study by Ratanasathien et al. (25), it was reported that Bis-GMA has a cytotoxic effect on fibroblasts when its concentration is above 4.78 ppm. On the other hand, increased estrogenic activity of HeLa cells was reported when the Bis-GMA concentration was above 5 ppm (26). In our study, the highest release amount of Bis-GMA was 34.8 ppd, which was considerably lower than those limits. According to these results, it may be assumed that the amount of Bis-GMA release does not reach toxic levels following orthodontic bonding.

Study Limitations

In our study, we found that light-curing composite adhesives, which are commonly used in orthodontic clinics, may be more disadvantageous in terms of biocompatibility compared to chemically curing counterparts. For this reason, manufacturers can plan studies to improve the chemical composition in order to reduce the amount of residual monomers of the light-curing composites. In addition, the use of chemically cured composite adhesives is more difficult clinically. Although fewer Bis-GMA release was found, the repositioning of incorrectly positioned brackets can cause the patients to be more exposed to additional residual monomers. Therefore, it can be suggested to use chemically curing composites with indirect bonding.

It is known that the residual monomer released in the first minutes following polymerization of adhesive resins is related to the oxygen inhibition layer, and the later release is related to residual monomers remaining in the adhesive resin matrix. One of the aims of our study was to check whether one-single rinsing was effective on evacuating the Bis-GMA immediately after orthodontic bonding. Thus, we only analyzed the pre-bonding, post-bonding and the second rinse samples. We proved that a single rinse was not effective in reducing the Bis-GMA concentration, but the number of effective rinsing was not presented. This might be considered as a limitation. Different rinsing solutions and the effective rinsing number may be determined with repeated rinsing procedures. Additionally, the amount of Bis-GMA can be monitored by long-term follow-up studies.

Conclusion

Bis-GMA release was observed immediately after orthodontic bonding in all groups. The highest amount of Bis-GMA was found in the Transbond group, whereas the lowest measurements were recorded in the Rely-A Bond group. The light-cured composite adhesives released higher amounts of residual monomer. The difference between the Bis-GMA levels in first and second rinses was statistically insignificant, meaning that a single rinse may not be effective in evacuating all residual monomers. However, mouth rinsing with water following orthodontic bonding may still be considered to moisten the oral mucosa and remove the possible residual acid.

Ethics

Ethics Committee Approval: Bezmialem Vakıf University (SBW_MF405-1210318105400.pdf).

Informed Consent: Obtained.

Peer-review: Externally peer reviewed.

Authorship Contributions

Surgical and Medical Practices: B.Ş.Y., Concept: E.G., B.Ş.Y., S.İ.R., Design: E.G., B.Ş.Y., S.İ.R., Data Collection or Processing: E.G., B.Ş.Y.,Analysis or Interpretation: E.G., B.Ş.Y., S.İ.R., Literature Search: E.G., B.Ş.Y., S.İ.R., Writing: E.G., B.Ş.Y., S.İ.R.

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Original Article

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The Effect of Iodised Poly-3-Hydroxy Butyrate on DNA and BSA

İyotlu Poli-3-Hidroksi Butiratın DNA ve BSA Üzerine Etkisi

Arzu EROL

Zonguldak Bülent Ecevit University Faculty of Sciences and Arts, Department of Molecular Biology and Genetics, Zonguldak, Turkey

ABSTRACT

Objective: An important criterion that radiopaque poly-3-hydroxy butyrate (PHB) derivatives synthesized and characterized by our study group do not interact with DNA and bovine albumin to be developed as an intravenous contrast agent for radiological imaging.

Methods: Interactions of radiopaque PHB derivatives between bovine serum albumin (BSA) and deoxyribonucleic acid (pUC19 DNA and polymerase chain reaction product) were investigated using absorption spectroscopy and agarose gel electrophoresis techniques.

Results: Absorption spectroscopy results showed that the radiopaque PHB derivatives did not show any interaction with BSA. Double-stranded annular DNA showed that the interaction between the double-stranded forms of linear DNA did not cause any damage.

Conclusion: It was determined that it did not interact with both BSA and various DNA forms.

Keywords: Albumin, DNA, iodinated poly-3-hydroxy butyrate, pUC19

ÖZ

Amaç: Çalışma grubumuz tarafından sentezi ve karakterizasyonu yapılan radyopak poly-3-hydroxy butyrate (PHB) türevlerinin, radyolojik görüntüleme kullanılacak, intravenöz kontrast madde olarak geliştirilebilmesi için, DNA ve sığır albumin ile etkileşim göstermemesi önemli bir kriterdir.

Yöntemler: Radyoopak PHB türevlerinin sığır serum albumin (BSA) ve deoksiribonükleik asit (pUC19 DNA'sı ve polimeraz zincir reaksiyonu ürünü) arasındaki etkileşimleri absorpsiyon spektroskopisi ve agaroz jel elektroforezi teknikleri kullanılarak incelenmiştir.

Bulgular: Absorpsiyon spektroskopisi sonuçları, radyoopak PHB türevlerinin BSA ile bir etkileşim göstermediği, absorpsiyon spektroskopisi ve agaroz jel elektroforezi sonuçları ise; çift iplikli halkasal DNA, çift iplikli lineer DNA formları arasın bir etkileşim ve hasara neden olmadığını göstermiştir.

Sonuç: Hem BSA hem de çeşitli DNA formları ile etkileşimi olmadığı tespit edilmiştir.

Anahtar Sözcükler: Albümin, DNA, iyotlu poli-3-hidroksibutirat, pUC19

Introduction

X-ray imaging using intravenous iodinated contrast agents, computed tomography, and fluoroscopy play important roles in medicine (1). The increased use of diagnostic radiological imaging has significantly increased radiation exposure and radiocontrast agent use in humans, and is associated with cancer risk. The chemotoxic and adverse effects of intravenous iodinated contrast agents, i.e., allergic reactions, kidney damage, and thyrotoxicosis are rare (2); however, they are well-known. In addition, intravenous iodinated contrast agents have shown to

Address for Correspondence: Arzu EROL, Zonguldak Bülent Ecevit University Faculty of Sciences and Arts, Department of Molecular Biology and Genetics, Zonguldak, Turkey
E-mail: erol.arzu@yahoo.com **ORCID ID:** orcid.org/0000-0002-4498-6663

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increase the risk of cancer following X-ray exposure by increasing deoxyribonucleic acid (DNA) damage after a certain dose. Since the late 1970s, studies showed that intravenous iodine contrast agent administration during X-ray imaging causes damage to blood cells (3). Results of other studies reported higher levels of DNA damage could also be explained by the potential effects of intravenous iodinated contrast agents. Most importantly, it is unclear whether the increase in DNA damage in blood cells triggers the risk of cancer if exposed to repeated doses with intravenous iodine contrast agents (4). This uncertainty adds to the risks of intravenous iodinated contrast media in addition to the risks of exposure to low-dose medical radiation, including risks from ongoing epidemiological studies (5).

To address this problem, a review of previously published studies investigating the effect of contrast agent on cell damage in diagnostic imaging was performed. Related articles, contrast radiotherapy, and studies investigating the dosimetric effects of elements with high atomic numbers in general were also collected (5). Parameters such as iodine concentration, time elapsed after exposure, and X-ray energy spectra vary between studies. In addition, intravenous iodinated contrast agents show low protein binding capacity (3-6).

Intravenous iodinated contrast agent may cause various types of DNA damage, including repeated DNA double helix breaks (6). DNA damage is normally repaired by the DNA repair system. However, genetic information may change, if errors are made in the repair of damaged DNA, thus leading to health effects such as cancer and vascular events (3). Therefore, it is important to examine the effects of increased intravenous iodinated contrast agent use, particularly the accurate quantification of the amount of DNA strand and double strand bonds that can be caused by intravenous iodinated contrast agent.

Here, we synthesized and characterized new radiopaque poly-3-hydroxy butyrate (PHB) derivatives previously reported by our study group (7). PHB is essential for *in vivo* medical applications to provide renewable, biodegradable, and biocompatible microbial polyester radiopacity. This study aimed to investigate the interaction of synthesized biodegradable and biocompatible polymers with DNA and plasma protein albumin. The synthesized polymers were named PHB-DEA-IB-6, PHB-DEA-IB-1, and PHB-DEA-IB-12 (7).

Methods

Preparation of Double-stranded DNA (pUC19)

pUC19 is a plasmid cloning vector. It is in the form of circular double-stranded DNA and has 2,686 base pairs. pUC19 is one of the most widely used vectors. In our laboratory, the pUC19 plasmid was isolated from *Escherichia coli* NEB5a, previously transformed with the plasmid by the Valipour group (8,9). The polymerase chain reaction (PCR) product double-stranded short DNA fragment was used by the Celik group, which was previously amplified from human genomic DNA. Figure 1 shows the sequence and schematized appearance of DNA in different forms according to the structure and size by agarose gel electrophoresis.

The pUC19 plasmid is in the form of supercoil. Using a factor that binds to the structure of a supercoil, the structural changes and agarose gel electrophoresis can be indicated by ultraviolet (UV) imaging. The treated polymer is nicked in the supercoil structure if it causes partial opening, which causes the supercoil structure to open completely, causing a linear form or fractures to create multiple images.

Measurement of pUC19 Plasmid DNA Using a UV-visible Spectrophotometer

The plasmid concentration of pUC19 was measured according to the Beer-Lambert Law, and the OD260 was measured after isolation. For samples containing a mixture of protein and nucleic acid for purity, the theoretical A260/A280 ratio is calculated using the following formula according to the Beer-Lambert Law (8,9):

$$A_{260} / A_{280} = \frac{(\varepsilon_{260,\,protein} \text{ X } (\%\text{Protein}) + \varepsilon_{260,\,DNA} \text{ X } (\%\text{ DNA})}{(\varepsilon_{280,\,protein} \text{ X } (\%\text{Protein}) + \varepsilon_{280,\,DNA} \text{ X } (\%\text{ DNA})}$$

Preparation of Radiopaque PHB Derivatives

For this, 1 mg of each of the radiopaque PHB-DEA-IB-6, PHB-DEA-IB-12, and PHB-DEA-3IB-1 polymers were ground to a powder. The powder was dissolved in a solvent system (chloroform, 1 mL). Samples were then allowed to stand for 4-5 h to completely dissolve. These solutions were filtered through Whatman filter paper, followed by microfiltration (0.2 umm). To verify the concentration of these polymers, the filtered solution was subjected to spectrophotometric analysis using the Beer-Lambert Law (8-10).

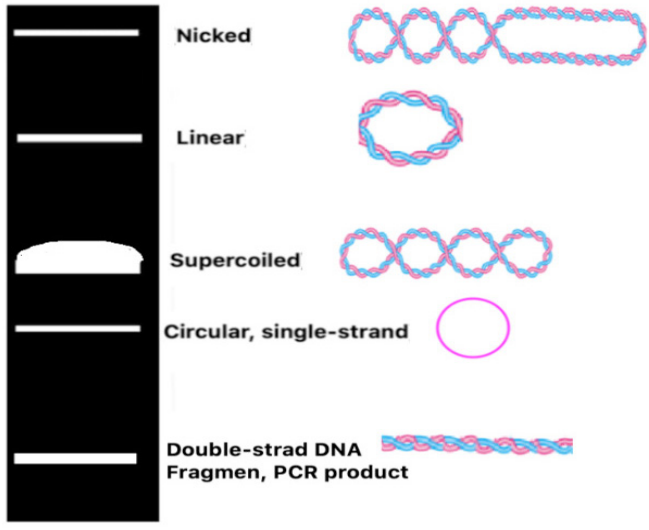

Figure 1. Band appearance and schematized DNA generated by agarose gel electrophoresis of the pUC19 plasmid and PCR product

PCR: Polymerase chain reaction

Preparation of Bovine Serum Albumin (BSA)

The absorbance value was measured on a spectrophotometer at a wavelength of 600 nm at varying concentrations of bovine serum albumin (BSA) with a stock solution of 2 mg/mL.

Preparation of BSA-radiopaque PHB Derivatives and pUC19-radiopaque PHB Derivatives

BSA and DNA solutions were prepared in a buffer solution of pH 7.4 prepared using 0.05 M Tris, 0.15 M sodium chloride, and hydrochloride. To prepare polymer solutions with a concentration between 0.01-0.10 μM, required amounts of the substance were taken from the stock solution and added to the solutions of BSA or pCU19 prepared at physiological pH [phosphate buffered saline (PBS)]. Then, the chloroform (the solvent of the PHB derivatives) was evaporated by leaving the tubes open for a while. Measurements were taken using samples prepared in this manner (BSA-radiopaque PHB derivatives and pCU19-radiopaque PHB derivatives).

Demonstration of the Interaction Between Radiopaque PHB Derivatives and pUC19 were Assessed by Agarose Gel Electrophoresis

Polymer solutions of different concentrations (20 μM, 2 μM, 0.2 μM, and 0.02 μM) and the pUC19 plasmid DNA prepared in order to demonstrate the effect of radiopaque PHB derivatives on pUC19 were incubated in PBS at 37 ºC for 50 min, then 1% agarose gel electrophoresis was carried out at 90 V for 60 min. PBS was used as the negative control, and the pUC19 plasmid without radiopaque PHB derivatives was used as the positive control.

Agarose Gel Electrophoresis Assessment of the Interaction Between Radiopaque PHB Derivatives and PCR Product DNA

A random PCR product obtained from different studies in the laboratory was incubated with different concentrations (20 μM, 2 μM, 0.2 μM, 0.02 μM) of radiopaque PHB derivatives at 37ºC for 50 minutes and carried out at 90 V for 40 minutes in 1% agarose gel electrophoresis. PBS was used as the negative control and the PCR product with and without chloroform and free of radiopaque PHB derivatives was used as the positive control.

Absorbance Spectra of Radiopaque PHB Derivatives with BSA

The absorption spectrum of BSA was found to have an absorption band with a maximum of 265 nm when examined. Changes in the BSA-containing medium after adding radiopaque PHB derivatives were monitored using the absorption spectra.

Results

Measurement and Calculation of the pUC19 Plasmid DNA Using UV-visible Spectrophotometry

UV-Visible spectrophotometry is an effective and easy-to-use method for evaluating interaction between DNA and molecules. It is known that DNA has the highest absorbance value at 260 nm with electron transitions due to chromophoric groups. Thus, molar absorptivity (ε) is at the level of 10^4 M^{-1} cm^{-1} and allows the measurement of DNA concentration. According to the Beer-Lambert Law, the concentration of pUC19 was 8 g/mL for optical density (OD) 260:0.008 after isolation. The A260/A280 and A260/A230 absorbance ratios are used to control the purity of DNA after isolation in various ways. The A260/A280 ratio was calculated according to the Beer-Lambert Law and the ratio of pUC19 plasmid DNA was found to be ~1.8.

Measurement and Calculation of Radiopaque PHB Derivatives Using UV-visible Spectrophotometry

Spectrophotometry measurements of radiopaque PHB derivatives were performed according to their molecular weight (0.001 μM) and dissolved in 1 mL of chloroform, then measured at wavelengths of 200-800 nm using UV-visible spectrophotometry, and 230 nm according to the resulting absorbance-wavelength graph (Figure 2). It was determined to be the preferred wavelength for these measurements.

According to the Beer-Lambert Law, the intensity (1) of light passing through a solution decreases with respect to the inlet (10). Extinction, a measure of OD, is directly proportional to the concentration (c) dissolved in the solution. As long as the average solute dimensions remain constant, a linear relationship is present between the optical density values and the solute density at lower limits of the OD. Absorbance was measured to calculate the extinction coefficient according to the equation "A = εcℓ," where "ε" is the extinction coefficient, "c" is the concentration of the solution, and "ℓ" is the path length. The value of "ℓ" is 1 cm since 1 ml cuvettes were used. "c" is the amount of polymer present in a volume of 1 mL (mL/cm^{-1} mol^{-1}). According to this equation, the calculation of the extinction coefficient of radiopaque PHB derivatives is shown in Table 1. The PHB-DEA-IB-6, PHB-DEA-IB-1, and PHB-DEA-IB-12 polymers were measured by preparing 1.0×10-3 M stock solutions in chloroform. In the continuation of the study, PHB-DEA-IB-6 is P1, PHB-DEA-IB-1 is P2, and PHB-DEA-IB-12 is P3.

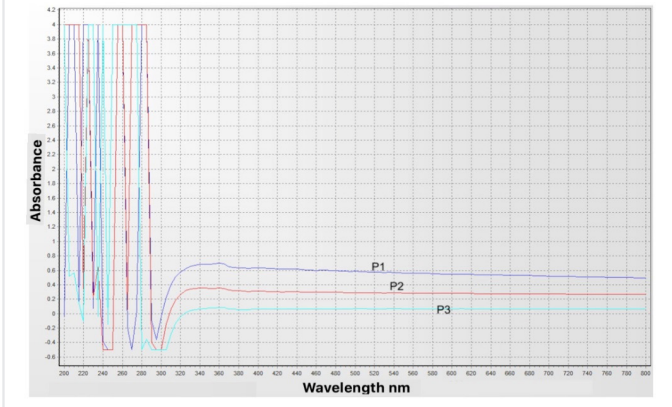

Figure 2. Measurement of P1, P2, and P3 at 200-800 nm with UV-visible spectrophotometry

UV: Ultraviolet

After the determination of the extinction coefficient, it was possible to calculate the absorbance-dependent concentration of radiopaque PHB derivatives without spectrophotometric measurements.

Measurement of BSA by UV-visible Spectrophotometry

BSA was used as the model protein. Albumin is the plasma protein with the highest concentration that will encounter radiopaque PHB derivatives delivered intravenously. BSA was used because of its availability and ease of operational standards. Structurally, human serum albumin (HSA) and BSA is very similar, as HSA contains only one tryptophan residue instead of two in BSA.

Absorbance values were measured by UV-visible spectrophotometry at 600 nm with varying concentrations of BSA from a stock solution of 2 mg/mL (Figure 3).

pUC19 Measurement of Radiopaque PHB Derivatives by UV-visible Spectrophotometry

Changes in the environment such as pH and ionic pressure create changes in molar absorptivity. Consequently, interactions between polymers and DNA result in an aggregating interaction between the aromatic chromophore and the DNA base pair. These interactions occur as hypochromic and bathochromic effects. The decrease in absorbance with increasing DNA concentration is hypochromism, and the increase in absorbance with increasing DNA concentration is hyperchromism. If DNA is treated with denaturing agents, the double helix is separated from the hydrogen bonds holding it together, and a single chain form arises and various bases can remain free. By decreasing the interaction between bases, the UV absorbance of the DNA increases. The extinction coefficient is calculated using the UV-visible spectrophotometry. The Beer-Lambert Law states that constant molar absorption depends on the concentration of dissolved substances in solution at a specific wavelength.

In this study, interactions between radiopaque PHB derivatives and pUC19 DNA were investigated using a UV-visible spectrophotometry. The absorbance properties of radiopaque PHB derivatives were determined both in non-DNA medium and in DNA containing medium in buffer solution (pH: 7.4). Radiopaque PHB derivatives were dissolved in chloroform to obtain a concentration of ~40 µM. Radiopaque PHB derivatives at 222 nm for P1 and 225 nm for P2 and P3 were kept constant, and 2 µL (10 times) of pUC19 DNA was added and incubated for 5 min with a 5 min incubation time. Figure 4 shows the absorption spectrum of media with radiopaque PHB derivatives and pUC19 DNA.

Considering Figure 4, no significant reduction or increase was found in the intensity of the absorption band of polymers at 222 and 225 nm with the addition of DNA. No significant difference was found in the measured values obtained by the addition of pUC19 DNA, thus the mean of the measurement results were plotted (Figure 4). The absorption spectra in the buffer medium clearly show no interaction between the radiopaque PHB derivatives and pUC19 DNA.

Demonstration of an Interaction Between Radiopaque PHB Derivatives and pUC19 by Agarose Gel Electrophoresis

Polymer solutions of different concentrations (20 µM and 2 µM) and pUC19 plasmid DNA prepared in order to demonstrate the effect of radiopaque PHB derivatives on pUC19 were incubated in PBS at 37 °C for 50 min, and subjected to agarose gel electrophoresis for 60 min at 90 V (Figure 5). After incubation with the P1, P2, and P3 polymers at 20 µM and 2 µM, c, no distinctive break in the structure of the super-stranded DNA sample was detected when the control group (c) containing chloroform was compared to the group (d) without chloroform. It was seen as a smear because no opening was found in the supercoil structure.

Table 1. Calculation of extinction coefficients of radiopaque PHB derivatives

	Absorbance (mm)	Concentration (µM)	Extinction coefficient (ε) mL/cm-1 mol-1
P1	0.535	0.001	535
P2	0.276	0.001	276
P3	0.066	0.001	66

PHB: Poly-3-hydroxy butyrate

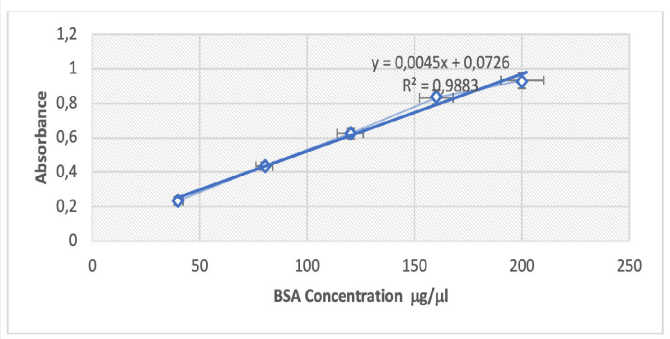

Figure 3. Concentration-dependent absorbance of BSA at 600 nm

BSA: Bovine serum albumin

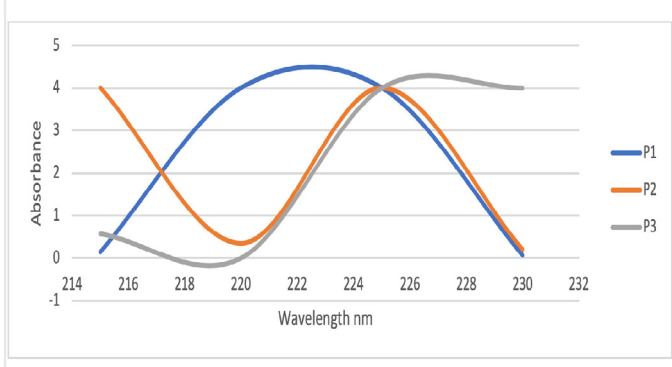

Figure 4. Average spectra of radiopaque PHB derivatives obtained by increasing the amount of pUC19 DNA

PHB: Poly-3-hydroxy butyrate

Agarose Gel Electrophoresis Image of Interaction of Radiopaque PHB Derivatives with the PCR DNA Product

The product obtained by CR is in the form of double-stranded short-chain linear DNA. Stability is poor compared to the circular and supercoil DNA forms. PCR products were treated with different concentrations (0.2 µM and 0.02 µM) of radiopaque PHB derivatives by incubating them together at 37 ºC for 50 min, then subjected to 1% agarose gel electrophoresis for 40 min at 90 V (Figure 5). After incubation with the P1, P2, and P3 polymers at 0.2 µM and 0.02 µM, comparison of the control group (a) containing chloroform and (b) without chloroform, the DNA sample was disrupted when the P1 and P3 polymers were used at 0.2 µM, which prevented band formation in the gel. No differences in DNA structure were detected in two concentrations of the P2 polymer. In addition, chloroform had an effect on DNA.

Investigation of the Binding Balance Between Radiopaque PHB Derivatives and BSA

BSA is a widely studied model protein due to its similar properties to HSA, medical importance, easy availability, low cost, ease of purification procedure, and stability. Many drugs are known to specifically bind to serum albumin. The effectiveness of drugs depends on their ability to bind to BSA. Many studies have been reported in important research areas (such as clinical medicine, life sciences, and chemistry) to clarify the structural properties of drugs after binding to BSA and determine the remedial efficacy of drugs. The absorption spectrum of BSA at physiological pH is presented in Figure 6.

In the absorption spectrum of BSA examination, an absorption band with a maximum of 265 nm was observed. Changes to the BSA medium following the addition of polymers were monitored by the absorption spectra. No significant changes were observed in the absorption spectra of BSA after the addition of radiopaque PHB derivatives. This is important in showing the interaction and binding between BSA and radiopaque PHB derivatives. Figure 7 shows the absorption spectrum of BSA in the presence of 10 µM polymer concentrations.

As shown in Figure 7, no significant increase was found in the intensity of the absorption band at 265 nm of the BSA with the addition of polymers. In addition, no significant increase or decrease in the absorption band at 265 nm of BSA as the polymer concentration increased.

Discussion

In this study, interactions between radiopaque PHB derivatives and BSA and DNA (pUC19 DNA and PCR products) were investigated using absorption spectroscopy and agarose gel electrophoresis.

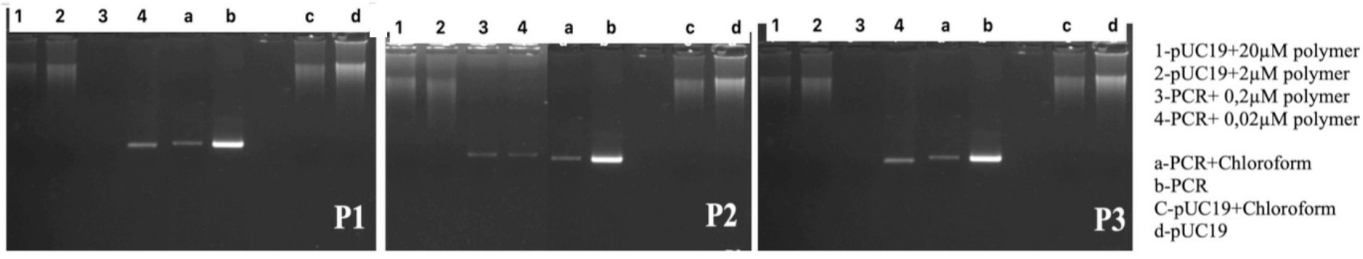

Figure 5. Effect of radiopaque PHB derivatives on pUC19 and PCR product
PHB: Poly-3-hydroxy butyrate, PCR: Polymerase chain reaction

Figure 6. Absorption spectrum of BSA at physiological pH
BSA: Bovine serum albumin

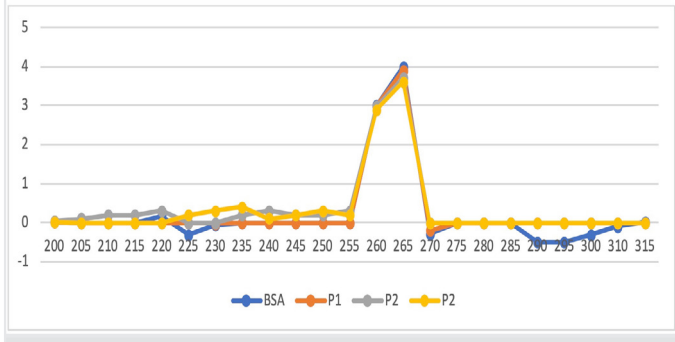

Figure 7. Absorption spectrum of 10 µM BSA concentration in the presence of radiopaque PHB derivatives
BSA: Bovine serum albumin, PHB: Poly-3-hydroxy butyrate

The absorbance properties of BSA were determined in PBS with a pH of 7.4. In the absorption spectrum of BSA examination, an absorption band with a maximum of 265 nm was found. Changes in the absorption spectra of BSA with the addition of radiopaque PHB derivatives are very important in terms of showing the interaction and binding between BSA and polymers. Changes to BSA medium with the addition of radiopaque PHB derivatives were monitored using the absorption spectrum. No significant increase or decrease in the intensity of the absorption band at 265 nm of BSA with the addition of polymers.

The spectral properties of pCU19 DNA were determined in buffer solution at pH 7.4. In the absorption spectrum of DNA examination, an absorption band with a maximum of 260 nm was observed. No significant reduction or increase was found in the intensity of absorption bands of the radiopaque PHB derivatives at 222 and 225 nm with the addition of DNA. This stability in absorbance intensity is indicative that the radiopaque PHB derivatives do not interact with DNA helix.

The pUC19 plasmid DNA and PCR DNA were incubated with iodinated poly-3-hydroxybutyrate and its derivatives, and the interaction of DNA with agarose gel electrophoresis was compared with the positive control, showing no denaturation both at the supercoiled form of plasmid DNA and the double-stranded DNA form of the PCR product. This also coincides with UV-visible spectrophotometry results.

Commercially available intravenous iodinated contrast agents are known as not binding to proteins. Similarly, iodinated poly-3-hydroxy butyrate and its derivatives, which are designed as intravenous iodinated contrast agents, are found to not interact with BSA (2). This information is important for the prediction that the possible interaction of iodinated poly-3-hydroxybutyrate and its derivatives with the intravenous administration of blood plasma proteins is low. At the same time, the lack of interaction with different DNA forms is an advantage over existing commercial products. Thus, it paves the way for intravenous administration *in vivo*.

Conclusion

Adverse reactions to intravenous iodinated contrast media are common; however, severe reactions are rare. The most severe acute reactions and contrast-induced nephropathy occur with the use of contrast-enhanced substances with high osmolarity ionic monomeric intravenous iodine. The best way to treat all adverse reactions is to prevent them from occurring. Identifying patients at risk for side effects and following preventive measures or developing an alternative intravenous iodine contrast agent may help to reduce the incidence of adverse effects. In addition, in situations requiring the use of intravenous iodinated contrast agents, addressing the adverse consequences and potential risks, benefits, and alternatives associated with this class of drug is necessary. For this purpose, interactions of iodized poly-3-hydroxy butyrate and its derivatives with DNA and BSA has been shown. It was found that it does not interact with DNA, unlike currently available contrast agents. No substance interferes with or with the structure of DNA, thus cells in contact with the radiopaque material have no effect on the genomic material. The lack of interaction with DNA forms is advantageous over existing commercial products.

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Ethics

Ethics Committee Approval: The study does not require ethics committee approval.

Peer-review: Externally peer reviewed.

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Original Article

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Bezmialem Science 2021;9(2):140-7

The Relationship Between Stress and Quality Life of Women Working in the Washing Tape of Magnesite Mine and Home Women

Ev Kadınlarında ve Manyezit Madeninin Yıkama Bandında Çalışan Kadınlarda Stres ve Yaşam Kalitesi Arasındaki İlişki

Burcu KÜÇÜKKAYA, Hatice KAHYAOĞLU SÜT

Trakya University Faculty of Health Science, Department of Nursing, Division of Women Health and Diseases Nursing, Edirne, Turkey

ABSTRACT

Objective: To explore the relationship between stress and quality life of women working in the washing tape of magnesite mine and home women.

Methods: This cross-sectional study was conducted in Eskisehir on total of 288 women that working women in the washing tape of magnesite mine (n=144, group 1) and home women who took municipal courses at two townhouses (n=144, group 2) completed structured questionnaire form with personeal features and work status, World Health Organization Short Form of Life- Turkish Version (WHOQOL-BREF-TR) and Depression-Anxiety-Stress scale (DASS-21).

Results: There was a statistically significant difference between physical, psychological, social and environmental dimensions that are sub-dimensions of WHOQOL-BREF-TR between groups and depression, anxiety and stress which are sub-dimensions of DAS-21 Short Form of groups. There was a correlation between physical, psychological, social and environmental sub-dimensions of WHOQOL-BREF-TR and a stress sub-dimensions score of DAS-21 Short Form of group 1 and 2.

Conclusion: While the quality of life of the women working in the washing band of magnesite mine was determined lower than the housewives, the stress status was higher.

Keywords: Home women, magnesite mining, Quality of life, stress, working women

ÖZ

Amaç: Bu çalışmada, ev kadınlarında ve manyezit madeninin yıkama bandında çalışan kadınlarda stres ve yaşam kalitesi arasındaki ilişkinin incelenmesi amaçlanmıştır.

Yöntemler: Kesitsel özellikteki bu araştırma Mayıs-Aralık 2018 tarihleri arasında Eskişehir'de bulunan bir manyezit madeninin yıkama bandında çalışan n=144 kadın (grup 1), Eskişehir'de bir belediyeye bağlı iki belde evinde kurs alan ve ev kadını olan n=144 kadın (grup 2) toplamda n=288 kadın üzerinde yürütülmüştür. Veriler anket formu, Dünya Sağlık Örgütü Yaşam Kalitesi Kısa Formu-Türkçe Versiyon (WHOQOL-BREF-TR) ve Depresyon- Anksiyete- Stres Ölçeği Kısa Formu (DASS-21) ile toplanmıştır.

Bulgular: Gruplar arasında DASS-21 Kısa Form alt boyut ve toplam puanları ile WHOQOL-BREF-TR alt boyut puan ortalamaları açısından istatistiksel olarak anlamlı farklılık belirlenmiştir. Grup 2'nin WHOQOL-BREF-TR'nin alt boyutları olan bedensel, ruhsal, sosyal ilişkiler ve çevre boyutları ile DAS-21 Kısa Formu'nun alt boyutları olan depresyon, anksiyete ve stres arasında ve WHOQOL-BREF-TR'nin alt boyutları olan bedensel ile ruhsal boyutları ve DAS-21 Kısa Formu toplam puanı arasında istatistiksel olarak anlamlı farklılık saptanmıştır. Grup 1'in WHOQOL-BREF-TR'nin alt boyutları olan bedensel, ruhsal, sosyal ilişkiler ve çevre boyutları ile DAS-21 Kısa Formu'nun toplam ve alt boyutları olan depresyon, anksiyete ve stres arasında istatistiksel olarak anlamlı farklılık bulunmuştur.

Sonuç: Manyezit madeninin yıkama bandında çalışan kadınların yaşam kalitesi ev kadınlarına göre daha düşük ve stres durumu daha yüksek bulundu.

Anahtar Sözcükler: Ev kadını, manyezit madeni, çalışan kadın, stres, yaşam kalitesi

Address for Correspondence: Burcu KÜÇÜKKAYA, Trakya University Faculty of Health Science, Department of Nursing, Division of Women Health and Diseases Nursing, Edirne, Turkey
E-mail: burcukucukkaya1992@gmail.com **ORCID ID:** orcid.org/0000-0002-3421-9794

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Introduction

According to the Turkey Statistical Institute, 49.8% of the population of Turkey was female as of 2017. This proportional balance between women and men varies in favor of women in the age group of 65 and over because of the longer life expectancy of women: 44% of the population in this age group is male and 56.0% is female (1). Women who cannot participate in the workforce because they are caring for their family members and performing housework are working in re-production on behalf of society (2).

As the position of women in society for centuries has bent consistently in favor of that of men, women are battling to find their place in the world today and forever. Women have the tremendous privilege of entering recreation in the progression of any nation. Because the existing intellectual and physical contact of women is much long-lasting and common. However, in connection with women's reproductive and hormonal cycles, the indicators of depression and anxiety are extremely sensitive. Given the situation resulting from the biological and physiological differences between the sexes, working conditions are thought to have a positive effect on women's employment (3,4).

Magnesite, a precious metal that contains magnesium in its composition, has both a very thick and a highly pure composition. These formations are in the form of metamorphic carbonates, varying in color and thickness, while they spread variably according to the metamorphic generations in which they are located (3). Magnesite contains MgO and CaO, and geochemical analyses are carried out to determine the proportion of the elements therein (3,5). In a magnesite mine, qualitative changes in the structure of unhealthy factors occur in the working environment with the formation of significant changes in raw material extraction technology characterized by a permanent tendency to increase in the magnesite is composed mechanism. These changes can be listed as dust, noise, microclimate, and changes in the working air (6). In this respect, the exposure status to these changes in women working in the magnesite mine varies *vis-à-vis* those of men.

Quality of life is defined by the World Health Organization (WHO) as an individual's own perceptions in the system of culture and values in their own life (7,8). Quality of life is not only a personal health situation but also a broader concept that includes personal well-being (8,9). The health and well-being of women working on the washing band of a magnesite mine are affected to different degrees in terms of physical, mental, social, and environmental aspects. The traditional indicators used to measure health today (illness, death, life expectancy, etc.) are taken into consideration when determining the affected situations and areas. Due to the inadequacy of definition of individuals' health levels, clarification of the issue from the perspective of quality of life is attempted (6). In this context, stress is one of the most important factors affecting quality of life. Stress is basically a physiological condition and progresses as a process (10). Stress is seen as a relationship between the person and the environment. All internal and external stimuli can be a source of stress. In general, human beings have roles in adaptation to physiological and psychological conditions. Therefore, the important point is the level of stress (11). Studies have shown that when stress is significantly complicated, it is primarily caused by physical diseases such as cardiovascular diseases as well as psychiatric disorders such as depressive disorders and anxiety disorders (12-15). Women working on the washing band of a magnesite mine are exposed to more magnesium individual need by both respiration and contact, compared with homemakers. Magnesite mine workers are exposed to factors that affect their physical and mental health more than individuals who are housewives and have handicrafts in their social life. Therefore, it is important for nurses specializing in women's health and disease to apply nursing care individually and appropriate to the needs of both housewives and women working on the washing band of magnesite mines.

Although it is thought that males work mostly in mines in our country and around the world, women work mostly on the washing band of mines. An examination of the literature did not find a relationship between stress levels and quality of life of mineral workers. In this study on the stress and quality of life of women working on the washing band of magnesite mines, compared with that of housewives, the relationship between stress and quality of life in women will be revealed.

Methods

Study Design

This cross-sectional type study aimed to investigate the relationship between stress and quality of life in women working on the washing tape of a magnesite mine (group 1) and homemakers (group 2). The study was conducted between May and December 2018 in the Tunalı and Esentepe Townhouses of the Eskişehir Tepebaşı Municipality with a magnesite mining company located in Eskişehir.

Research Questions

R.Q.1. Is there a difference is stress between the groups?

R.Q.2. Is there a difference in quality of life and its sub-dimensions between the groups?

Conceptual Framework

In Turkey and around the world, although there is no clear data on the number of women working in mines, it is believed that fewer women than men work in mines. For this reason, it is determined that the study will be carried out in a magnesite mine because it contains Mg, which represents the universe, has a real effect on women's health, both through respiratory exposure and contact when passing through in the mine, and is used in medical treatments. Required written permission for the study was obtained from the magnesite mining company designated for the study. In addition, townhouses are regionally qualified course areas that support the development of individuals through a variety of educational programs, have permission from government agencies, and have a specific vision and mission, which is based on lifelong learning.

Participants

The required sample number was calculated as sufficient to test the two-way hypothesis, with a significance level of α=0.05, a power of 0.95, and an effect size of 0.75. The effect sample size was verified by the study on quality of life among married working women and housewives by Ahmad and Khan (16). The number of samples calculated for each group was 140 in the present study. However, 144 women each group were included as a precaution against possible missing data. The whole sample was divided into two groups by online computer randomization software (17).

The criteria for inclusion in group 1 were as follows: (1) employment in a magnesite mining company located in Eskişehir; (2) age over 18 years and consent given to participate in the study; (3) presence in a magnesite mining company located in Eskişehir during the study period and; and (4) comprehension of the questions of questionnaire form and scales and ability to without problems. The criteria for inclusion in group 2 were as follows: (1) occupation as a homemaker; (2) age over 18 years; (3) consent given to participate in the study; (4) enrollment in courses in Tunalı and Esentepe Townhouses connected to Eskişehir Tepebaşı Municipality during the study period; and (5) comprehension of the questions on the questionnaire form and scales and ability to communicate without problems.

Data Collection

A notice on a bulletin board in the magnesite mining company in Eskişehir, Eskişehir Tepebaşı Municipality, and Tunalı and Esentepe Townhouses was used to recruit participants during the recruitment period from May to December 2018. During the research, the researcher confirmed that participants were eligible to be on the participant list. If they were on the list, the researcher explained the study in detail, and participants agreed and provided oral informed consent. The survey took approximately 30 minutes to complete.

Ethical Approval

To preserve the rights of the participants in the present study, this study was conducted after ethical committee approval was obtained from the Committee for Evaluation of Scientific Research of Trakya University, Faculty of Medicine (BAEK 2018/214), the magnesite mining company in Eskişehir, Eskişehir Tepebasi Municipality, and Tunalı and Esentepe Townhouses gave written permission. Each participant gave oral consent after receiving an explanation of the study goals, questionnaire form, and scales.

Measures

Questionnaire Form: The literature prepared by the researchers consisted of a total of 25 questions that included personal characteristics (gender, educational status, employment status, income level, place of residence, smoking status, use of alcohol, physical activity/exercise status, marital status, spouse; age, educational status, employment status, duration of marriage, type of family, being satisfied with life, how he felt about himself recently, condition, and duration of discomfort), 18 questions about features of their work (daily working hours, weekly working hours, working conditions, working conditions in a safe environment, satisfaction with working conditions, inclusion in work area, frequent pressure, and stressful situations at work), and seven other questions (18-23).

World Health Organization Quality of Life Brief Form-Turkish (WHOQOL-BREF-TR)

The WHOQOL-BREF-TR is an instrument developed by the WHO that allows subjective assessment of quality of life (9). The validity and reliability of the WHOQOL-BREF-TR, composed of 26 questions, were tested in 1999 by Eser et al. (23). The WHOQOL-BREF-TR is spread across four domains, including physical health, psychological health, and social and environmental relationships. The physical domain focuses on the capability of performing daily activities, medicines and treatment compliance, liveliness, pain, inconvenience, sleep, rest, and fatigue. The psychological domain evaluates positive and negative feelings, self-esteem, body image, personal beliefs, and interest. The social relationships domain measures relations with other individuals, friendship, social support, and sex life. Finally, the environmental domain includes questions concerning the domestic environment, physical security and safety, economic status, procurement of healthcare services, opportunities for leisure activities, and the physical environment. The questionnaire is scored from 1 to 5, and each domain score can range between 4 and 20 (as in this study) or between 0 and 100. An overall score is not calculated, and higher score indicates a better QoL (9). In the present study, Cronbach's coefficients of total, physical, psychological, social, and environmental sub-dimensions of the WHOQOL-BREF-TR were 0.96, 0.95, 0.89, 0.93, and 0.92, respectively.

Depression Stress and Anxiety Scale Short Form (DASS-21 Short Form)

The long form was developed by Lovibond and Lovibond in 1995. The short form of the scale was written by Henry and Crawford in 2005 and by Mahmoud et al. (26) in 2010 and is taken from studies conducted by Yilmaz et al. (27) in 2017. The reliability and validity of the Turkish version were determined (24,25). The DASS-21 Short Form is very well-known tool consisting of 21 symptoms divided into 3 subscales (depression, anxiety, and stress) of 7 items each and has excellent reliability estimates. The scores obtained from each of the dimensions depression, anxiety, and stress indicates that the individual is uncomfortable with the problem. There are no negative elements of the scale, the score on each sub-dimension is between 0 and 21, and the total score is 63 (27). For stress, a score between 0 and 7 is normal, a score between 7.01 and 9 is light, between 9.01 and 12.5 is medium, one point forward between 12.51 and 16.5 and one point forward between 16.51 and above are considered stress at very advanced levels (13,28). In the present study, Cronbach's coefficients of total, depression, stress, and anxiety domains of the DASS were 0.99, 0.99, 0.98, and 0.99, respectively.

Data Analyses

Descriptive statistics, independent t-tests, and chi-square tests were used to identify the homogeneity of participants' characteristics and outcome baseline variables between groups. The chi-square test was used for the comparison of categorical data between the groups. The Student's t-test was used to compare the changes between the groups after calculating the changes in mean scores on the WHOQOL-BREF-TR and DASS-21 Short Form. Pearson's Correlation was used for the relationship between the WHOQOL-BREF-TR and DASS-21 Short Form fort the purpose of the study. A value of $p<0.05$ was accepted as the threshold for statistical significance. In the data analysis, SPSS 23.0 statistical software (IBM Corp. Released 2011, IBM SPSS Statistics for Windows, Version 20.0 Armonk, NY: IBM Corp.) was used.

Results

Table 1 and Table 2 present descriptive statistics for general characteristics and work conditions, as well as a comparison of the results of the DASS-21 Short Form and WHOQOL-BREF-TR and the differences between the respective groups. The average ages were 42.1 years [standard deviation (SD) =7.0; group 1)] and 41.9 years (SD =6.3; group 2). There existed no statistically significant differences between the two groups in terms of age, spouse's age, duration of marriage (years), education, income status, family type, and working status of spouse. There existed a statistically significant difference between the two groups in terms satisfaction with life and the feeling recently more (Table 1). Table 2 reveals that the mean stress level of group 1 was 18.57 (SD =5.67) in contrast to 4.55 (SD =5.36) for that of group 2. The difference was statistically significant (t=21.57, p=0<0.001), which was a significant difference in the average of the total and sub-dimension score on the DASS-21 Short Form of the two groups. Moreover, the mean of the physical, psychological, social, and environmental sub-dimensions of the WHOQOL-BREF-TR was 13.32 (SD=2.03), 12.68 (SD=2.53), 10.58 (SD=4.24), and 11.07 (SD=2.85) in group 1, respectively, and 16.43 (SD=2.31), 17.72 (SD=2.79), 17.78 (SD=3.38), and 16.99 (SD=2.89) in group 2, respectively. The difference was statistically significant (t=-12.14, p=0<0.001; t=-16.06, p=0<0.001; t=-15.94, p=0<0.001; and t=-17.45, p=0<0.001, respectively), which was a significant difference in the average of physical, psychological, social, and environmental sub-dimension scores of the WHOQOL-BREF-TR of the two groups.

Table 3 shows the correlation between the WHOQOL-BREF-TR and DASS-21 Short Form in both groups. There was a correlation between the average scores on the physical, psychological, social, and environmental sub-dimensions of the WHOQOL-BREF-TR and the average total scores on the DASS-21 and the depression, anxiety, and stress sub-dimensions of the DASS-21 in group 1 ($p<0.001$). There was a correlation between the average scores on the physical, psychological, social, and environmental sub-dimensions of the WHOQOL-BREF-TR and the average total score on the DASS-21 and on the depression, anxiety, and stress sub-dimensions of the DASS-21 in homemakers ($p<0.001$) (Table 3).

Research Question Testing

R.Q.1. It was found that group 1 perceived their stress level to be 14.03 points higher than that of group 2 ($p<0.001$). Thus, this hypothesis was supported, as shown in Table 2.

R.Q.2. It was found that the level of physical, psychological, social, and environmental sub-dimensions of quality of life in group 1 were, respectively, 3.11, 5.04, 7.19, and 5.92 points lower than those of group 2 ($p<0.001$). Thus, this hypothesis was supported, as shown in Table 2.

Discussion

To our knowledge, this was the first study comparing perceived stress and QoL in women working in on the washing band of a magnesite mine and homemakers. The findings contribute to the evidence that managing the stresses of working conditions and improving quality of life can play a significant direct role in decreasing the negative effect of perceived stress on QoL and that identifying working conditions and recent feelings may be a major priority to improve QoL in women working on the washing band of magnesite mines.

In the study, it was determined that women working on the washing band of a magnesite mine experienced more stress than homemakers (Table 2). The study of Abbas et al. (29), which examined social support for the stress of working and non-working women in Pakistan, reported that working women experienced more stress than non-working women. Studies of Rathod (30) reported that working women have higher stress levels than non-working women. The study by Sharma and Mishra (31) reported that while work, children, and household tasks are the most common stress factors in working women, children, articles and stress constructors are the stress factors in non-working women, and working women have higher stress levels compared with non-working women. Studies of Shukla et al. (32) reported that working women have higher stress levels than non-working women. In the study of Patil (33), which examined the stress levels of working and non-working women, it was reported that working women experienced more stress than non-working women. A study by Anindita and Vijaya (34), which examined the relationship between working and non-working women's modernization status and stress, included 100 women and 100 non-working women. They found that working and non-working women differed significantly in terms of modernization, and employees had higher scores on the stress scale than those who did not work (34). In contrast to our study, the study of Arslan and Ceviz (35), in which housewives and working women evaluated the prevalence of obesity and healthy lifestyle behaviors, did not find a significant difference between housewives and working women in terms of stress management. Since the stress level of working women was higher than that of non-working women, our study findings show parallelism with the literature. Due to the difficult working conditions of women

Table 1. The general characteristics and working conditions of the two study groups

Variables	Group 1 n (%) or M ± SD	Group 2 n (%) or M ± SD	x^2 or t (p)
Age	42.11±6.95 (min: 24, max: 58)	41.90±6.30 (min: 23, max: 56)	0.29 (0.790)
Age of the spouse	45.40±6.84 (min: 24, max: 60)	46.31±7.10 (min: 28, max: 61)	-1.09 (0.276)
Duration of marriage (yrs)	21.25±7.95 (min: 1, max: 39)	21.30±6.85 (min: 4, max: 37)	-0.06 (0.953)
Working time (per month)	81.9±58.4 (min: 24, max:58)	-	-
Weekly working time (hrs)	53.30±0.00	-	-
Education			
Primary and lower	121 (84.0)	116 (80.6)	0.60 (0.440)
High school and upper	23 (16.0)	28 (19.4)	
Income status			
Less income than expense	6 (4.2)	9 (6.3)	0.63 (0.426)
Income equal to expense	138 (95.8)	135 (93.8)	
Family type			
Nuclear family	137 (95.1)	137 (95.1)	0.00 (1.000)
Extended family	7 (4.9)	7 (4.9)	
Working status of spouse			
Working	90 (62.5)	105 (72.9)	2.97 (0.226)
Not working	5 (3.5)	7 (4.9)	
Retired	43 (29.9)	32 (22.2)	
Satisfaction with life			
Satisfied	56 (38.9)	136 (94.4)	100.00 (<0.001)
Not satisfied	88 (61.1)	8 (5.6)	
Predominant recent feeling			
Happy	36 (25.0)	121 (84.0)	104.75 (<0.001)
Unhappy	21 (14.6)	2 (1.4)	
Depressed	0 (0.0)	1 (0.7)	
Stress	76 (52.8)	17 (11.8)	
Tension	8 (5.6)	2 (1.4)	
Excitement	3 (2.1)	1 (0.7)	
Before working in the magnesite mine, take any precautions for Occupational Safety			
Yes	132 (91.7)	-	-
No	12 (8.3)	-	
General tests (blood, lung film, breathing tests) before starting work on magnesite mine			
Yes	144 (100.0)	-	-
The status of thinking that there is a level of noise that will affect you in your workspace			
Yes	144 (100.0)	-	-
Speaking out loud when communicating in your daily life			
Yes	120 (83.3)	-	-
No	24 (16.7)	-	
Feeling under stress often at work			
Yes	133 (92.4)	-	-
No	11 (7.6)	-	
If you feel stressed at work often, do you think the reason is			
New job status	13 (9.7)	-	
The situation of not fully recognizing the mine	13 (9.7)	-	
The case of not wanting to be absent from the mine to increase the burden on other people on the tape	88 (65.7)	-	
The other people on the tape are not willing to work	16 (11.9)	-	
Professional staff	4 (3.0)	-	

M: mean, SD: Sstandard deviation, n: Number, min: Minimum, max: Maximum
[a]x^2: Chi-square test, t: Independent two-sample t-test

working in magnesite mines, people in social and business life can be said to experience more stress than those at home.

In this study, it was determined that the quality of life of homemakers was higher than that of working women on the washing band of a magnesite mine (Table 2). A study by of Ahmad and Khan (16) that examined the quality of life of married women who were working and housewives reported that the quality of life of working women was lower in terms of social and environmental relations. Research has shown a significant difference between working women and homemakers in terms of physical, mental, social, and environmental quality of life (36). In contrast to our study, that of Bhattacharya and Ganguli (37) examined attitudes toward money, material value, and quality of life expressed by working and non-working women, and there was no significant difference in quality of life between working and non-working women. A study by Mukerji and Sharma (38) indicated that the quality of life of working women was higher than that of non-working women because of having a motivating job, feeling safe, feeling confident and satisfied with it, and feeling close to other people who exist in individual lives. Our study findings and the literature showed that the quality of life of housewives is higher than that of working women.

In the study, it was found that the quality of physical, mental, social, and environmental life decreased as depression, anxiety, and stress levels both of working women on the washing band of a magnesite mine and of homemakers increased (Table 3). A study by Rusli et al. (39) has showed that stress in working women is directly related to anxiety and depression, physical and social relationships, and decreased environmental quality of life. It can be said in this direction that stress levels in both working women and homemakers are thought to have a negative effect on quality of life by increasing anxiety and depression.

Study Limitations

With regard to the limitations of this research, this study used a cross-sectional design specific to the targeted participants only. This study focused on assessing the time duration for the development of stress and quality of life among women working on the washing band of a magnesite mine and homemakers. Moreover, it is advisable to test other scales to calculate working stress and health issues in working women in various mining organizations in Turkey. The researchers collected data from women working on the washing tape of the magnesite mine for the current research sample; therefore, the results cannot be generalized to women working in other mines. Thus, for

Table 2. Comparison and results of the DASS-21 Short Form And WHOQOL-BREF-TR between groups

Variables M ± SD		Group 1 M ± SD	Group 2	Differences	t (p)
DASS-21 short form	Depression	16.00±6.62	0.73±2.68	15.27±0.60	25.65 (<0.001)
	Anxiety	15.75±6.96	1.53±3.27	14.23±0.64	22.17 (<0.001)
	Stress	18.57±5.67	4.55±5.36	14.03±0.65	21.57 (<0.001)
	Total	50.48±18.18	6.81±9.55	43.67±1.71	25.48 (<0.001)
WHOQOL -BREF-TR	Physical	13.32±2.03	16.43±2.31	-3.11±0.26	-12.14 (<0.001)
	Psychological	12.68±2.53	17.72±2.79	-5.04±0.31	-16.06 (<0.001)
	Social	10.58±4.24	17.78±3.38	-7,19±0.45	-15.94 (<0.001)
	Environmental	11.07±2.85	16.99±2.89	-5.92±0.34	-17.45 (<0.001)

WHOQOL-BREF-TR: World Health Organization Short Form of Life- Turkish Version, DASS-21: Depression-Anxiety-Stress Scale, M: Mean, SD: Standard deviation, at:Independent two-sample t-test

Table 3. Correlation between the WHOQOL-BREF-TR and DASS-21 short form between groups

Variables		Group 1				Group 2			
DASS-21 short form		WHOQOL-BREF-TR				WHOQOL-BREF-TR			
Physical		Psycholo-gical	Social	Environmental	Physical	Psycholo-gical	Social	Environmental	
Depression	r	-0.38	-0.51	-0.22	-0.45	-0.67	-0.57	-0.63	-0.60
	p	<0.001	<0.001	0.010	<0.001	<0.001	<0.001	<0.001	<0.001
Anxiety	r	-0.35	-0.50	-0.25	-0.43	-0.67	-0.59	-0.64	-0.43
	p	<0.001	<0.001	0.003	<0.001	<0.001	<0.001	<0.001	<0.001
Stress	r	-0.46	-0.72	-0.50	-0.73	-0.69	-0.75	-0.71	-0.75
	p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Total	r	-0.42	-0.61	-0.34	-0.56	-0.80	-0.79	-0.79	-0.80
	p	≤0.001	0.004	0.263	0.128	<0.001	<0.001	<0.001	<0.001

WHOQOL-BREF-TR: World Health Organization Short Form of Life- Turkish Version, DASS-21: Depression-Anxiety-Stress Scale, [a]p: Pearson's correlation

future research, we suggest that, in order to evaluate the effect of magnesite mining on quality of life and stress, it is compared with working women in the same institution but who are not in charge of the mine washing band, and descriptive or experimental techniques should be used to investigate the coping mechanisms of working women in the magnesite mine to measure mental, physical, and psychological health issues. Hence, large samples are advisable for a more extensive assessment of magnesite mining workers and non-working women.

Conclusion

In the study, women working on the washing band of a magnesite mine experienced more stress than homemakers; the quality of life of homemakers was higher than that of working women on the washing band of a magnesite mine; it was determined that both women working on the washing band of a magnesite mine and homemakers experienced increased depression, anxiety, and stress levels, as their physical, mental, social, and environmental quality of life decreased.

According to the findings, we recommend that working conditions should be improved for women working on the washing band of magnesite mines to reduce their stress levels and improve their quality of life, that homemakers have more time allocated to themselves, and that women should take advantage of courses and activities in a shorter time frame so that working women may obtain quality, stress-reducing efforts; moreover, women's health and disease nurses should organize education programs on methods of coping with stress and improving quality of life with a holistic approach to both women working on the washing band of magnesite mines and homemakers, conduct seminars and practices on complementary and alternative treatment methods for coping with stress, and provide follow-up of individuals with tele-nursing practices.

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Ethics

Ethics Committee Approval: To preserve the rights of the participants in the present study, this study was conducted after ethical committee approval was obtained from the Committee for Evaluation of Scientific Research of Trakya University, Faculty of Medicine (BAEK 2018/214).

Informed Consent: Obtained.

Peer-review: Externally peer reviewed.

Authorship Contributions

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

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Original Article

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Effectiveness of Radiofrequency Ablation Therapy in Osteoid Osteoma: Our 5 Years Experience

Radyofrekans Ablasyon Tedavisinin Osteoid Osteomadaki Etkinliği: 5 Yıllık Tecrübemiz

Temel Fatih YILMAZ

Bezmialem Vakıf University Faculty of Medicine, Department of Radiology, İstanbul, Turkey

ABSTRACT

Objective: To evaluate the results of treatment with computed tomography (CT) guided percutaneous radiofrequency ablation (RFA) for osteoid osteomas (OO) and to evaluate imaging findings.

Methods: Sixty-three patients (18 female, 45 male) with OO included. Patients were diagnosed by clinic and radiologic findings. Mean age was 18.2 (4-51). Ablation was performed under sedoanalgesia and local anestesia in CT room. Eleven-fifteen gauge coaxial needle was introduced to nidus and 17 gauge RF probe was placed to nidus trough coaxial system. Ablation duration was 6 minutes.

Results: Mean nidus size was measured as 8.3 mm (2-16 mm). As a result of statistical analysis, as the size of the nidus increased, the chance of detecting nidus increased on both plain radiography and magnetic resonance imaging ($p<0.001$). In 63 patients who underwent RFA, only 3 patients were operated for relapse. One patient received RFA for the second time because of recurrence. Our primary success rate in the treatment of osteoid osteoma was 93.6% (59/63) and overall success rate was 95.4%.

Conclusion: RFA is a minimally invasive, safe and effective treatment option and has many advantages in the treatment of osteoid osteoma. RFA should be the method of choice for the treatment of osteoid osteoma.

Keywords: Radiofrequency ablation, osteoid osteoma, MRG, nidus

ÖZ

Amaç: Osteoid osteomlar (OO) için bilgisayarlı tomografi (BT) eşliğinde perkütan radyofrekans ablasyon (RFA) ile tedavi sonuçlarını değerlendirmek ve görüntüleme bulgularını değerlendirmek.

Yöntemler: OO tanısı olan 63 hasta (18 kadın, 45 erkek) çalışmaya dahil edildi. Hastalara klinik ve radyolojik bulgular ile tanı konuldu. Yaş ortalaması 18,2 (4-51) idi. BT odasında sedoanaljezi ve lokal anestezi altında ablasyon yapıldı. On bir-on beş gauge koaksiyel iğne nidusa yerleştirildi. Koaksiyel sistem içerisinden 17 gauge RF probu lezyona ulaştırıldı. Ablasyon süresi 6 dakika idi.

Bulgular: Ortalama nidus boyutu 8,3 mm (2-16 mm) olarak ölçüldü. İstatistiksel analiz sonucunda, nidusun boyutu arttıkça, hem düz radyografi hem de manyetik rezonans görüntülemede nidus saptama şansı artmıştır ($p<0,001$). RFA uygulanan 63 hastada sadece 3 hasta nüks için opere edildi. Bir hasta rekürrens nedeniyle ikinci kez RFA aldı. Osteoid osteoma tedavisinde primer başarı oranımız %93,6 (59/63) ve genel başarı oranımız %95,4 idi.

Sonuç: RFA minimal invaziv, güvenli ve etkili bir tedavi seçeneğidir ve osteoid osteomanın tedavisinde birçok avantaja sahiptir. RFA, osteoid osteomanın tedavisi için tercih edilen yöntem olmalıdır.

Anahtar Sözcükler: Radyofrekans ablasyon, osteoid osteoma, MRI, nidus

Address for Correspondence: Temel Fatih YILMAZ, Bezmialem Vakıf University Faculty of Medicine, Department of Radiology, İstanbul, Turkey
E-mail: temelfatihyilmaz@gmail.com **ORCID ID:** orcid.org/0000-0002-3752-8234

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Introduction

Osteoid osteoma (OO), first described by Jaffe in 1935, accounts for 10% of all benign bone tumors. OO is a benign osteoblastic lesion characterized by an osteoid tissue nidus. OO is seen in children and young adults (1).

The most important clinical symptom is pain that is more severe at night and responds well to salicylates or other nonsteroidal anti-inflammatory drugs. This is due to the secretion of different amounts of prostaglandins at the tumor side (1).

There are basically three different approaches to treatment. These are surgery, conservative treatment and percutaneous ablation therapy.

Traditional surgical treatment can be very difficult for both the patient and the surgeon. It can be difficult to recognize the tumor during surgery, and incomplete removal can lead to recurrence. Resection of the weight-bearing bone may limit activities for a long time (2).

Approximately 3 years after the onset of symptoms, invasive treatment becomes a necessity in the majority of patients receiving drug therapy. The reason for this is intolerance and side effects that develop as a result of long-term use of drugs. Surgical excision was the preferred method of treatment in the past, and less invasive techniques were developed due to difficult access, risk of complications and long-term postoperative recovery.

Recently, computed tomography (CT)-guided percutaneous radiofrequency ablation (RFA) treatment has become the most frequently used treatment option (3). In this article, we will report the imaging findings of OO and the results of RFA treatment performed in patients with OO in our center.

Methods

Patient Selection

Sixty three patients who received percutaneous RFA treatment in our interventional radiology clinic between January 2015 and December 2019 were included in our study. Patients' data were analyzed retrospectively. Ethics committee approval was obtained prior to the study. Before the procedure, informed consent was obtained from the patients who underwent the procedure. The diagnosis of OO was made in the light of both clinical and radiological findings, and CT imaging was performed in all patients.

Forty five of the patients were male and 18 of them were female. The mean age was 18.2 years (4-51). The nidus measurement of OO was made by CT and the longest nidus size was measured.

CT scans were available in all patients, and direct radiography and magnetic resonance (MR) imaging could not be obtained in all patients. Fifty of the patients had direct radiography and 54 had MR imaging. Presence of nidus and periost reaction with existing imaging modalities were evaluated. Histopathological verfication was not applied to the patients before processing, and the diagnosis was made in the light of clinical and radiological findings.

Procedure

The procedure was performed by two interventional radiologists with 15 years and 8 years of experience in the CT room. The procedure was performed under sedoanalgesia under the supervision of an anesthesiologist, and the patients' complete blood count and coagulation parameters were checked before the procedure.

The intervention was made in the shortest and easiest way to provide the stability of the coaxial system to the lesion, away from vascular and neural structures. After aseptic cleaning and dressing, 3 to 5 cc of local anesthetic agent (prilocaine hydrochloride 2%) was injected into the area to be treated and local anesthesia was applied. With the help of a 11-15 gauge coaxial needle-charged drill, the lesion was reached by passing through the skin, subcutaneous tissue, muscle plans, periosteum and cortex. Meanwhile, in order to show the accuracy of the needle direction, 1 mm thick multi-slice CT imaging was performed on the lesion area. After deciding the correctness of the localization, 17 Gauge "cooled type" RF probe and RF generator were connected in the coaxial system. Care was taken to place the RF probe in the middle of the nidus (Figure 1 and 2). Nidus lesions with a long diameter of 7 mm or below, with a 7 mm active tip, and lesions with a nidus of up to 12 mm long, were ablated with a RF probe with 10 mm active tip. The lesions with a long dimension of more than 12 mm were entered in their polar parts separately and ablated in two parts. The ablation energy was determined automatically by the generator according to the capacitance of the RF probe, and it usually reached a temperature of 900C. Ablation time was 6 minutes. After ablation, the RF probe and coaxial system were carefully removed so as not to damage the bone structure. After the procedure, our patients were followed up by anesthesiologists in our outpatient service for recovery, and they were hospitalized in the interventional radiology service after the recovery. After the procedure, 15 mg/kg paracetamol was given to the patients for pain control. The patients were discharged the next day after staying in the hospital for one night. The patients were recommended bed rest for 1 week and activity restriction for 1 month. The patients were called for a control examination 3 months later.

Table 1. Process and imaging results

Number of patients	63
Male	45
Female	18
Nidus visibility	
CT	100%
MR	77.8%
DG	54%
Mean age	18.1 (4-51)
Primary procedure success	93.6%
Total procedure success	95.4%

CT: Computed tomography, MR: Magnetic resonance

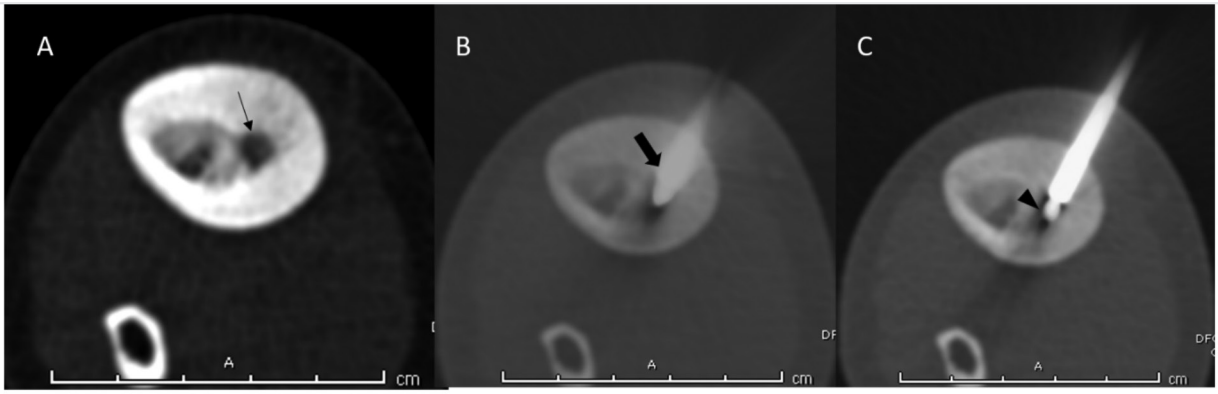


Figure 1 A-C. An 11-year-old male patient was admitted to the orthopedic clinic due to right leg pain that increased at night and continued for 6 months, and an OO lesion with a cortical nidus (black arrow) was found in the right tibial diaphyseal section. The patient underwent CT-guided radiofrequency ablation, and under sedoanalgesia, the 15-gauge coaxial system was placed in the nidus under local anesthesia (thick arrow). The RF probe with a 17 gauge 10 mm active tip was placed in the nidus through the coaxial system (arrowhead), and the lesion was ablated for 6 minutes at 90 C

OO: Osteoid osteoma, CT: Computed tomography

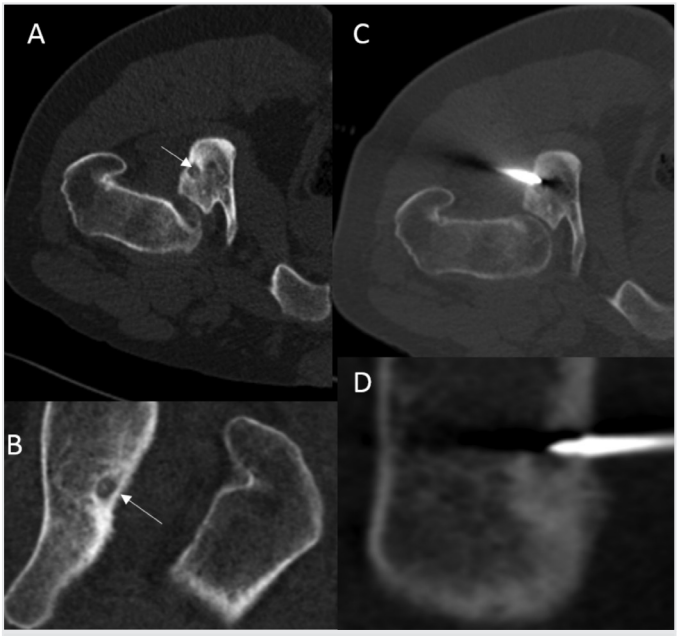
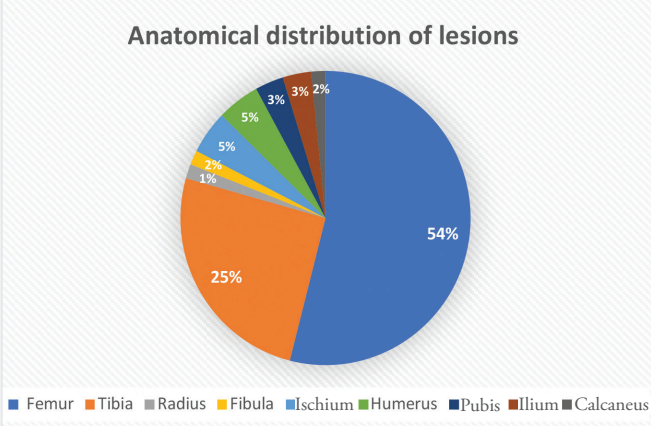


Figure 2 A-D. In the examinations of 33-year-old male patient who was admitted with right hip pain, a lesion compatible with an osteoid osteoma with a 7 mm nidus located in the ischium in the posterior part of the acetabulum in axial (A) and coronal (B) CT sections (arrow). The patient was placed in the prone position and the lesion was entered with a CT-guided 11-gauge coaxial needle. Ablation procedure was performed with 17 gauge RFA probe in the coaxial system (C, D)

CT: Computed tomography, RFA: Radiofrequency ablation

Statistical Analysis

Basic clinical and radiological features of the patients were evaluated with the help of Microsoft Excel program. The relationship between nidus visibility and nidus size in X-ray and MR imaging was evaluated using the Mann-Whitney U test with the help of the SPSS version 20 package program.



Graphic 1. Anatomical distributions of osteoid osteomas

Results

Lesion locations were femur in 34 patients (54%), tibia in 16 patients (25%), radius in 1 patient (1.6%), fibula in 1 patient (1.6%), humerus in 3 patients (5%), and ischium in 3 patients (5%). It was pubis in 2 patients (3.1%), iliac bone in 2 patients (3.1%), and calcaneus in 1 patient (1.6%).

The mean nidus size was measured as 8.3 mm (2-16 mm).

As a result of the statistical analysis, as the size of the nidus increased, the chance of detecting nidus on both X-ray and MRI increased ($p<0.001$).

Nidus was detected in 27 (54%) of 50 patients who underwent X-ray. In 28 (77.8%) of 36 patients with periosteal reaction detected by CT imaging, periosteal reaction was detected by X-ray. This rate was calculated as 74% by MR imaging.

Medullary bone marrow edema was detected in 52 (96.3%) of 54 patients with MRI. Nidus was detected in 42 (77.8%) of 54 patients by MR imaging.

Nidus was present in all patients with CT. In 57% of patients with OO, periosteal reaction was found (36/63).

In 63 patients who underwent RFA, only 3 patients were operated due to recurrence. One patient received RFA therapy for the second time due to relapse. Our primary success rate in OO treatment was found to be 93.6% (59/63), and our overall success was calculated as 95.4%.

After the procedure, fracture developed in 1 patient after cortical lesion ablation in the femoral neck, and the fracture was fixed with open surgery.

In one patient, RFA probe breakage occurred in a corticomedullary lesion in the distal part of the left femur after the procedure, and removal was achieved by surgery. Our complication rate was found to be 3.2% (2/63).

Our mean follow-up period was calculated as 30.1 months (1-60 months).

Discussion

Conservative approach, surgical and percutaneous local treatment options are available for treatment of OO (4). Although OO is treated with nonsteroidal anti-inflammatory drugs, spontaneous regression can be observed in some patients (2,5). In patients in whom conservative treatment fails, the classical treatment approach is surgery (6). However, in order to completely excise the tumoral tissue with surgical treatment, wide resection can be performed from normal bone tissue, as a result, patients have weakness in the bone structure. Accordingly, prolonged activity restriction, prolonged recovery and increased treatment complications occur (7-9). Surgical complications increase in anatomically difficult parts such as acetabulum, glenoid femoral head and neck (10). RFA is the preferred treatment modality because it significantly reduces the duration of hospital stay and healing time compared to surgery. Success rates with this treatment method have been reported between 70% and 100% (11). Chahal et al. (12) reported the primary success rate as 89.7% in their study. Mahnken et al. (3) reported a success rate of 92% (11 out of 12 patients) after one or two procedures using bipolar RFA. Rosenthal et al. (13) reported a primary success rate of 91% in a study performed on RFA for OO using monopolar RF ablation (3). In our study, our primary success rate was 93.6%. Our total success rate was 95.4%.

In our study, we performed RFA treatment with sedoanalgesia and local anesthesia. Some authors recommend treatment under general anesthesia for pain control and position stabilization (4,5). However, we did not have any difficulties in position stabilization and pain control during the procedure. In addition, we did not experience complications due to anesthesia.

Complications such as skin burns, skin and fat necrosis, soft tissue infections, vasomotor instability, tendinitis, and hematoma were reported in previous studies (14-16), and femoral neck fracture developed in 1 patient in our study. Surgical removal was performed in 1 patient due to RF probe breakage. Our complication rate was found to be 3.2% (2/63).

Theoretically, the main disadvantage of this method is that the diagnosis is not histopathologically confirmed. However, some authors argue that the diagnosis is predominantly made clinically and radiologically, that histopathological confirmation is not required, and that its absence does not interfere with the clinical outcome (17). Hoffman et al. concluded that biopsy before treatment was not mandatory because of the considerable amount of false-negative findings in patients with clinically and morphologically uncertain OO. They concluded that 14 (48%) of 29 patients were diagnosed as having OO with biopsy (18).

Typical radiographic findings of OO include cortical nidus with varying degrees of mineralization and cortical thickening, and reactive sclerosis. The nidus is round or oval and usually smaller than 2 cm (19). Bone density can be reduced due to pain-related use. Although the nidus is very well seen on CT, it appears as an oval or round-shaped hypodense focus. Reactive sclerosis is prominent and can range from mild to large periosteal recession that may obscure the nidus.

Various studies have shown that MR imaging has a limited value in showing the nidus compared to CT (20). In our study, while the nidus was detected at a frequency of 100% with CT, this rate remained at 77.8% with MRI. With X-ray, this rate remained at 54%.

Small niduses may not be detected with MRI. In our study, a statistically significant positive correlation was found between the increase in nidus size and visibility of nidus with MRI.

CT emerges as the best imaging method that can be chosen to show the nidus. In particular, multiplanar imaging can show nidus in all patients (20). In our study, the nidus was visible in all 63 patients. Recent studies have reported that the diagnostic value of MR imaging has increased, especially in OO with medullary localization. In our study, medullary OO was present in 1 patient. Its nidus was detected after it was diagnosed with MRI. In addition, bone marrow and soft tissue edema, joint effusion, and synovitis are better demonstrated in MR imaging than CT. In our study, bone marrow was detected at a frequency of 96.3% in patients with OO (21-25).

Study Limitations

The small number of our patients, lack of standardization of X-ray, CT and MRI images of the patients referred to our center for treatment from an external center are not standardized, and therefore, the inability to obtain homogeneous imaging findings were among the limitations of our study.

Conclusion

In conclusion, RFA is a minimally invasive, safe and effective treatment option with low complication rate, and has many advantages in the treatment of OO. For these reasons, RFA should be the method of choice for the treatment of OO.

Ethics

Ethics Committee Approval: Approval was obtained from Bezmialem Vakıf University Non-interventional Research Ethics Committee (date: 16.02.2021).

Informed Consent: Retrospective study.

Peer-review: Externally peer reviewed.

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Original Article

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Carbonic Anhydrase Isoenzymes I and II Inhibition Potentials of *Leiotulus dasyanthus* (K. Koch) Pimenov&Ostr. and *Ferulago pauciradiata* Boiss.&Heldr. (Apiaceae)

Leiotulus dasyanthus (K. Koch) Pimenov&Ostr. ve *Ferulago pauciradiata* Boiss.&Heldr.'nın (Apiaceae) Karbonikanhidraz İzoenzimleri I ve II İnhibisyon Potansiyelleri

Songül KARAKAYA[1], Zeynebe BİNGÖL[2], İlhami GÜLÇİN[3], Fatmagül DELİMUSTAFAOĞLU[4], Hayri DUMAN[5], Ceyda Sibel KILIÇ[4]

[1]Atatürk University Faculty of Pharmacy, Erzurum, Turkey
[2]Gaziosmanpaşa University, Vocational School of Health Services, Tokat, Turkey
[3]Atatürk University Faculty of Science, Erzurum, Turkey
[4]Ankara University Faculty of Pharmacy, Ankara, Turkey
[5]Gazi University Faculty of Science, Ankara, Turkey

ABSTRACT

Objective: Members of the Apiaceae family have many biological and pharmacological effects like anticancer, antidiabetic, antioxidant, anti-inflammatory, anti-hypertensive, anticoagulant, antimicrobial, and anticholinesterase. Also, some of them are consumed as vegetables, pickles, and spices. Carbonic anhydrase has a remarkable role in diseases like osteoporosis, glaucoma, edema, cancer, epilepsy, and obesity. The inhibition effects of methanol extracts and sub-extracts (n-hexane, dichloromethane, ethyl acetate, n-butanol, and aqueous residue) of *Leiotulus dasyanthus* and *Ferulago pauciradiata* roots on carbonic anhydrase I and II isoenzymes were investigated in this research.

Methods: *Leiotulus dasyanthus* and *Ferulago pauciradiata* were collected in 2017 from Erzurum and in 2013 from Nevşehir (Turkey), respectively. The roots of *L. dasyanthus* and *F. pauciradiata* were macerated with methanol, dispersed with methanol: distilled water (1:9), and fractionated with n-hexane, dichloromethane, ethyl acetate, and n-butanol, in turn. The hCA I and II isoenzymes were

ÖZ

Amaç: Apiaceae familyasının üyeleri antikanser, antidiyabetik, antioksidan, anti-enflamatuvar, antihipertansif, antikoagülan, antimikrobiyal ve antikolinesteraz gibi pek çok biyolojik ve farmakolojik etkiye sahiptir. Ayrıca, bu üyelerin bazıları sebze, turşu ve baharat olarak tüketilmektedir. Karbonik anhidraz osteoporoz, glokom, ödem, kanser, epilepsi ve obezite gibi hastalıklarda önemli bir role sahiptir. Bu çalışmada *Leiotulus dasyanthus* ve *Ferulago pauciradiata* köklerinden hazırlanan metanol ekstreleri ve alt ekstrelerinin (n-hekzan, diklorometan, etil asetat, n-butanol ve sulu artık) karbonik anhidraz I ve II izoenzimleri üzerindeki inhibisyon etkileri araştırılmıştır.

Yöntemler: *Leiotulus dasyanthus* ve *Ferulago pauciradiata* sırasıyla 2017 yılında Erzurum'dan ve 2013'te Nevşehir'den (Türkiye) toplanmıştır. Bitkilerin kökleri metanol ile masere edildi, metanol:su (1:9) ile çözüldükten sonra sırayla n-hekzan, diklorometan, etil asetat ve n-butanol ile fraksiyonlanmıştır. hCA I ve II izoenzimleri, taze insan kanının eritrositlerinden Sepharose-4B-L-Tirozin-

Address for Correspondence: Songül KARAKAYA, Atatürk University Faculty of Pharmacy, Erzurum, Turkey
E-mail: songul.karakaya@atauni.edu.tr **ORCID ID:** orcid.org/0000-0002-3268-721X

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isolated from erythrocytes of fresh human blood via Sepharose-4B-L-Tyrosine-sulfanilamide affinity chromatography. The analysis of hCA I and II isoenzymes was actualized by the Verpoorte method.

Results: The hCA I and II were influentially inhibited by the dichloromethane and ethyl acetate sub-extracts of *F. pauciradiata* root with IC_{50} values of 1.694 μg/mL towards hCA I and 1.677 μg/mL towards hCA II, respectively. Whereas dichloromethane sub-extract and methanol extract of *L. dasyanthus* root inhibited hCA I and II isoenzymes with IC_{50} values of 1.828 μg/mL towards hCA I and 1.852 μg/mL towards hCA II, respectively.

Conclusion: The hCA I and II were influentially inhibited by the dichloromethane and ethyl acetate sub-extracts of *Ferulago pauciradiata* root.

Keywords: Apiaceae, carbonic anhydrase, *Ferulago*, *Leiotulus,* hCA I, hCA II

sülfanilamid afinite kromatografisi yoluyla izole edilmiştir. hCA I ve II izoenzimlerinin analizi Verpoorte metodu ile gerçekleştirilmiştir.

Bulgular: hCA I ve II, *F. pauciradiata*'nın köklerinden hazırlanan diklorometan ve etil asetat alt ekstreleri tarafından IC_{50} değerleri hCA I'e karşı 1.694 μg/mL ve hCA II'ye karşı ise 1.677 μg/mL ile etkili bir şekilde inhibe edildi. *L. dasyanthus* köklerinden hazırlanan diklorometan alt ekstresi ve metanol ekstresi hCA I ve II izoenzimlerini hCA I'e karşı 1.828 μg/mL ve hCA II'ye karşı 1.852 μg/mL IC_{50} değerleriyle inhibe etmiştir.

Sonuç: hCA I ve II, *F. pauciradiata*'nın köklerinden hazırlanan diklorometan ve etil asetat alt ekstreleri tarafından etkili bir şekilde inhibe edilmiştir.

Anahtar Sözcükler: Apiaceae, karbonik anhidraz, *Ferulago*, *Leiotulus,* hCA I, hCA II

Introduction

Enzymes are biological molecules liable for a lot of metabolic processes that are synthesized through the living cells and speed up chemical reactions along with the metabolism in living organisms. Carbonic anhydrases (CAs) are systematized via five gene families such as α-, β-, γ-, δ-, and ζ-CA. The β-CA is found in herbs and several prokaryotes (1). CA is a family of metalloenzymes that are ubiquitous alive organisms. It has considerable pathological and physiological roles like gluconeogenesis, fluid balance, respiration, calcification, tumorigenicity, carbon dioxide (CO_2) and ion transport, carboxylation reactions, pH regulation, acid-base balance, lipogenesis, and many pathophysiological processes. The bicarbonate (HCO_3^-) and proton catalyzed the reversible conversion of CO_2 and water (H_2O) by CA. The clinical utilization of CA inhibitors has been identified for the treatment of glaucoma, neurological disorders such as epilepsy, osteoporosis, cancer, edema, gastric and duodenal ulceration, inflammatory illnesses, and obesity (2-5).

For ages, CA has been known to present in a lot of photosynthetic organisms, and a significant act is expected in their photosynthetic processes. Several organisms might possess various kinds of CAs in different cellular places, and each kind of CA might have a different act in photosynthetic processes. The evolution in our realization of CA function has been reported in a range of photosynthetic organisms, containing micro and macroalgae from marine and freshwater habitats, and cyanobacteria, terrestrial, and aquatic higher herbs (6).

In this study, the "*Leiotulus dasyanthus* (K. Koch) Pimenov & Ostr. and *Ferulago pauciradiata* Boiss. & Heldr. (Apiaceae)" were chosen as the resource of plant extracts. Apiaceae (Umbelliferae) is one of the biggest families containing about 450 genera and 3.700 species in the world. The taxons of Apiaceae are well-known such as medical, culinary, and vegetable herbs. Members of Apiaceae generally have a characteristically strong or aromatic fragrance due to the existence of essential oil or oleoresin. Plants of this family have varied components with many biological and pharmacological activities such as hepatoprotective, vaso-relaxant, cyclooxygenase inhibitory, antibacterial, and antitumor (7). A total of 104 genera and 486 species belonged to the Apiaceae family in Turkey (8). In 1994, *Malabaila dasyantha* has confirmed as a synonym of *L. dasyanthus* (K. Koch) Pimenov & Ostr. In Turkey, *M. dasyantha* is known as "dudakpatlatan" (9) and "mandak" (10). It is utilized in the Turkish conventional medicine for hemorrhoids, nail disorders, and stomachache treatment (11). *Malabaila* Hoffman. genus is represented by seven species in Turkey (9). Likewise, leaves and stems of these species are used for roasting, and making soup and pickle (12). *Ferulago* Koch. genus is represented by 34 species in Turkey (9). In Turkey *F. pauciradiata* Boiss. & Heldr. (Apiaceae) is known as "etekli kişniş"(9). *F. pauciradiata* was first identified in Diagn. Pl. Orient. ser. 1, 10: 37 (1849) (9). *Ferulago* species are traditionally utilized as an antiflatulent, digestive, aphrodisiac, sedative, vermicidal, and treatment against cephalalgia, ulceration, snake bite, hemorrhoid, and skin disorders (10,13).

This study aimed to assess the human CA isoenzymes I and II (hCA I and II isoenzymes) inhibition of roots extracts and sub-extracts of *L. dasyanthus* and *F. pauciradiata*.

Methods

Plant Materials

L. dasyanthus and *F. pauciradiata* were collected in 2017 from Erzurum and in 2013 from Nevsehir (Turkey), respectively and were qualified by Prof. Dr. Hayri Duman. Voucher specimens were stored at the Atatürk University, Pharmacy Faculty Herbarium (AUEF 1284) and Ankara University, Pharmacy Faculty Herbarium (AEF 26360).

Extraction and Fractionation

The roots of *L. dasyanthus* (80 g) and *F. pauciradiata* (80 g) were pulverized and macerated with methanol by a mechanical mixer at 150 rpm. Extracts of *L. dasyanthus* and *F. pauciradiata* roots were filtered and evaporated, which then dispersed with methanol and H_2O solution with 1:9 ratio and fractionated

with 150 mL of n-hexane, dichloromethane, ethyl acetate, and n-butanol, in turn. The combined n-hexane, dichloromethane, ethyl acetate, n-butanol, and aqueous residue sub-extracts were evaporated, respectively, and then weighed. Sums of gained samples are exhibited in Table 1.

Purification and Inhibition Assays of Carbonic anhydrase Isoenzymes

The analysis of hCA I and II isoenzymes was actualized using the Verpoorte method with slight modifications. The isoenzymes hCA I and II were isolated from erythrocytes of fresh human blood via Sepharose-4B-L-Tyrosine-sulfanilamide affinity chromatography. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was utilized to check the purity of isoenzymes. (14). The enzyme unit of CA esterase effect is detected within one min as the hydrolysis of 1 μmol of *p*-nitrophenyl acetate to *p*-nitrophenol and acetate (15). Using the spectrophotometer, the *p*-nitrophenol content is determined at maximum absorbance at 348 nm (UV-1800 Shimadzu, Kyoto, Japan). Protein ingredient was estimated by the Bradford method at 595 nm (16). Bovine serum albumin was utilized as the reference protein. Acetazolamide (AZA) was used as a positive control for isoenzymes hCA I and II. IC_{50} values against extracts and sub-extracts parcel activity (%) were figured out. Three distinctive concentrations were utilized to attain Ki values. Lineweaver-Burk graphics were used for measurements (17).

Results

The methanol extracts of *L. dasyanthus* and *F. pauciradiata* roots were made to liquid-liquid partitioning with hexane, dichloromethane, ethyl acetate, butanol, and H_2O. The CA inhibitory activity of extracts and sub-extracts of *L. dasyanthus* and *F. pauciradiata* roots was carried out. Two physiologically relevant CA isoforms (hCA I and II) were utilized. Findings of enzymes inhibition values were demonstrated in Table 2. Findings attained from this study expressly display the efficacious inhibition activities of these samples toward cytosolic isoforms hCA I and II with low micromolar range.

Extracts and sub-extracts connect to hCA I in a micromolar range. IC_{50} values are ranging from 1.694±0.9828 to 2.783±0.9816 μg/mL for hCA I isoenzyme. Otherwise, AZA as a broad-specific

Table 1. Sums of gained samples from *Leiotulus dasyanthus* and *Ferulago pauciradiata* roots

Extracts/sub-extracts	*Leiotulus dasyanthus*	*Ferulago pauciradiata*
MeOH (g)	8.35	9.02
Hexane (g)	0.91	0.99
CH_2Cl_2 (g)	2.95	3.12
EtOAc (g)	0.88	0.97
BuOH (g)	1.37	1.53
Aqueous residue (g)	1.77	1.97

Table 2. Human carbonic anhydrase isoenzymes (hCA I and II) inhibition values of *Leiotulus dasyanthus* and *Ferulago pauciradiata* roots

Extracts and sub-extracts	IC_{50} (μg/mL for extracts and sub-extracts and μM for AZA)			
	hCA I	r^2	hCA II	r^2
MLR	2.287	0.9824	1.852	0.9817
HLR	2.510	0.9847	2.851	0.9775
DLR	1.828	0.9822	2.294	0.9715
ELR	2.112	0.9804	2.165	0.9755
BLR	1.838	0.9817	2.440	0.9862
WLR	2.483	0.9817	2.200	0.9780
MFR	2.158	0.9778	2.574	0.9802
HFR	2.242	0.9900	2.475	0.9851
DFR	1.694	0.9828	2.475	0.9722
EFR	2.132	0.9841	1.677	0.9833
BFR	2.783	0.9816	2.911	0.9851
WFR	2.179	0.9702	1.878	0.9872
AZA-	1.008	0.9935	0.222	0.9943

MLR: Methanol extract of *Leiotulus dasyanthus* root, HLR: hexane sub-extract of *L. dasyanthus* root, DLR: dichloromethane sub-extract of *L. dasyanthus* root, ELR: ethylacetate sub-extract of *L. dasyanthus* root, BLR: butanol sub-extract of *L. dasyanthus* root, WLR: H_2O sub-extract of *L. dasyanthus* root; MFR: methanol extract of *Ferulago pauciradiata* root, HFR: sub-extract extract of *F. pauciradiata* root, DFR: dichloromethane sub-extract of *F. pauciradiata* root, EFR: ethylacetate sub-extract of *F. pauciradiata* root, BFR: butanol sub-extract of *F. pauciradiata* root, and WFR: H_2O sub-extract of *F. pauciradiata* root.

CA inhibitor, displayed IC_{50} value of 1.008±0.9935 μg/mL toward hCA I due to its extended inhibition of CAs. Among these extracts and sub-extracts, dichloromethane sub-extract of *F. pauciradiata* root indicated the best inhibition toward hCA I with 1,694 μg/mL IC_{50} value. In addition, the butanol sub-extract of *F. pauciradiata* root indicated the lowest inhibition against hCA I with 2.783 μg/mL IC_{50} value. IC_{50} values range from 1.677±0.9833 to 2.911±0.9851 μg/mL for hCA II isoenzyme. Otherwise, AZA as a broad-specific CA inhibitor, displayed IC_{50} value of 0.222±0.9943 μM toward hCA II due to its extended inhibition of CAs. Among these extracts and sub-extracts, ethyl acetate sub-extract of *F. pauciradiata* root indicated the best inhibition against hCA II with 1.677 μg/mL IC_{50} value. Moreover, the butanol sub-extract of *F. pauciradiata* root indicated the lowest inhibition against hCA II with 2.911 μg/mL IC_{50} value. Inhibition effects of whole samples are strongly high when in proportion to the AZA. Furthermore, dichloromethane sub-extract and methanol extract of *L. dasyanthus* root inhibited hCA I and II isoenzymes with IC_{50} values of 1.828 μg/mL toward hCA I and 1.852 μg/mL toward hCA II, respectively.

Discussion

Whole CAs are zinc metalloenzymes that catalyze the conversion of CO_2 and HCO_3^-. Enzymes are omnipresent in nature and are an exemplification of convergent evolution, thus a great number of structurally and sequentially different families of CA were discovered. Plants possess three types of CA (α, β, and γ) (18).

Many natural and synthetic compounds could influence live metabolism via changing enzyme effects and influencing metabolic pathways at low concentrations. The inhibition activities of several antioxidant phenolics and polyphenolic compounds such as hydroquinone, catechol, quercetin, resorcinol, simple phenol, and pyrogallol have been investigated particularly, as well. Moreover, a series of active natural phenolic compounds containing curcumin, resveratrol, dobutamine, catechin, and silymarin were researched for the inhibition of whole catalytically active mammalian CA isoenzymes (1).

The main components of the Apiaceae family are coumarins and essential oils (19). Coumarins acted as classical CA inhibitors with resolved inhibition mechanisms (20). Coumarin compounds such as bergapten, pimpinellin, and umbelliferone were isolated from the roots of *L. dasyanthus* (21). In addition, other coumarin compounds such as prantschimgin and felamidin were isolated from the roots of *F. pauciradiata* (9). Thus, we estimate that CA activity is caused by coumarins in these plants. This paper is the first study on CA inhibition potentials of *L. dasyanthus* and *F. pauciradiata.*

Conclusion

Extracts and sub-extracts of *L. dasyanthus* and *F. pauciradiata* roots were carried out for their isoenzymes hCA I and II inhibition activity. The isoenzymes hCA I and II were influentially inhibited by the dichloromethane and ethyl acetate sub-extracts of *F. pauciradiata* root.

Ethics

Ethics Committee Approval: There is no need for an Ethics Committee Approval.

Informed Consent: The authors were informed about this research, and the informed consent form was signed.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: S.K., Z.B., İ.G., Design: S.K., İ.G., Data Collection or Processing: S.K., F.D., H.D., C.S.K., Analysis or Interpretation: S.K., Z.B., İ.G., Literature Search: S.K., İ.G., Writing: S.K., İ.G.

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Original Article

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Can the Nasal Schirmer Test be Used as a Topographic Test in Facial Paralysis Patients?

Nazal Schirmer Testi Fasiyal Paralizi Hastalarında Topografik Bir Test Olarak Kullanılabilir mi?

Muhammet YILDIZ[1], Ahmet BAKİ[2]

[1]Antalya Training and Research Hospital, Clinic of Otolaryngology, Antalya, Turkey

[2]Üsküdar State Hospital, Clinic of Otolaryngology, İstanbul, Turkey

ABSTRACT

Objective: Mucociliary clearance is an important defense mechanism of the respiratory system. One of the main factors determining mucociliary clearance is the ciliary activity of the respiratory epithelium. The amount of nasal secretion can be easily determined by the recently used nasal Schirmer test. The aim of this study was to investigate the amount of nasal secretion in patients with facial paralysis.

Methods: A total of 50 patients with unilateral facial paralysis were included in the study. Eye and nasal Schirmer test were applied to the patients. The eye Schirmer test results were calculated after 5 minutes and the nasal Schirmer test results were calculated after 10 minutes, in millimeters according to the amount of wetting of the test papers. Schirmer test results of paralyzed and nonparalyzed sides were compared with each other.

Results: The mean paralytic and non-paralytic side eye Schirmer test values of the patients with facial paralysis were 11.14±4.80 and 19.54±4.01, respectively. Mean paralytic and non-paralytic side nasal Schirmer test values were 10.92±4.82 and 20.54±4.31, respectively. Eye and nasal Schirmer test values of the paralytic side were significantly lower than the non-paralytic side ($p<0.05$). In the right facial paralysis group; the right eye and nasal Schirmer test results were significantly lower than the left side results ($p<0.05$). In the left facial paralysis group; the left eye and nasal Schirmer test were significantly lower than the right side results ($p<0.05$). Nasal

ÖZ

Amaç: Mukosiliyer klirens solunum sisteminin önemli bir defans mekanizmasıdır. Mukosiliyer klirensi belirleyen ana faktörlerden biri solunum epitelinin siliyer aktivitesidir. Nazal sekresyon miktarı son zamanlarda sık kullanılan nazal Schirmer testi ile kolaylıkla belirlenebilmektedir. Bu çalışmanın amacı fasiyal paralizisi olan hastalarda nazal sekresyon miktarını araştırmaktır.

Yöntemler: Çalışmaya tek taraflı fasiyal paralizisi olan toplam 50 hasta dahil edildi. Hastaların paralizisi olan ve olmayan taraflarına öncelikle göz Schirmer ve daha sonra nazal Schirmer testleri uygulandı. Göz Schirmer test sonuçları 5 dakika sonra ve nazal Schirmer test sonuçları 10 dakika sonra test kağıtlarının ıslanma miktarına göre milimetre cinsinden hesaplandı. Paralizi olan ve olmayan tarafların test sonuçları birbirleriyle karşılaştırıldı.

Bulgular: Fasiyal paralizi hastalarının paralitik ve paralitik olmayan taraf göz Schirmer değerleri ortalaması sırasıyla 11,14±4,80 ve 19,54±4,01 olarak saptandı. Paralitik ve paralitik olmayan taraf nazal Schirmer değerleri ortalaması sırasıyla 10,92±4,82 ve 20,54±4,31 olarak saptandı. Paralitik taraf göz ve nazal Schirmer değerleri paralitik olmayan tarafa göre istatistiksel olarak anlamlı düzeyde düşük bulundu ($p<0,05$). Sağ fasiyal paralizi grubunda, sağ taraf göz ve nazal Schirmer değerleri sol tarafa göre istatiksel anlamlı düşük saptandı ($p<0,05$). Sol fasiyal paralizi grubunda, sol taraf göz ve nazal Schirmer değerleri sağ tarafa göre istatiksel anlamlı düşük saptandı ($p<0,05$). Grade 2-3 ile Grade 4-5 fasiyal paraliziye sahip

Address for Correspondence: Ahmet BAKİ, Üsküdar State Hospital, Clinic of Otolaryngology, İstanbul, Turkey
E-mail: dr.ahmet170@gmail.com **ORCID ID:** orcid.org/0000-0003-2851-0849

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Schirmer test results of Grade 2-3 and Grade 4-5 facial paralysis patients were not statistically significant (p>0.05).

Conclusion: In this study, the amount of nasal secretion on the paralysis side of the patients with unilateral facial paralysis was lower than the contralateral side. Further studies are needed to determine whether decreased secretion improves in long-term follow-up and the prognostic significance of nasal dryness in facial paralysis.

Keywords: Facial paralysis, intranasal Schirmer test, nasal secretion

hastaların nazal Schirmer test sonuçlarında istatiksel anlamlı farklılık saptanmadı (p>0,05).

Sonuç: Bu çalışmada tek taraflı fasiyal paralizisi olan hastaların paralizi tarafında karşı tarafa göre daha düşük nazal sekresyon miktarı tespit edildi. Daha geniş hasta serilerinde azalmış sekresyonun düzelip düzelmediğini ve nazal kuruluğun fasiyal paralizide prognostik önemini ortaya koyacak yeni çalışmalara ihtiyaç vardır.

Anahtar Sözcükler: Fasiyal paralizi, nazal Schirmer test, nazal sekresyon

Introduction

Mucociliary clearance is an important protection mechanism of the upper and lower respiratory tract. The removal of foreign particles and pathogens from the inhaled air is achieved by the mucociliary function maintained by the collective work of the cilia, mucus cover, and mucus-producing glands (1). Nasal secretion protects the airway epithelium from the harmful effects of the external environment and contributes to the maintenance of normal physiology by keeping the mucosa moist (2,3). Many environmental and individual factors affected the amount of nasal secretion (4,5).

Nasal mucosal secretion and its contents are controlled by the autonomic nervous system. In addition, the parasympathetic activity increases both the amount of secretion and nasal congestion. Any reduction in the parasympathetic activity leads to a more viscous secretion that may alter the mucociliary clearance rate of the nasal mucosa. The parasympathetic innervation of the nasal mucosa is controlled by the superior salivatory nucleus located in the pons. The parasympathetic fibers then continue in the nervus intermedius and coexist with the internal acoustic canal and fallopian canal with the facial nerve (6,7).

Bell's paralysis, also called idiopathic facial paralysis, is the most common form of facial paralysis. The annual incidence varies between 15 and 40 per 100,000. It is most commonly seen in 15-45 age groups, without difference in terms of gender and race. Typically, it is a peripheral sub-motor neuron paralysis that is self-limiting, with acute-onset and of unknown cause, affecting all muscle groups on only one side of the face (8-10). Any condition that affects facial nerve function may also affect the nervus intermedius. The greater petrosal nerve, a branch of N. intermedius, provides parasympathetic innervation of the lacrimal glands, and the Schirmer test is used as a topographic test to demonstrate a reduction in the amount of tears in Bell's palsy (11). The greater petrosal nerve also carries parasympathetic fibers to the nasal cavity and plays a significant role in the amount of nasal secretion and mucociliary clearance. The nasal Schirmer test is an inexpensive, simple, effective, and non-adverse method used to objectively determine the amount of nasal secretion. The amount of nasal secretion was first shown by Saunte, and the effects of a parasympathetic drug (pilocarpine) on tears, saliva, and nasal secretions were determined (12).

No article investigating the effect of Bell's paralysis on nasal mucosal secretion has been reported. Thus, this study aimed to investigate the changes in the amount of nasal secretion between the paralyzed side and normal facial functions in patients with unilateral Bell's paralysis using the nasal Schirmer test.

Method

This study was planned as a prospective study by the otorhinolaryngology clinic and started after the approval of the ethics committee. Participants were informed about the study, and informed consent was obtained. The study was carried out in accordance to the principles of the Helsinki Declaration. A total of 67 patients with peripheral facial paralysis underwent detailed otorhinolaryngologic examination and nasal endoscopic examination. Inclusion criteria for patients with Bell palsy include the following: presence of unilateral facial palsy for <2 weeks without previously medical treatment for facial palsy, no known nasal-paranasal sinus symptoms or nasal-paranasal sinus surgery, and normal nasal examination findings. Exclusion criteria for Bell palsy patients includes the following: patients with facial palsy duration of >2 weeks, previous medical treatment for Bell palsy disease, nasal and paranasal disease symptoms such as allergic rhinitis and rhinosinusitis, nasal septal deviation, nasal polyps, nasal mucosal abnormalities and other structural nasal abnormalities, diabetes mellitus, hepatic impairment, chronic renal failure, hypo or hyper thyroidism, history of topical or systemic drug use affecting the nasal physiology (topical or oral decongestant, antihypertensive, antidepressant, antipsychotic, etc.), smoking and alcohol consumption. In addition, patients with recent head trauma, psychiatric disorder, autoimmune disease, neurodegenerative disease, previous radiotherapy to the head and neck region, and with signs and symptoms of upper respiratory tract infection on the day of the test were also excluded. Steroids and other treatments were started after the eye and nasal Schirmer tests. Facial nerve function was evaluated according to the House Brackmann staging system. A total of 17 patients were excluded from the study. Therefore, 50 patients with facial palsy participated in the study. Patients were divided into two groups as 23 right and 27 left peripheral facial paralysis groups. Age, gender, facial paralysis degree, and admission days of patients were recorded.

Eye and Nasal Schirmer Test

After the patients were adapted to the hospital environment for 15-30 min, they were taken to the Schirmer test room. Environmental temperature was recorded as 20.25 °C ± 0.87 °C

(18.3 °C - 21.9 °C) and humidity as 45.34±14.26 (23%-68%). For eye and nasal Schirmer test, standard Schirmer test paper of 35 mm length and 5 mm width was used (ERC SCHIRMER Tear Test Strip, Turkey). This paper was first curled about 5 mm at one end and inserted into the lower fornix between the middle third and the outer third of the two eyes. Care was taken not to touch the cornea. The test was performed in a dimly lit environment, and the patient was asked not to look at any light source to avoid reflex stimulation. The amount of wetting on the paper was recorded in millimeters (mm) after waiting for 5 minutes. The nasal Schirmer test paper was folded at an angle of 45 degrees from an area of approximately 5 mm from one end, and then placed bilaterally with the help of a speculum and bayonet in parallel with the nasal dorsum and anterior nasal septum after an anterior rhinoscopic examination. The 5 mm portion of the test paper was placed in contact with the anterior nasal mucosa and the rest of the test paper overflowing from the nostril. During this period, care was taken not to touch the turbinates on the lateral nasal wall. After waiting for 10 minutes, the amount of wetting of the paper was recorded in mm.

Statistical Analysis

Statistical Package for the Social Sciences 22 program was used for statistical analysis. The suitability of parameters to normal distribution was evaluated by Shapiro-Wilks test. Descriptive statistical methods (mean and standard deviation) were determined when evaluating the study data. Wilcoxon Signed Rank test was used to evaluate the dependent non-parametric data. One-way Analysis of variance test was used to compare the groups in terms of stages. P<0.05 was considered statistically significant.

Results

Ages of patients ranged from 18 to 79 years, and the mean age of patients was 42.88±14.93 years. 60% of patients were female and 40% were male. A total of 23 patients had right facial paralysis and 27 patients had left side facial paralysis. On the first day, 78% of patients applied, whereas 10% on the second day, 6% on the third day, 4% on the fifth day, and 2% on the seventh day. It was found that 46% of patients presented at the 2nd stage, 38% at the 3rd stage, 8% at the 4th stage, and 8% at the 5th stage (Table 1).

The paralytic eye Schirmer values of patients with facial paralysis ranged from 5 to 26 and the mean value was 11.14±4.80. The eye Schirmer values of the non-paralytic side ranged between 14 and 30 and the mean value was 19.54±4.01. Paralytic side nasal Schirmer values ranged from 4 to 23 and the mean value was 10.92±4.82. The nasal Schirmer values of the non-paralytic side ranged between 13 and 34 and the mean value was 20.54±4.31. The eye Schirmer values on the paralytic side were significantly lower than the non-paralytic side (p=0.001) (Table 2). Nasal Schirmer values on the paralytic side were significantly lower than the non-paralytic side (p=0.001) (Table 2).

The right eye Schirmer values of 23 patients with right facial paralysis ranged from 8 to 26, with a mean value of 13.13±6.01. Schirmer values of the left eye ranged from 15 to 30, with a mean value of 20.82±4.98. Right nasal Schirmer values ranged from 5 to 22, with a mean value of 11.30±5.14. Left nasal Schirmer values ranged from 15 to 34, with a mean value of 21.00±4.35. In the right facial paralysis group, Schirmer results of the right eye were significantly lower than that of the left eye (p=0.001) (Table 3). The right nasal Schirmer results were found to be significantly lower than that of the left nasal cavity (p=0.001) (Table 3).

The left eye Schirmer values of 27 patients with left facial paralysis ranged from 5 to 14, with a mean value of 9.44±2.53. Schirmer values of the right eye ranged from 14 to 25, with a mean value of 18.44±2.56. Left nasal Schirmer values ranged from 4 to 23, with a mean value of 10.59±4.60. The right nasal Schirmer values ranged between 13 and 32, with a mean

Table 1. Demographic data of patients with facial paralysis

Male/female (number patients)	20/30
Age (years)/mean ± SD	42.88±14.93
Facial paralysis side (%)	
Right	23 (46%)
Left	27 (54%)
Stage (House-Brackmann) (%)	
Stage 2	23 (46%)
Stage 3	19 (38%)
Stage 4	4 (8%)
Stage 5	4 (8%)
Applied day (number-%)	
1st day	39 (78%)
2nd day	5 (10%)
3th day	3 (6%)
5th day	2 (4%)
7th day	1 (2%)

SD: Standard deviation

Table 2. Comparison of eye and nasal Schirmer test values of sides with and without facial paralysis

Schirmer test	Paralytic side mean ± SD (min-max) (mm)	Non-paralytic side mean ± SD (min-max) (mm)	p
Eye (n=50)	11.14±4.80 (5-26)	19.54±4.01 (14-30)	0.001
Nose (n=50)	10.92±4.82 (4-23)	20.54±4.31 (13-34)	0.001

Wilcoxon test p≤0.05, SD: Standard deviation, n: Number of patients, min: Minimum, max: Maximum

value of 20.14±4.32. In the left facial paralysis group, the left eye Schirmer results were found to be statistically lower than that of the right eye (p=0.001) (Table 3). Left nasal Schirmer results were significantly lower than that of the right nasal cavity (p=0.001) (Table 3).

According to House Brackmann staging, no statistically significant difference were found in nasal Schirmer test results of patients with Grade 2-3 and Grade 4-5 facial paralysis (p>0.05) (Table 4).

Discussion

In this study, patients with unilateral facial paralysis were evaluated objectively using the eye and nasal Schirmer tests, and lower Schirmer test results were obtained on the paralyzed side than the non-paralyzed side.

The fact that the nasal mucosa is rich in vascular and glandular structures plays an important role both for mucus layer and mucociliary movement. Any factor affecting the mucus layer also has an effect on mucociliary clearance. Nasal glandular secretion is important for nasal mucociliary clearance (13).

Functional problems seen in the lacrimal gland in facial paralysis patients may also be seen in the nasal glands. Changes in the amount and content of nasal secretion affect mucociliary clearance (14). Based on this idea, we planned to investigate the changes in the amount of nasal secretion in patients with unilateral Bell's paralysis with the nasal Schirmer test.

Schirmer test is frequently used in cases where lacrimal function provides information and decreases the amount of tears for various reasons (15). Modified intraoral Schirmer test is used to determine the amount of saliva in cases of salivary gland hypofunction (16). The nasal Schirmer test is an inexpensive and easy to use method that has recently been used and does not cause discomfort to determine the amount of nasal secretion. Server et al. (17) showed that intranasal Schirmer test is a simple and practical method that can be used to determine the amount of nasal secretion in the Turkish population. In addition, the mean value of normal values for the Turkish population was 19±7 mm, and no statistically significant difference was found between test results in terms of gender. In our study, we obtained the Schirmer test results similar to this study in nasal cavities without facial paralysis.

Lindemann et al. (18) performed a nasal Schirmer test for the quantitative assessment of the amount of nasal secretion on 159 non-smokers and 30 smokers. The nasal Schirmer test results of the volunteers who smoked were significantly lower than the control group. In our study, patients who are smoking were excluded from the study to avoid confusing factors.

Boynuegri et al. (19) examined the nasal mucociliary clearance times of patients that are paralyzed and non-paralyzed in 38 patients diagnosed with Bell palsy rhinoscintigraphically. They found a statistically significant increase in clearance time on the paralyzed side compared to the opposite side. The increase of the clearance time is due to reduced parasympathetic activity in the nasal cavity on the paralysis side. This situation was explained by mucosal dryness resulting from decreased mucus secretion as a result of decreased parasympathetic activity. Thus, our study aimed to evaluate the amount of nasal secretion in patients with unilateral facial paralysis using the nasal Schirmer test. Nasal Schirmer test showed a statistically significant decrease in the amount of nasal secretion on the side with facial paralysis compared to the side without paralysis.

Table 3. Comparison of nasal and eye Schirmer test values according to facial paralysis side

Group		Right Schirmer Mean ± SD (min-max) (mm)	Left Schirmer Mean ± SD (min-max) (mm)	p
Right facial paralysis (n=23)	**Eye**	13.13±6.01 (8-26)	20.82±4.98 (15-30)	**0.001***
	Nose	11.30±5.14 (5-22)	21.00±4.35 (15-34)	**0.001***
Left facial paralysis (n=27)	**Eye**	18.44±2.56 (14-25)	9.44±2.53 (5-14)	**0.001***
	Nose	20.14±-4.32 (13-32)	10.59±4.60 (4-23)	**0.001***

Wilcoxon test p≤0.05, SD: Standard deviation, n: Number of patients, min: Minimum, max: Maximum

Table 4. Comparison of nasal Schirmer test values of patients according to Hause-Brackmann stage

House-Brackmann stage	Paralytic side nasal Schirmer mean ± SD (min-max) (mm)	p	Non-paralytic side nasal Schirmer mean ± SD (min-max) (mm)	p
Grade 2-3 (n=42)	11.42±5.04 (4-23)	0.766	21.11±4.29 (15-34)	0.638
Grade 4-5 (n=8)	8.25±2.05 (6-12)		17.50±3.16 (13-21)	

One-way analysis of variance test p≤0.05, SD: Standard deviation, n: Number of patients, min: Minimum, max: Maximum

The cornea needs to be protected in facial paralysis as a result of weakness in eye closure and a decrease in tear amount (20). Tear drops and eye ointments are often used for this purpose. Sterile eye closure or the use of eye protection glasses is recommended to eliminate these possible risks in the cornea (21). In our study, dryness of the eye Schirmer values on the side that developed facial paralysis was found to be statistically significant compared to the other side, thus, preventive treatments and precautions were recommended to these patients. Due to facial paralysis, the sphincter function of the orbicularis oris muscle is impaired as a result of this condition oral incontinence impaired, thus abrasion and ulceration of the lip and cheek mucosa can be observed. These complications can be prevented by developing strategic eating habits (using pipettes for liquids, turning to a soft diet, etc.) and using dental spacers to prevent buccal mucosal irritation during chewing (22). In this study, a decrease in the amount of nasal secretion in the nasal cavity on the side with facial paralysis was found. In addition, impairment of the mucociliary clearance due to facial paralysis has been shown in the literature. The use of nasal moisturizers to promote a nasal mucociliary activity or the development of other treatment modalities may help in preventing nasal or paranasal complications. New clinical studies are needed to detect and manage various nasal and paranasal complications that may develop due to facial paralysis.

Study Limitations

Limitations of this study were the low number of patients and the inability to investigate whether the decreased nasal Schirmer values changed after the treatment. In addition, the fact that the prognostic value of decreased nasal Schirmer values on the paralyzed side during the follow-up of patients was not investigated is another limitation.

Conclusion

In unilateral facial paralysis, a statistically significant decrease was found in the nasal Schirmer test results on the side with paralysis compared to the side without paralysis. Further studies are needed to investigate the effects of reduced nasal secretion caused by unilateral facial paralysis on the nasal mucosa and paranasal sinuses.

Ethics

Ethics Committee Approval: Approval for the study was granted by the Zeynep Kamil Women and Children Diseases Training and Research Hospital Ethics Committee (Protocol Number: 89).

Informed Consent: Written informed consent was obtained from the patients.

Peer-review: Externally peer reviewed.

Authorship Contributions

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

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Original Article

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Kainic Acid and MPP+ Induce Upregulation of GLT-1 in Neuroblastoma and Glia Cells

Kainik Asit ve MPP+ GLT-1'in İfade Artışını Neuroblastoma ve Glia Hücrelerinde Düzenler

Rabia UYARICI, Ceylan AK, Gizem DÖNMEZ YALÇIN

Aydın Adnan Menderes University Faculty of Medicine, Department of Medical Biology, Aydın, Turkey

ABSTRACT

Objective: Glutamate is the major excitatory transmitter in the brain. The excessive glutamate will lead to excitotoxicity. Glutamate transporter-1 (GLT-1) is the major transporter that performs 95% of the glutamate clearance contributing to normal neuronal function and preventing excitotoxicity. In this study, we investigated the effect of two toxins, kainic acid and MPP^+ (1-methyl-4-phenylpyridinium), on GLT-1 expression and excitotoxicity in neuroblastoma and glia cells (immortalized human astrocytes).

Methods: We treated neuroblastoma and glia cells with kainic acid and MPP^+, applied MTT assay to measure the cell viability. We identified the mRNA and protein levels of GLT-1 and also analyzed released glutamate levels using glutamate assay.

Results: The mRNA level of GLT-1 increased in neuroblastoma cells as a result of kainic acid or MPP^+ treatment while the protein expression of GLT-1 increased in glia cells after the treatment with MPP^+. Excess glutamate was found to be decreased after 12 h MPP^+ treatment. However, this decrease was no more prominent with further MPP^+ treatment.

Conclusion: Our results show that GLT-1 levels are elevated as a result of kainic acid or MPP^+ treatment as a survival mechanism to prevent excitotoxicity.

Keywords: Excitotoxicity, GLT-1, glutamate, MPP^+, kainic acid, mRNA

ÖZ

Amaç: Glutamat, beyindeki majör uyarıcı transmiterdir. Fazla glutamat eksitotoksisiteye yol açar. GLT-1 (Glutamat Transportır 1) beyindeki glutamatın %95'ini temizleyerek nöron fonksiyonunu sağlar ve eksitotoksisiteyi önler. Bu çalışmada kainik asit and MPP^+ (1-methyl-4-phenylpyridinium) isimli iki toksinin nöroblastoma ve glia (immortalize edilmiş insan astrositleri) hücrelerinde GLT-1 ve eksitotoksisite üzerindeki etkilerini incelemektir.

Yöntemler: Nöroblastoma ve glia hücreleri kainik asit ve MPP^+ ile muamele edilip hücre canlılığını ölçüldü. GLT-1'in mRNA ve protein ifadesi tespit edildikten sonra glutamat assay ile birikmiş Glutamate miktarı belirlendi.

Bulgular: GLT-1'in mRNA seviyesi kainik asit ve MPP^+ ile muamele sonucu nöroblastoma ve glia hücrelerinde artmıştır. GLT-1'in protein seviyesi de glia hücrelerinde MPP^+ muamelesi sonucu artmıştır. Salınan glutamatın 12 saatlik MPP^+ muamelesinden sonra azaldığı gözlenmiştir. Ancak bu azalış daha sonra geçerli olmamıştır.

Sonuç: Bulgularımız göstermektedir ki GLT-1 ifadesi kainik asit veya MPP^+ muamelesi sonucu yaşamsal bir mekanizma olarak eksitotoksisiteyi önlemek için artmaktadır.

Anahtar Sözcükler: Eksitotoksisite, GLT-1, glutamat, MPP^+, kainik asit, mRNA

Address for Correspondence: Gizem DÖNMEZ YALÇIN, Aydın Adnan Menderes University Faculty of Medicine, Department of Medical Biology, Aydın, Turkey
E-mail: gizem.yalcin@adu.edu.tr **ORCID ID:** orcid.org/0000-0002-5121-8232

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Introduction

Glutamate is one of the major neurotransmitters in the central nervous system. It helps to regulate the principal functions of the brain, among which are learning and memory (1). Glutamate transporter-1 (GLT-1), also known as excitatory amino acid transporter 2 (EAAT2), is the major GLT, performing 95% of glutamate clearance, contributing to normal neuronal function, and protecting against excitotoxicity (2). There is no other extracellular mechanism of glutamate catabolism. After glutamate is taken up by astrocytes, it is either catabolized by glutamate dehydrogenase and the tricarboxylic acid cycle, or it is converted to glutamine by glutamine synthetase. Glutamine is then transported out of the astrocyte, enters the glutamatergic neurons, and is converted to glutamate via the enzyme glutaminase (3). Glutamate interacts with ionotropic and metabotropic receptors on postsynaptic neurons following its release from glutamatergic neurons (4).

Excitotoxicity is observed in age-dependent neurodegenerative diseases and also in stroke, epilepsy, and brain trauma (5). Preventing excitotoxicity will help to slow or halt the progression of these diseases. Excitotoxicity occurs as a result of excessive release of glutamate to the synapse after increased nerve cell stimulation. Excessive glutamate is taken up by GLT-1 present on astrocytes. Dysfunction or reduced expression of GLT-1 has been observed in many neurodegenerative disorders. Therefore, GLT-1 may be a therapeutical target for excitotoxicity (6).

Excitotoxicity may lead to neuronal death via apoptosis or necrosis (7). Overstimulation of glutamate receptors will trigger the entry of excessive Ca^{+2} and Na^{+} into cells via ion channels. Together with the Ca^{+2} released from mitochondria, this influx of Ca^{+2} ions will cause an overdose, resulting in necrosis. Excess Ca^{+2} influx will also lead to endoplasmic reticulum stress. Neuronal death may cause epileptogenic activities. Excitotoxicity is one of the major causes of epilepsy, which is a neurological disease associated with abnormal electrical activity in the brain, resulting in seizures and neuronal death (8). It may be diagnosed at all ages. Kainic acid is an AMPA/kainate receptor agonist and leads to excitotoxicity. Kainic acid is used as a model for status epilepticus in both cell (9) and animal models (10). It causes seizures, increased levels of glutamate in synapses, and neuronal death. It is also associated with neurodegeneration, behavioral phenotypes, oxidative stress, inflammation, mitochondrial dysfunction, and endoplasmic reticulum stress. Kainic acid is an agonist of kainate-class ionotropic glutamate receptors, which are generally excitatory (11). Excess stimulation by kainic acid induces excitotoxicity, which leads to apoptosis and epileptic seizures.

Parkinson's disease (PD) is one of the most common neurodegenerative diseases, in which patients display motor deficit symptoms such as tremor, rigidity, bradykinesia, and akinesia (12). The pathology of PD includes progressive nigrostriatal dopaminergic degeneration and the presence of Lewy bodies. Excitotoxicity is one of the underlying molecular mechanisms of PD (12,13). Current studies indicate the decreased expression and function of EAATs in animal PD models (14). Additionally, increased expression and function of EAATs were shown to attenuate the death of dopamine neurons in the substantia nigra and striatum and ameliorate motor dysfunction in PD animal models. EAATs are potential effective drug targets for PD (14,15).

MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) is a reliable and reproducible chemical model for PD (16,17). It produces damage of the nigrostriatal dopaminergic pathway after systemic administration. After passing through the blood–brain barrier, MPTP is converted to a toxic metabolite called 1-methyl 4-phenylpyridinium (MPP^{+}) by MaoB enzyme in glial cells. Afterwards, MPP^{+} is taken up by the dopamine transporter (17) into dopaminergic neurons, where it inhibits complex I in mitochondria leading to the generation of extensive ROS (17) and oxidative stress.

Neuroblastoma cells are used as a possible model in the study of glutamate receptors (18) and therefore used with kainic acid treatment in order to investigate the excitotoxicity and the molecular mechanism of epilepsy. Neuroblastoma cells are also used with MPP^{+} to investigate PD. Glial cells are also commonly used as a cellular model for elucidating the underlying mechanisms of neurodegenerative diseases.

Both neuroblastoma (N2A) and glial cell types are capable of glutamate release and uptake. Glial cells were shown previously to express kainate receptors (19-21). Kainic acid was demonstrated to destroy both oligodendrocytes and neurons and show its effects on both glia and neurons (22). The vast majority of cell lines, including glioma and oligodendritic cells, have been shown to contain ionotropic glutamate receptors co-existing with CySS/glutamate antiporters and metabotropic glutamate receptors (23).

In this study, we investigated the effect of two different toxins, kainic acid and MPP^{+}, on the expression of GLT-1 using two different cell lines.

Methods

Ethical Approval

Neither humans nor animals were used in the research; therefore, ethical approval was not required.

Cell Culture

Neuroblastoma (N2A) cells were obtained from ECACC. N2A cells were maintained in MEM with 10% FBS, 100 μg/mL penicillin/streptomycin, and 2 mM L-glutamine in 37 °C incubators with 5% CO2. Glial cells (IHA-immortalized human astrocytes) (24) were obtained from Dr. Yavuz Oktay at the IBG (Izmir Biomedicine and Genome Institute) and grown in DMEM with 10% FBS, 100 μg/ml penicillin/streptomycin, and 2 mM L-glutamine.

Kainic Acid and MPP^{+} Treatment

Both kainic acid and MPP^{+} were dissolved in H_2O. Vehicle-treated cells were used as a control. N2A cells were treated with 50 μM, 200 μM, and 1000 μM kainic acid for 24 h. For the

MPP^+ experiment, N2A cells were treated with 10 μM, 50 μM, 200 μM, and 500 μM MPP^+, and IHA cells were treated with 50 μM, 500 μM, and 1000 μM MPP^+ for 24 h.

MTT Cell Viability Assay

The MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Bromide) Cell Viability Assay (Biotum) is a standard test to determine the number of living cells. MTT is a tetrazolium salt metabolized by living cells and converted to purple formazan crystals. It is dissolved in DMSO and measured at 570 nm. The assay was performed according to the manufacturer's guidelines.

Glutamate Assay

The Glutamate-Glo assay (Promega) is a bioluminescent assay for selective and sensitive detection of glutamate in a biological sample. The assay couples glutamate oxidation and NADH production with a bioluminescent NADH detection system and does not measure glutamic acid in the structure of peptides or proteins. The assay was performed according to the manufacturer's guidelines, and the measurement was conducted using a luminometer.

Western Blotting

Protein was isolated from whole cell extracts using RIPA Lysis buffer according to the manufacturer's guidelines (VWR). Protease and phosphatase inhibitors (MedChem Express-100X) were added. Cell extracts were loaded onto 4%-15% gradient SDS-PAGE gels (Bio-Rad) following incubation with loading buffer (126 mM Tris HCl pH 6.5, 25% glycerol, 5% SDS, 5% β-mercaptoethanol, 0.25% bromophenol blue at 95°C for 5 min. Proteins were immunoblotted with anti-GLT-1 (Elabscience) and anti-tubulin (Elabscience) antibodies at 1:1000 and 1:5000 dilutions, respectively. The membranes were then probed with horseradish peroxidase-conjugated secondary antibodies (Elabscience) and developed with enhanced chemiluminescence western blotting substrate (Thermo Scientific). Blots were exposed to HyBlot autoradiography film or visualized with a detection system. The protein bands were quantified by densitometry analysis using ImageJ software (NIH, Washington, DC, USA).

RNA Isolation, cDNA Synthesis, and Real-Time PCR

Total RNA was isolated from N2A cells using the RiboEx solution (GeneAll) following the manufacturer's standard protocols. RNA concentration and purity were determined and confirmed via spectrophotometry. RNA (1 μg) was reverse transcribed using the high-capacity RNA to cDNA kit (WizBio) according to the manufacturer's protocol and used in RT-PCR reactions.

The cDNA was subjected to quantitative PCR analysis with GLT-1- (GLT-1 fwd: AACAATATGCCCAAGCAGGT, GLT-1 rev: CTCCCAGGATGACACCAAAC) and β-actin-specific primers (β-actin fwd: AACTGGGACGACATGGAGAA, β-actin rev: GAAGGTCTCAAACATGATCTGG) using the SYBR Green Gene Expression Assay from GeneAll, and quantifications were performed according to the manufacturers' instructions. The relative abundance of mRNA was obtained by normalization to β-actin mRNA levels.

Statistics Analysis

All statistical analyses were performed using statistical software (GraphPad Software, Inc., San Diego, USA). Specifically, "unpaired, one-tail, equal variance and two-sample t-tests" were performed using Prism 5 software. Significant differences are shown by asterisks indicating *$p<0.05$. Error bars on figures represent the standard error of the mean.

Results

Kainic Acid and MPP^+ Treatment Leads to a Decrease In Cell Viability in N2A Cells

To determine whether kainic acid induces excitotoxicity and cell death, we analyzed cell viability using the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay. We treated N2A cells with 50 μM, 200 μM, and 1000 μM kainic acid for 24 h and observed a significant decrease in cell viability with the 1000 μM concentration (Figure 1A). These results showed that kainic acid-induced excitotoxicity leads to the lowest cell viability at the 1000 μM concentration after a 24-h treatment, when compared with the control.

We also treated N2A cells with 10 μM, 50 μM, 200 μM, and 500 μM MPP^+ for 24 h and observed a significant decrease in cell viability with both 200 μM and 500 μM concentrations (Figure 1B). These results showed that MPP^+-induced excitotoxicity leads to the lowest cell viability at the 500 μM concentration after a 24-h treatment, when compared with the control.

Elevation of GLT-1 mRNA Levels in N2A Cells After Kainic Acid or MPP^+ Treatment

To analyze GLT-1 expression in neuroblastoma cells, we treated N2A cells with kainic acid or MPP^+, isolated total protein, and checked for GLT-1 protein levels. However, we could not visualize GLT-1 protein via western blotting, although we tried several different antibodies. We believe that this was due to the lower level of GLT-1 expression in neuroblastoma cells, compared with that in glial cells. GLT-1 is mainly expressed on astrocyte membranes in order to take up excess glutamate. It is also expressed in neuroblastoma cells but at much lower levels, compared with that in glial cells. Therefore, we isolated total RNA from N2A cells after treatment with 1000 μM kainic acid or 500 μM MPP^+ for 24 h. We identified GLT-1 mRNA expression using real-time quantitative PCR (q-PCR) using β-actin as a reference gene. We observed that both MPP^+ and kainic acid treatment induced much higher expression of GLT1 mRNA in N2A cells than control untreated cells (Figure 1C and 1D).

MPP^+ Treatment Leads to a Decrease in Cell Viability in IHA Cells

To determine whether MPP^+ induces excitotoxicity and cell death, we analyzed cell viability using the MTT assay. We treated IHA cells with 50 μM, 500 μM, and 1000 μM MPP^+ for 24 h and observed a significant decrease in cell viability. The 1000 μM concentration of MPP^+ resulted in the lowest cell viability (Figure 2A).

Elevation of GLT-1 Protein Levels in IHA Cells After MPP^+ Treatment

In order to investigate the effect of MPP^+ treatment on GLT-1 expression, we isolated total protein from IHA cells and analyzed GLT-1 protein levels by western blotting. We treated IHA cells with 1000 μM MPP^+ for 24 h, isolated total protein, and checked for GLT-1 expression. In two different experiments, we observed that GLT-1 levels increase significantly after MPP^+ treatment in IHA cells, compared with those in control cells (Figure 2B).

Elevation of GLT-1 Levels Delays Excitotoxicity in Cells After MPP^+ Treatment

We then analyzed the excess glutamate released in both N2A and IHA cells after MPP^+ treatment as a result of excitotoxicity via a glutamate assay, which measures only secreted extracellular glutamate. Glutamate dehydrogenase uses glutamate and NAD^+ to produce α-ketoglutarate and NADH. The assay couples glutamate oxidation and NADH production with a bioluminescent NADH detection system. We treated N2A cells with 500 μM MPP^+, collected the cell culture media at certain time points such as 0 h, 12 h, 24 h, and 48 h and measured glutamate with the glutamate assay (Figure 3A). At the 12-h time point, the glutamate levels of MPP^+-treated cells were even lower than those of non-treated control cells (Figure 3A). At the 24-h time point, this difference in levels of secreted glutamate between non-treated and MPP^+-treated N2A cells was no longer present. At the 48-h time point, the levels of secreted glutamate of MPP^+-treated N2A cells started to increase and tended to be higher than those of control cells, although the difference was not significant (Figure 3A).

Similarly, we treated IHA cells with 1000 μM MPP^+, and then, at 0, 12, 24, and 48 h, cell culture media was collected, and the excess glutamate level was assayed (Figure 3B). Similar to N2A cells, at the 12-h time point, the glutamate levels of MPP^+-treated cells were even lower than those of non-treated control cells (Figure 3B). At the 24-h time point, this difference in levels

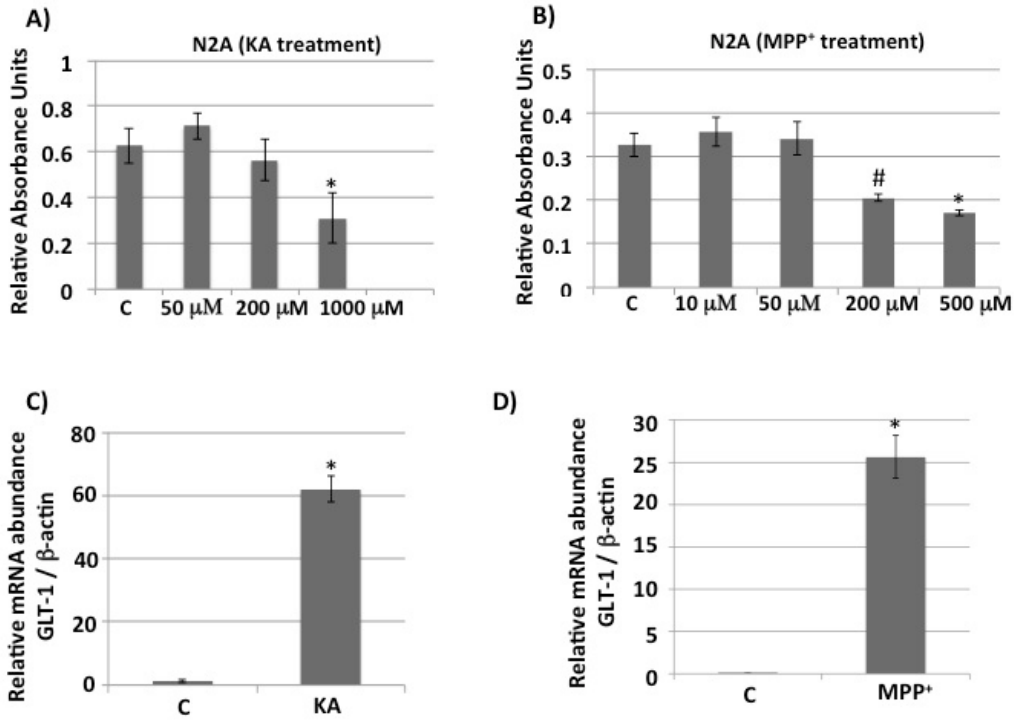

Figure 1. The effect of kainic acid and MPP^+ on cell viability and GLT-1 mRNA levels. Cell viability was measured using an MTT assay. The absorbance at 570 nm is expressed as arbitrary units. The values significantly different from the relative controls are indicated with symbols indicating $p<0.05$. **a)** N2A cells were treated with the indicated concentrations of kainic acid for 24 h. * denotes the comparison between control (C) and 1000 μM kainic acid treatment. **b)** The effect of MPP^+ on cell viability. Cell viability was measured using an MTT assay. The absorbance at 570 nm is expressed as arbitrary units. The values significantly different from the relative controls are indicated with symbols (*, #) indicating $p< 0.05$. N2A cells were treated with the indicated concentrations of MPP^+ for 24 h. * denotes the comparison between control (C) and 500 μM MPP^+ treatment. # denotes the comparison between control (C) and 200 μM MPP^+ treatment. **c)** GLT-1 mRNA expression in N2A cells. GLT-1 mRNA expression detected by q-PCR in non-treated or kainic acid treated N2A cells $p<0.05$. The numbers on y axis show the fold increase relative to β-actin levels. C: non-treated cells KA: Kainic acid treated cells. * denotes the comparison between C and KA. **d)** GLT-1 mRNA expression detected by q-PCR in non-treated or MPP^+ treated N2A cells $p<0.05$. The numbers on y axis show the fold increase relative to β-actin levels. C: non-treated cells MPP^+: MPP^+ treated cells. * denotes the comparison between C and MPP^+. The graph shows the average of three independent experiments

of secreted glutamate between non-treated and MPP+-treated IHA cells was no longer present. At the 48 h time point, the levels of secreted glutamate of MPP+-treated IHA cells started to increase as in the case of N2A cells (Figure 3A) and tended to be higher than those of control cells, although the difference was not significant (Figure 3B).

This result demonstrates that GLT-1 expression levels are elevated as a result of kainic acid or MPP+ treatment as a survival mechanism to take up excess glutamate and prevent excitotoxicity. This may be the reason why the levels of secreted glutamate of MPP+-treated IHA cells after the 12-h time point is even lower than that of control non-treated cells. However, if the insult persists, this transient elevation of GLT-1 might not be sufficient to take up excess glutamate as in the case of the 24 h and 48 h time points (Figure 3A and 3B). Another reason might be that, due to the death of cells after 12 h of treatment, the cell number decreases; therefore, the amount of glutamate secreted is decreased.

Discussion

These results show that, after an insult to the cell that increases excitotoxicity, such as MPP+ or kainic acid, the cell automatically increases GLT-1 expression as a survival mechanism in order to take up excess glutamate. This was the case in both cell types. When we analyzed the levels of secreted glutamate with the glutamate assay in N2A and IHA cells after MPP+ treatment, we observed that, in both cell types, glutamate levels showed a tendency to increase in MPP+-treated cells after 48 h of treatment.

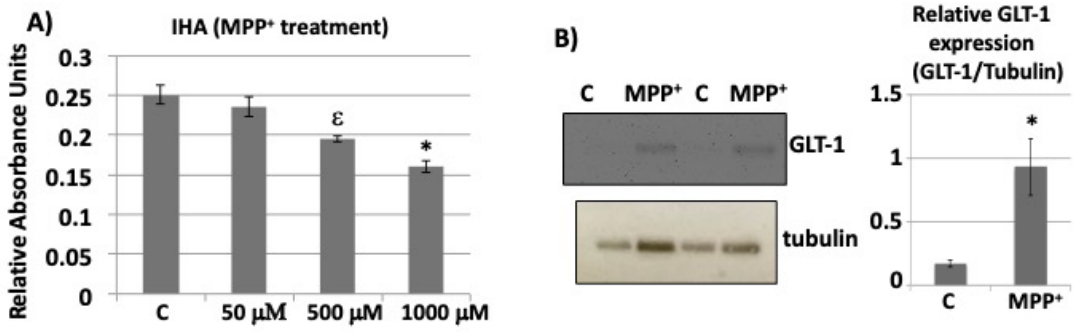

Figure 2. The effect of MPP+ treatment on IHA cells and GLT-1 protein expression level. **a)** IHA cells were treated with the indicated concentrations of MPP+ for 24 h. The values significantly different from the relative controls are indicated with symbols (*, ε) indicating p<0.05. * denotes the comparison between control (C) and 1000 µM MPP+ treatment. ε denotes the comparison between control (C) and 500 µM MPP+ treatment. **b)** A western blot of non-treated (C) or MPP+ treated IHA cell extracts show expression levels of GLT-1, with tubulin as a loading control. Relative protein levels from the blot (the average of two independent experiments) are quantified on the right. *p<0.05. C: non-treated cells MPP+: MPP+-treated cells. * denotes the comparison between C and MPP+

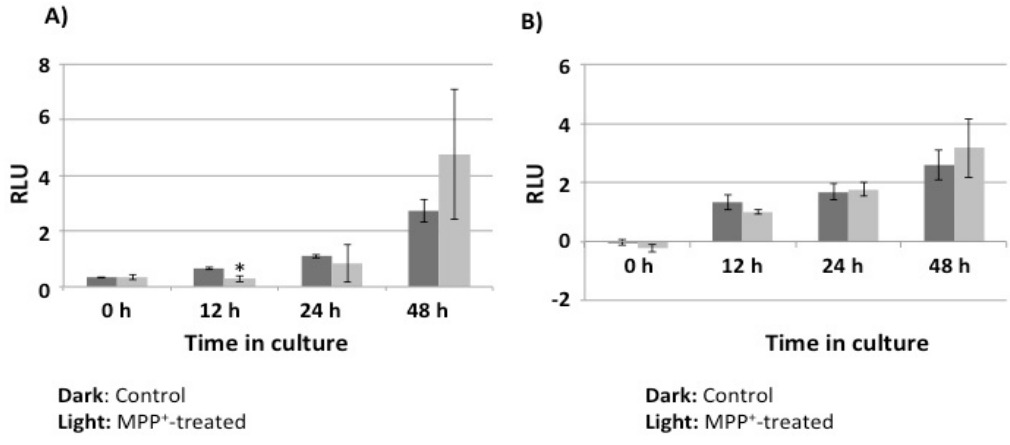

Figure 3. The measurement of secreted glutamate levels in MPP+-treated cells. **a)** The excess glutamate levels were measured from cell media using glutamate assay in non-treated and MPP+-treated N2A cells at indicated time points. p<0.05. C: non-treated cells. MPP+: MPP+-treated cells. * denotes the comparison between C and MPP+ at 12 h time point. RLU: Relative Luminescence Units. **b)** The excess glutamate levels were measured from cell media using glutamate assay in non-treated and MPP+-treated IHA cells at indicated time points. C: non-treated cells. MPP+: MPP+-treated cells. RLU: Relative Luminescence Units. Dark: Control (C), Light: MPP+-treated

After 12 h of treatment, the levels of secreted glutamate in MPP^+-treated cells were lower than those in control cells. At the 24-h time point, this difference was no longer prominent. This shows that, in the first 24 h of MPP^+ or kainic acid treatment, GLT-1 levels increased immediately in treated cells. Therefore, glutamate levels were even lower in MPP^+-treated cells, compared with non-treated cells due to the increase in GLT-1 following treatment. However, after 24 h, first, the difference was no longer present (24-h time point), and then the glutamate levels started to increase in MPP^+-treated cells (48-h time point). The reason for this might be that the transient increase in GLT-1 expression might have returned to normal levels. Another reason for the reduction in glutamate levels after 12 h might be cell death. Since the number of living cells decreases, the amount of secreted glutamate is reduced.

We could not detect GLT-1 expression in the total proteins we isolated from N2A cells, although we tried different antibodies. The explanation for this might lie the fact that neurons express much less GLT-1 protein than glial cells.

Our experimental system does not distinguish between the secreted and absorbed glutamate, nor does it measure dynamics. The glutamate assay only measures the secreted and accumulated glutamate in the medium. Obviously, using a neuron-glia mixed culture could be ideal for studying this system; however, since we are interested in the change in expression of GLT-1, which is expressed by both cell types, using one cell type enabled us to analyze the response of that particular cell line to kainic acid or MPP^+.

Any stress to the cell will lead to an activation of the stress-response mechanisms (25). Administration of a toxin to a cell line is also a stress and triggers certain defense mechanisms. Both kainic acid and MPTP are known to induce oxidative stress (26,27). After the induction of stress in the cells due to treatment with kainic acid or MPP^+, glutamate release increases. As a response to this event, GLT-1, whose task is to clear up the excess glutamate, automatically increases. Since the upregulation of GLT-1 increases glutamate uptake (28), the cell attempts to get rid of the excess glutamate by increasing the expression of GLT-1 after the administration of kainic acid or MPP^+. However, the elevation of GLT-1 levels might not be adequate to cope with the increase in excitotoxicity due to the continuation of the stress.

A previous study showed that when kainic acid was administered (intraperitoneally 25 mg/kg) to SIRT4 KO mice, they displayed increased seizure activity (29). This result shows that the lack of SIRT4 in the brain increases the excitotoxicity induced by kainic acid. The molecular mechanism was found to be the decrease in glutamate uptake and also the decrease in the cell surface expression of GLT-1. GLT-1 is known to be internalized from the cell surface when there is no excess glutamate and degraded in the cell via ubiquitination (30-32). Its surface expression increases when there is an increase in extracellular glutamate levels. When the excitotoxicity decreases, GLT-1 might be internalized via vesicles and degraded (30-32).

Study Limitations

In the future studies, glutamate could be measured before the 12-h time point to capture the level of glutamate secretion before cell death. In this way, we can rule out a reduction in glutamate secretion due to the decrease in cell number.

Conclusion

Our study showed that both cell types, neuronal and glial, showed an autonomous survival mechanism as a result of an excitotoxic insult (MPP^+ or kainic acid) by significantly increasing GLT-1 expression levels in order to take up the excess glutamate to protect the cell. GLT-1 is the major transporter that performs 95% of the glutamate clearance contributing to normal neuronal function and protecting against excitotoxicity. Therefore, increasing GLT-1 expression may protect from excitotoxicity and cell death. Memantine, an FDA-approved drug for Alzheimer's Disease and other dementias, targets glutamate excitotoxicity (33). Rilutek (Riluzole) was the first drug approved by the U.S. Food and Drug Administration (FDA) in 1995 to treat amyotrophic lateral sclerosis. The exact mechanism of Rilutek is not known. However, it is believed that the drug blocks the release of glutamate from nerve cells (34). Therefore, targeting molecular pathways regarding the increase of GLT-1 expression or activity may lead to therapeutics of neurodegeneration and pave the way to enlarging our understanding of brain diseases on the cellular level.

Ethics

Ethics Committee Approval: Neither humans nor animals were used in the research; therefore, ethical approval was not required.

Peer-review: Externally peer reviewed.

Authorship Contributions

Concept: G.D.Y., Design: G.D.Y., Data Collection or Processing: R.U., C.A., Analysis or Interpretation: R.U., C.A., G.D.Y., Literature Search: R.U., C.A., G.D.Y., Writing: G.D.Y.

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

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Original Article

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Effectiveness of Diffusion Tensor Imaging in the Microstructural Evaluation of Corpus Callosum and Brain Parenchyma in Children with Neurofibromatosis Type I

Nörofibromatosis Tip 1'li Çocuklarda Korpus Kallozum ve Beyin Parankiminin Mikroyapısal Değerlendirilmesinde Difüzyon Tensör Görüntülemenin Etkinliği

Dilek Hacer ÇEŞME

Bezmialem Vakıf University Faculty of Medicine, Department of Radiology, İstanbul, Turkey

ABSTRACT

Objective: To compare tractional anisotropy (FA) and mean diffusivity (MD) values obtained from corpus callosum (CC), basal ganglion, thalamus, frontal and parietal white matter in NF1 patients compared to the control group and to investigate the correlation with CC volume.

Methods: Thirty three cases diagnosed with NF1 and 21 healthy control groups were included in the study. CC volume was measured in both groups. Using tensor imaging (DTI), MD and FA values were calculated from CC genu and splenium, globus pallidum, caudate nucleus, putamen, thalamus, parietal and frontal white matter.

Results: CC volume increased significantly in cases with NF1. There was a significant difference in MD and FA values obtained from CC genu and splenium compared to the control group. MD values obtained from frontal and parietal white matter, globus pallidum, putamen, thalamus and caudate nucleus were significantly higher than the control group. FA values decreased in caudate nucleus and putamen while FA values in globus pallidum were higher than control group. There was a negative correlation between CC volume and MD values obtained from KK splenium, putamen, thalamus and caudate nucleus

Conclusion: Increased MD in areas of involvement in NF1 cases can be explained by impaired myelination and demyelination. Heterogeneity in FA values suggests that it is caused by

ÖZ

Amaç: NF1'li olgularda korpus kallozum (KK) hacmi ile bazal ganglionlar, talamus, frontal ve parietal beyaz cevherden elde olunan fractional anisotropy (FA) ve mean diffusity (MD) değerlerinin sağlıklı kontrol grubu ile farklılık gösterip göstermediğinin araştırılmasıdır.

Yöntemler: NF1 tanısı almış 33 olgu ve 21 sağlıklı kontrol grubu çalışmaya dahil edildi. Her iki grupta KK hacmi ölçüldü. Difüzyon tensör görüntüleme (DTG) ile KK genu ve splenium, globus pallidum, kaudat nükleus, putamen, talamus, parietal ve frontal beyaz cevherden MD ve FA değerleri hesaplanarak karşılaştırma yapıldı.

Bulgular: NF1'li olgularda KK hacmi belirgin artmıştı. KK genu ve spleniumdan elde olunan MD ve FA değerlerinde kontrol grubuna göre anlamlı farklılık saptandı. Frontal ve parietal beyaz cevher, globus pallidum, putamen, talamus ve kaudat nukleus MD değerleri kontrol grubuna göre anlamlı yüksekti. Kaudat nükleus ve putamende FA değerleri azalırken, globus pallidum FA değerleri ise kontrol grubuna göre yüksekti. KK hacmi ile KK splenium, putamen, talamus ve kaudat nükleus MD değerleri arasında negatif korelasyon vardı.

Sonuç: NF1'li olgularda tutulum alanlarında MD artışı miyelinizasyonda bozulma ve demiyelinizasyon ile açıklanabilir. FA değerlerindeki heterojenite miyelin kılıflarının parçalanması

Address for Correspondence: Dilek Hacer ÇEŞME, Bezmialem Vakıf University Faculty of Medicine, Department of Radiology, İstanbul, Turkey
E-mail: dhcesme@bezmialem.edu.tr **ORCID ID:** orcid.org/0000-0002-3869-0524

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microstructural differences resulting from the breakdown of myelin sheaths or axonal disruption in different locations of the brain. In cases with NF1, DTI findings will help us to understand the occurrence of the disease and the physiopathology of clinical findings in more detail.

Keywords: Neurofibromatosis type I, corpus callosum, diffusion tensor imaging, MD, FA

veya aksonal bozulmaya bağlı mikroyapısal değişiklikler sonucu görülebilir. NF1'li olgularda DTG bulguları hastalığın gelişim sürecini ve klinik bulguların fizyopatolojisini daha detaylı anlamamıza yardımcı olacaktır.

Anahtar Sözcükler: Nörofibromatozis tip 1, korpus kallosum, difüzyon tensor görüntüleme, MD, FA

Introduction

Neurofibromatosis type 1 (NF1) is a neurocutaneous syndrome known as von Recklinghausen's disease. It is caused by mutation or deletion in the *NF1* gene on the 17th chromosome, which is inherited autosomal dominantly and results in decreased production of neurofibromin, a tumor suppressor protein (1,2). Diagnostic criteria of NF1 are; cafe-au lait macules, neurofibromas, freckles in the axillary or inguinal areas, optic path gliomas, Lisch nodules, bone lesions, central nervous system neoplasms, cognitive problems and learning difficulties (1).

Hamartomatous lesions observed as hyperintense on T2-weighted images in the brain parenchyma are called unidentified bright objects (UBOs). The most common areas of involvement are; globus pallidum, brainstem, thalamus, cerebellum and subcortical white matter. The prevalence of UBOs in childhood varies between 55-90% (3). Although the structure of hamartomas is not fully understood; it is known that heterotopia, gliosis, intramyelinic edema, atypical glial infiltrates, microcalcification foci, and even dysplasia are transformed into low-grade astrocytomas and as a result, spongy or vacuolar change develops in the white matter (2,4,5).

The corpus callosum (CC) is the largest commissure in the human brain and is responsible for the communication between both hemispheres. Macrocephaly has been reported in 38% of the patients with NF1 (6). Although the pathogenesis of CC enlargement in patients with NF1 is not clearly understood yet; it is thought that it may be associated with abnormal neurofibromin and Ras protein activity (4,6). It is thought to be associated with an increase in axon size and number due to myelin disorder (4,6).

Diffusion Tensor Imaging (DTI) shows the relationship between white matter structures and neural functions. Both mean diffusity (MD) and fractional anisotropy (FA) values provide information about microstructural changes in white matter. It provides useful information in detecting abnormalities in white matter structures and in white matter commissures such as corpus callosum in children with NF1 (1).

In our study, we aimed to compare CC volume and FA and MD values obtained from basal ganglia, thalamus, frontal and parietal white matter in patients with NF1 with healthy control group and to investigate the relationship between them.

Methods

Thirty three patients (22 males and 11 females, mean age; 8.87±3.44) who were followed up with a diagnosis of NF1, and 21 age-matched healthy controls (10 males and 11 females, mean age; 9.61±4.20) were included in this retrospective study. Ethical approval was obtained from our institution. Routine MRI was applied to all patients with NF1 and control group. The following parameters were used in the imaging protocol; Axial and sagittal T2 images (TR/TE: 4,280/91 ms matrix: 384x211; NSA: 1; slice thickness 5 mm), axial T1 images (TR/TE: 500/87 ms; matrix: 256x125; NSA: 1; slice thickness 5 mm), axial and coronal FLAIR images (TR/TE/TI: 8.000/118/23.687 ms; NSA: 1; slice thickness 5 mm), contrast-enhanced axial and coronal T1 images (TR/TR: 448/87; matrix: 256x134; NSA: 1), and 3D T1 postcontrast sagittal images (R/TE: 476/86; matrix: 256x154; NSA: 1; slice thickness 1 mm). In 3D T1 images, the boundaries of CC with region of interest (ROI) were drawn manually from a total of 3 sections on the midsagittal line. The CC volume was measured with the aid of a Siemens Syngo-via workstation (Figure 1). DTIs were obtained in the axial plan and

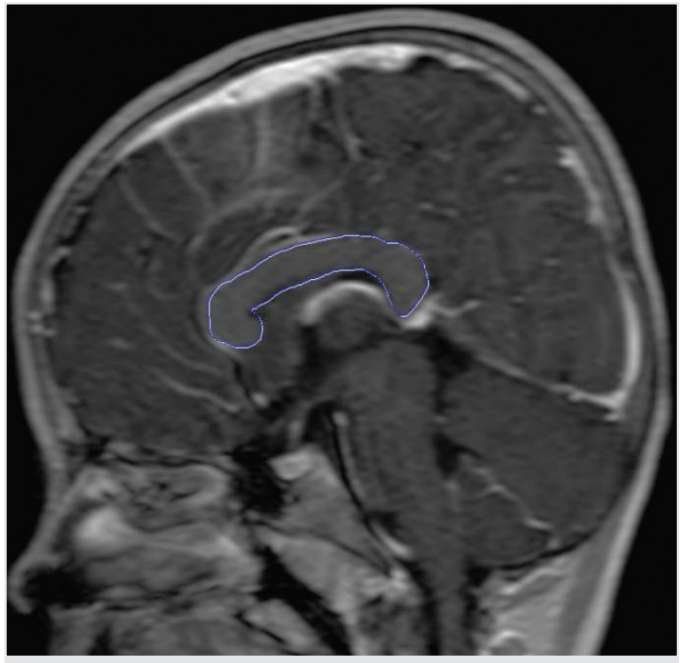

Figure 1. In post-contrast 3D T1 images, the corpus callosum boundaries are determined with ROI and its volume is measured

ROI: Region of interest

the protocol determined was applied (TR=6,000 ms, TE=89 ms, 30 directional, b=1000 s/mm^2, 5-mm section thickness, 230 mm FOV and matrix: 128x128). MD and FA values were calculated by placing ROI on CC genu and splenium, caudate nucleus, globus pallidum, putamen, thalamus, frontal white matter and parietal white matter in Siemens Leonardo workstation (Figures 2a and 2b).

Statistical Analysis

All statistical analyzes were performed with IBM SPSS 19.0. Whether the groups showed normal distribution was evaluated with the Kolmogirov-Smirnov test. Comparisons between the patients with NF1 and the healthy control group were made using the Mann-Whitney U test. The correlation between the CC volume and DTI parameters was investigated with Spearman's correlation test. P values less than 0.05 were considered significant.

Results

The MD and FA values and CC volumes in different localizations of the brain belonging to the patients with NF1 and the healthy control group are presented in Table 1.

There was a significant difference between the CC volume of the patients with NF1 and the healthy control group. CC volume was larger in patients with NF1 (p=0.01).

The MD and FA values obtained from the CC genu and splenium in patients with NF1 showed a significant difference compared to the control group (p<0.001 for each). MD and FA values were higher in patients with NF1.

In patients with NF1, MD values obtained from frontal and parietal white matter appearing normal were significantly higher than healthy controls (p<0.001 for each). However, there was no significant difference between FA values in both locations.

Globus pallidum, putamen, thalamus and caudate nucleus MD values were significantly higher than the control group (p<0.001 for each).

There was a significant difference in globus pallidum, putamen and caudate nucleus FA values of patients with NF1 compared with the healthy control group (p=0.047, p=0.001, and p=0.003, respectively). While FA values decreased in the caudate nucleus and putamen, globus pallidum FA values were higher in patients with NF1 than in the control group.

There was a negative correlation between CC volume and MD value of putamen, thalamus, caudate nucleus and CC splenium in patients with NF1 (p=0.015, r=-0.418, p=0.010, r=-0.444; p=0.004, r=-0.491, p=0.001, and r=-0.600, respectively).

Discussion

The UBOs begin to be seen in the childhood age group, especially at the age of 3, and continue to increase in number and size until the end of the first decade. They become invisible radiologically as spontaneously regressing from the second decade (7). The loss of signal increases in T2-weighted images with age may reflect

Table 1. The MD and FA values and corpus callosum volumes in different localizations of the brain belonging to the patients with NF1 and the healthy control group

	Patients with NF1 (n=33)	Control (n=21)
CC volume	12.97±3.30	10.44±2.87
CC genu MD	904.06±50.12	703.47±83.77
CC genu FA	699.51±66.33	430.66±171.32
CC splenium MD	901.90±60.43	700.09±76.24
CC splenium FA	730.97±58.15	463.52±138.66
Frontal white matter MD	885.06±56.12	732.85±57.53
Frontal white matter FA	331.18±73.45	352.14±93.80
Parietal white matter MD	917.24±81.09	722.52±37.08
Parietal white matter FA	368.54±79.56	350.90±92.78
Globus pallidum MD	834.27±78.04	657.14±68.39
Globus pallidum FA	437.36±134.64	364.95±147.59
Putamen MD	810.21±58.53	695.095±49.17
Putamen FA	240.39±112.49	330.81±96.13
Thalamus MD	876.84±53.54	698.38±59.83
Thalamus FA	311.60±76.50	349.04±102.11
Caudat nucleus MD	814.69±44.86	711.57±64.57
Caudat nucleus FA	240.42±104.49	304.23±63.54

MD: Mean diffusity (x10^{-6}), FA: Fractional anisotropy, CC: Corpus callosum, NF1: Neurofibromatosis type 1

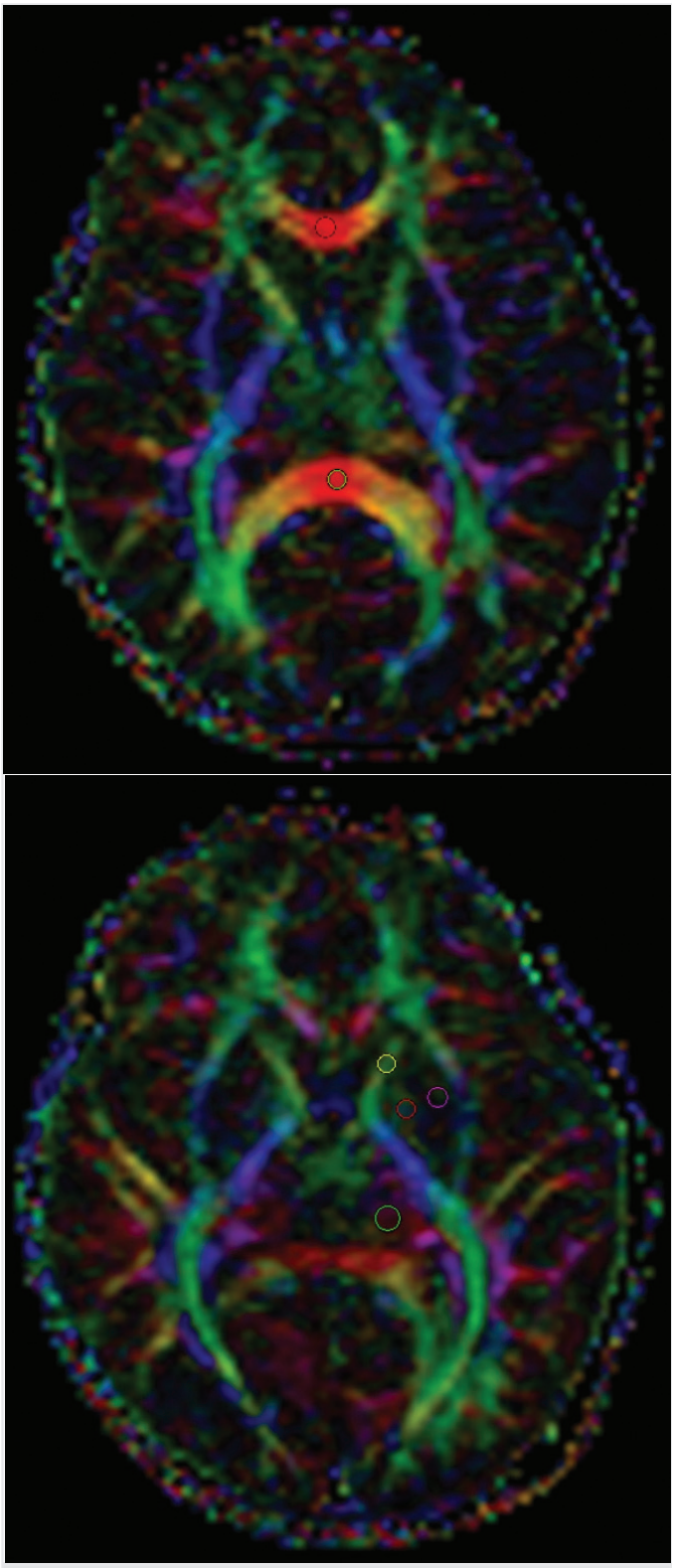

Figure 2. In the colored FA map, MD and FA values are calculated by placing the ROI on the corpus callosum genu and splenium (a), and on the caudate nucleus, globus pallidum, putamen and thalamus (b)

MD: Mean diffusity, FA: Fractional anisotropy

the change in the water content of the brain parenchyma (2). Considering the available pathological data on NF1, it is seen that there is a relationship between UBOs and myelin vacuoles, which can represent evidence of the demyelination process (2). The lesions appearing hyperintense in T2-weighted series represent focal areas with severe disease within the areas showing diffuse myelin disorder.

The CC is the largest axonal pathway in the central nervous system. It provides interhemispheric integration, which is an important function for creativity and intelligence (8). When the CC reaches adult size is controversial. In some studies, it has been reported that growth continues until the age of 15 (8). The generally accepted theory is; it slows down after a rapid growth in the first 4 years of life, and it becomes compatible with the size of adult age group at the age of 10-12. The topography of the CC is well defined. Fibers from the inferior frontal lobes and inferior parietal lobes pass through the CC genu. The fibers in the remaining parts of the frontal and parietal lobes pass through the CC body. Temporal and occipital lobe fibers intersect in the splenium (8).

It has been shown that the increased total brain volume in patients with NF1 is primarily due to the increase in white matter volume. The expanded CC volume in patients is generally greater than the increase in brain volume (1). Structural brain abnormalities such as enlargement of the CC in children with NF1 have been associated with cognitive impairment (1). In our study, the CC volume increased significantly compared to the control group. Total volume was measured without dividing the CC into sections. While the CC volume in the age-matched healthy control group was 10.44±2.87 cm^3, it was 12.97±3.30 cm^3 in the patients with NF1. One of the most likely explanations for CC enlargement in children with NF1 is the production of more commissural fibers due to delay or decrease in apoptosis (6,8,9). The decrease in apoptotic process is due to the relation between the regulation of the *NF1* gene product neurofibromin and the Ras protein activation pathway. Activated Ras proteins have been shown to have beneficial or harmful effects in the regulation of apoptosis depending on cell type and other factors (8). Other possible causes have been reported as excessive myelination, increased extracellular fluid, vacuolar myelinopathy, increased axon number and width, and/or a combination of these factors (1,10).

It is known that there is a negative relationship between CC enlargement and IQ (1,3,6,11). Pride et al. (11) found a relationship between the enlargement of the CC body and isthmus and decreased cognitive performance. Contrary to this hypothesis, Kayl et al. (12) claimed that children with more attention problems had a smaller total CC area. Aydin et al. (4) reported that the CC genu may be affected more in children with NF1 with neurocognitive disabilities. In general, the anterior part of the CC is often enlarged in children, while the posterior and isthmus parts of the adult patients with NF1 are predominantly enlarged (6).

DTI is an advanced neuroimaging method used to obtain FA and MD maps of white matter pathways. Mutations in NF1 cause microstructural changes in white matter, resulting in motor and cognitive dysfunctions (3). FA is the quantitative measurement of the diffusion of water molecules along the axon direction. It is a very sensitive but nonspecific indicator of the microstructure of white matter. FA reflects fiber density, axonal diameter and myelination in white matter. MD values increase with increasing tissue fluid amount such as inflammation or edema, and MD values decrease with hypercellularity (4,13). In demyelinating diseases, loss of normal myelin structure and axonal loss increase MD by causing enlargement of the extracellular space. The high MD values in UBOs indicate a relatively high molecular movement in these regions compared to the normal brain parenchyma (2). Detection of higher MD values in regions with UBO in patients with NF1 compared to regions without UBO supports the hypothesis that the microstructure of the brain tissue is impaired due to fluid accumulation and vacuolization (3).

Decrease in FA and increase in MD in patients with NF1 indicate disruption of myelin sheaths and/or axonal disruption (10). Diffusion changes result from myelin disorder (7). Sheikh et al. (14) reported that the number and/or size of myelin vacuoles were increased. Alkan et al. (2) stated that it might be related with demyelination. Eastwood et al. (15) explained it with myelin disorders such as demyelination or increased myelin turnover with decreased amount of myelin. However, the observed changes in brain diffusion appear more likely to indicate dysmyelination in patients with NF1. Zamboni et al. (7) claimed that it was the result of widespread and fundamental changes in the cerebral microstructure caused by the underlying gene mutation. In our study, MD values obtained from the CC genu and splenium were significantly higher. We thought that the changes in the CC microstructure such as disruption in myelinization, demyelination, axonal loss and fluid accumulation in the extracellular space caused increase in MD values, as in UBOs in patients with NF1. In addition, frontal and parietal white matter MD values were significantly higher. Despite the normal appearance of white matter, high MD values might be associated with demyelination. Likewise, the detection of high MD values in the basal ganglia (caudate nucleus, putamen, globus pallidum) and thalamus could be explained by the disruption of myelin structure and demyelination. In our study, while CC volume increased in patients with NF1, MD values decreased in the putamen, caudate nucleus and CC splenium . One of the most important hypotheses explaining CC enlargement in patients with NF1 is the production of more commissural fibers due to delay or decrease in apoptosis. The result of this is an increase in the size and number of axons due to myelin disorder. We think that as the CC volume increases, the relative MD decrease in spenlium, putamen and caudate nucleus can be explained by this hypothesis.

FA can be affected by factors such as axon packing, relative membrane permeability of water, internal axon structure, myelination, and tissue water content (16,17). It is not possible to eliminate the possibility of the anatomical changes due to the dominance of white matter tracts or gray matter in the studied areas, varying intensity of microstructural damage, and regional differences. In patients with NF1, the decrease in FA values may indicate general microstructural changes and dysmyelination (7,16). Increased FA has been associated with decreased axonal caliber and increased axonal density (4). In a study, it was reported that the decrease in FA values in the CC genu in adult patients with NF1 may have been caused by disruptions in axon or myelin and the decrease in the density of myelinated axons (1). Low FA in any white matter region indicates a decreased myelin content and therefore less efficient axonal transmission (3). In our study, the FA values of the CC genu and splenium were significantly higher than in the patients with NF1 than the healthy control group. This increase in FA could be explained by decreased axonal caliber and increased axonal density in CC.

The thalamus has widespread connections with many cortical regions and enables different sensory inputs to be transmitted and transformed to the cortex (18). The nuclei of the thalamus play a role in sensory and motor information, memory and executive functions, and it has been shown that there is impairment in the integration of these competencies in patients with NF1 (18-21). The caudate nucleus is the nucleus of the basal ganglia and plays a role in sensorimotor coordination and targeted behaviors (18,22). It is widely associated with the frontal lobe and especially the dorsolateral prefrontal cortex, which plays a role in working memory and executive function. Demyelination in the thalamus and basal ganglia due to the disruption of myelin structure can be explained by the presence of clinical symptoms that develop in patients with NF1. In our study, there were significant differences in terms of FA values in the basal ganglia between the patients with NF1 and the healthy control group. In patients with NF1, FA values in the caudate nucleus and putamen decreased, while FA values in globus pallidum were higher than in the healthy control group. The decreased FA values observed in the caudate nucleus and putamen suggested dysmyelination due to microstructural changes. The decreased FA values in the basal ganglia supported the view that the disruption in the axon or myelin may have resulted from decrease in the density of myelinated axons. In our study, increased FA values in the globus pallidum may be associated with the revitalization of an area with myelin impairment or with intramyelinic edema and microcalcification secondary to the repair process (16). We are of the opinion that the hyperintensity that occurs in T1-weighted images, especially during the remyelination phase, may be related to myelin repair and microcalcification. Especially in the basal ganglia, the heterogeneity due to increased and decreased FA values supports the view that it may be due to the presence of microstructural differences caused by fragmentation of myelin sheaths or axonal disruption (3).

Study Limitations

The first limitation of our study was the low number of patients. The second limitation was that clinical and neurocognitive tests were not performed in patients with NF1 and the correlation between CC volume and DTI findings were not investigated.

Our third limitation was that DTI measurements were made using manual ROI. Using voxel-based measurement methods would minimize the margin of error.

Conclusion

Our findings showed that the increase in MD values observed in patients with NF1 might be due to impaired myelinization, demyelination, axonal loss and fluid accumulation in the extracellular space. The heterogeneity in FA values suggested that it was caused by microstructural differences that occured as a result of myelin sheaths or axonal disruption in different locations of the brain. We believe that DTI findings obtained in patients with NF1 will help us to understand the formation of the disease and the physiopathology of clinical findings in more detail at the microstructural level.

Ethics

Ethics Committee Approval: No: 2020/7229.

Informed Consent: Informed consent was not obtained as the study design was retrospective.

Peer-review: Externally peer reviewed.

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Original Article

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In Vitro Evaluation of the Shear Bond Strength of Different Luting Cements on Zirconium Oxide Specimens in Primary Teeth

Süt Dişlerinde Farklı Yapıştırma Materyalleriyle Uygulanan Zirkonyum Oksit Örneklerin Bağlanma Dayanımının *İn Vitro* İncelenmesi

Zekiye EFE, Zeynep Aslı GÜÇLÜ

Erciyes University Faculty of Dentistry, Department of Pedodontics, Kayseri, Turkey

ABSTRACT

Objective: Nowadays in pediatric dentistry clinics, zirconia crowns have become widespread restoration choice of primary teeth with extensive decay. Aim of this study was to evaluate shear bond strength of different luting cements on zirconium oxide (zirconia) specimens in primary teeth.

Methods: Eighty extracted primary molar teeth were selected for the study and teeth were randomly divided into four groups. Eighty zirconium oxide specimen were fabricated using CAD/CAM technology. Samples were cemented with zinc polycarboxylate cement (PK), conventional glass ionomer cement (GCIS), resin modified GCIS (RMCIS) and self adhesive resin cement (RS). After cementation, samples were kept in distilled water for three days at room temperature. Then speciemens were subjected to 1000 cycles thermal cycle process. Shear bond strength was determined using universal testing machine, debonded surfaces were examined using stereomicroscope. Tamhane's test was used for pairwise comparisons of the mean bonding forces of the groups. Pearson chi-square test was used to compare the debonded surfaces. Significance level (p) was accepted as 0.05 in the application of the tests.

Results: Mean bond strength of RMCIS group was statistically lower than RS, GCIS and PK cements ($p<0.05$). There wasn't statistically significant difference between mean bonding strength of RS, GCIS and PK cement group ($p>0.05$). There wasn't statistically significant difference between debonded surfaces of zirconia specimen after cementation with different bonding cements ($p>0.05$).

ÖZ

Amaç: Son yıllarda çocuk diş hekimliğinde aşırı madde kaybı görülen süt dişlerinin restorasyonunda zirkonya kronların kullanımı yaygınlaşmaktadır. Çalışmamızda zirkonyum oksit (zirkonya) örneklerin farklı yapıştırma materyalleriyle kullanımlarının süt dişlerine bağlanma kuvvetine etkisinin değerlendirilmesi amaçlandı.

Yöntemler: Çalışmada 80 adet çekilmiş süt azı dişi, her grupta yirmi diş olacak şekilde rastgele dört gruba ayrıldı. Seksen adet zirkonya örnek CAD/CAM kullanılarak üretildi. Örnekler çinko polikarboksilat siman (PK), geleneksel cam iyonomer siman (GCIS), rezin modifiye cam iyonomer siman (RMCIS) ve self adeziv rezin siman (RS) ile simante edildi. Simantasyon sonrası örnekler, distile su içerisinde oda sıcaklığında üç gün bekletildi. Sonrasında 1000 devir termal döngü işlemine tabi tutuldu. Termal döngü sonrası, bağlanma dayanımları üniversal test cihazında ölçüldü. Kopma yüzeyleri, ışık mikroskobunda incelendi. Grupların ortalama bağlanma kuvvetlerinin ikili karşılaştırılmasında Tamhane's testi kullanıldı. Kopma yüzeylerinin karşılaştırılmasında ise Pearson ki-kare testi kullanıldı. Testlerin uygulanmasında anlamlılık düzeyi ($p>0,05$) olarak kabul edildi.

Bulgular: Çalışma sonunda RMCIS grubunun ortalama bağlanma kuvveti RS, GCIS ve PK siman göre daha düşük bulundu ($p<0,05$). RS, GCIS ve PK siman grubunun ortalama bağlanma kuvveti arasında istatistiksel olarak fark bulunmadı ($p>0,05$). Zirkonya örneklerin, farklı yapıştırma simanlarıyla simantasyonu sonrası kopma yüzeyleri arasında istatistiksel olarak anlamlı farklılık bulunmadı ($p>0,05$).

Address for Correspondence: Zekiye EFE, Erciyes University Faculty of Dentistry, Department of Pedodontics, Kayseri, Turkey
E-mail: zekiyehidayet@hotmail.com **ORCID ID:** orcid.org/0000-0001-5621-5279

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Conclusion: We think that it may be more advantageous to adhere zirconia crowns with self adhesive RS in pediatric patients whom are difficult to provide moisture control.

Keywords: Luting cement, retention, posterior primary teeth, zirconia

Sonuç: Nem kontrolünün sağlanması zor olan çocuk hastalarda zirkonya kronların self adeziv rezin siman ile yapıştırılmasının daha avantajlı olabileceğini düşünüyoruz.

Anahtar Sözcükler: Retansiyon, süt azı dişleri,yapıştırma simanı, zirkonya

Introduction

The choice of restorative materials in deciduous dentition differs compared to permanent dentition. Primary teeth are smaller in size compared to permanent teeth, and the thickness of enamel and dentin is thin, causing the remaining support structure to become weaker after the treatment (1,2). Therefore, the possibility of microleakage and brittleness increases in the long term in large-restorated deciduous teeth (3). The application of resin-based materials in large masses in the restoration of primary teeth with excessive material loss causes an increase in polymerization shrinkage and deterioration of restoration compliance (4). In order to eliminate these negative situations, it is important that the physical and mechanical properties of the restorative material are close to the natural tooth structure, biocompatible and do not require regeneration until the time of physiological fall (4,5).

In clinical studies, the use of prefabricated crowns in the restoration of primary teeth with excessive material loss has significantly reduced the causes of failure seen in other restorative materials in the long term (4,6). Nowadays, stainless steel crowns (STC) and zirconium oxide (zirconia) crowns are used as prefabricated crowns in pediatric dentistry.

In recent years, the use of zirconia crowns, which do not have aesthetic concerns instead of STC and have characteristics close to natural tooth structure, has become widespread (5). Studies have reported that zirconia crowns are clinically successful restorations and their use increases aesthetic satisfaction (7,8). The fact that zirconia restorations are biocompatible and exhibit excellent aesthetic and mechanical properties have enabled them to be used in pediatric dentistry as well as in many areas of dentistry (9).

The most important cause of clinical failure of prefabricated crowns is microleakage due to marginal edge opening (10). Although the contour harmony of the STC at the gum level is provided with various forceps, gaps between the tooth and the crown may remain. These openings are covered with adhesive cements. In a study, it has been shown that microleakage occurs in the area close to the gingiva border, even in STC that are perfectly matched to the extracted teeth and adhered with traditional glass ionomer cement (11). The microleakage resistance of the bonding cement used directly affects crown retention (12). The presence of an ideal cementation material that provides adaptation between the restoration and the tooth and increases the bond strength is important in the clinical success of prefabricated crowns (13,14). Although it has been reported in the literature that zirconia crowns can be cemented with different bonding materials, there is no definitive explanation about the ideal cementation material to be used in primary teeth (13,14). Therefore, in our study, it was aimed to evaluate the bonding strength of zirconia samples cemented with different bonding cements to primary teeth.

Methods

The study was approved by the Erciyes University Faculty of Medicine Ethics Committee on 20.06.2018 and the decision number was 2018/314. This study was supported by the Scientific Research Projects Unit of Erciyes University with the project coded TDH-2018-8332.

Selection of Primary Teeth Suitable for Inclusion Criteria

Inclusion Criteria in the Study

The extracted teeth belonging to the children of the parents who read and accepted the working conditions and signed the consent form were included in the study. In the study, primary molars without caries and/or with enamel caries with an indication of extraction due to periodontal or orthodontic reasons and near physiological fall time were used.

The Criteria for Exclusion from the Study

Teeth belonging to children whose parents did not sign the consent form were not included in the study. Among the teeth belonging to the children of the parents who accepted to participate in the study, primary molars with hypoplasia or deep caries, and cracked or broken primary molars were excluded from the study.

Collection and Preparation of Primary Teeth

Eighty extracted primary molars meeting the inclusion criteria were kept in 0.5% chloramine solution for one month in order to provide disinfection. Teeth were embedded in pressure-cured white acrylic (Orthocryl® EQ, Dentaurum, Germany) in standard molds (2.5x1.5x1.5 cm) with their buccal or lingual surfaces exposed. Acrylic hardening was achieved under heat and pressure. The exposed surface of each specimen was then smoothed under water in a sanding and polishing device (Tegrapol-11, Struers, Denmark) with 220 and 400 grit silicon carbide to obtain a flat dentine surface parallel to the long axis of the tooth. Afterwards, the polishing process was completed with 600 grit silicon carbide to ensure the formation of a standard smear layer in each sample.

Preparation of Zirconia Specimens

The zirconia structures used in the study were obtained from pre-sintered Katana zirconia blocks (Kuraray Noritake Inc., Kurashiki, Japan). The dimensions of the zirconia samples were designed to be 3 mm in diameter and 6 mm in length after sintering. Designs were prepared using DWOS® (Dental Wings Open System) computer-aided design (CAD) software. Eighty samples were designed and produced by using computer aided manufacturing (CAM) (CAM Yenadent DC40, Yenadent Ltd., Istanbul, Turkey) (Figure 1, 2). Since the zirconia structures showed 20% shrinkage linearly after the sintering process, the samples were designed at this rate than the specified dimensions. Afterwards, it was sintered at 1500 °C for 2 hours in the sintering furnace (Programat CS4, Ivoclar Vivadent, Germany) in line with the manufacturer's recommendations.

Figure 1. Side view of zirconia sample produced with CAM

Cementation Procedures

Each group was divided into four groups as 20 randomly distributed zirconia samples and 20 prepared teeth. Samples divided into four groups were cemented under finger pressure with conventional glass ionomer luting cement (GCIS), resin modified GCIS (RMCIS), zinc polycarboxylate cement (PK) and self-adhesive resin cement (RS) prepared in accordance with the manufacturer's instructions (Figure 2). Table 1 contains information about the luting cements used.

Thermal Cycling of Samples

Samples whose hardening were completed after cementation were stored in distilled water in their own closed collection containers for three days at room temperature. Subsequently, the samples in each group were placed in thin water-permeable sheaths and their mouths were tied with ribbons of different colors. The covers were placed horizontally in the water tanks. They were

Figure 2. The cementation surface of the zirconia sample before sintering and the side view of the cemented sample

Table 1. Luting cements used in the cementation process

Material class	Materiel, manufacturer	Lot number	Method of application
Zinc polycarboxylate cement	Adhesor Carbofine, Spofa Dental, Germany	6009542 - 1	Two parts powder and five drops of liquid were mixed for 30 seconds. Working time at room temperature was 2 minutes, hardening time was 5-8 minutes.
Conventional glass ionomer luting cement	Meron, Voco, Cuxhaven, Germany	1410392	One part powder and one drop of liquid were mixed for 30 seconds. Working time at room temperature was approximately 3 minutes, hardening time was 5-7 minutes. is.
Resin modified glass ionomer luting cement	Meron Plus, Voco, Cuxhaven, Germany	1810042	One part powder and two drops of liquid were mixed for 30 seconds. Working time at room temperature was about 2-4 minutes, hardening time was 3-5 minutes. is.
Self adhesive resin cement	R & D Series Nova Resin Cement, Imicryl, Konya, Turkey	18087	The cement, which was squeezed equally from the base and catalysis syringes, was mixed for 15 seconds. After removing the excess cement applied, it was polymerized by applying visible light for 20 seconds from each surface of the samples.

subjected to 1000 cycle thermal cycling process (Jubalo FT400, Seelbach, Germany) with a waiting time of 20 seconds and a transfer time between tanks of 10 seconds in tanks containing water at 5±1 °C and 55±1 °C. After the thermal cycle, samples were kept in distilled water in collection containers for 24 hours at room temperature.

Measurement of Bonding Strength

The long axis of the teeth were fixed between the holding arms of the universal testing device (Instron 3345, Instron Corp., Norwood, USA) parallel to the direction of the applied force (Figure 3). The blade edge of the device was positioned in contact with the fixed sample (Figure 4). The crosshead speed of the device was set at 0.5 mm/min (15,16). Shear force was applied until separation occurred from the midpoint of the bonding area between the zirconia specimen and the dentin surface. The maximum force value obtained at the end of the test was recorded in Newton (N). The same procedure was applied for all samples in four groups.

Evaluation of Debonded Surfaces

The debonded surfaces were examined by the same investigator at x40 magnification under a light microscope (Leica Optical Microscope, Leica Cambridge Ltd., Cambridge, UK). The debonded surfaces were classified as adhesive, cohesive and adhesive-cohesive (mixed) type debonding.

Statistical Analysis

Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA) Windows 16.0 program was used for the statistical analysis of the data. The mean, standard deviation, minimum and maximum values of each experimental group were calculated. Kolmogorov-Smirnov test was used to examine the normal distribution and it was found that the data were not normally distributed (p=0.002). Therefore, the nonparametric Kruskal-Wallis test was used to evaluate whether there was a difference between the mean bond strengths of the groups. The Tamhane's test was used for pairwise comparison of the average bond strengths of the groups. Pearson's chi-square test was used to compare the debonded surfaces. The significance level (p) in the application of the tests was accepted as 0.05.

Results

Bonding Strength Results

Table 2 shows the average bond strength, standard deviation, minimum and maximum values of each experimental group (Table 2). According to the results of the Kruskal-Wallis test, a statistically significant difference was found between the means of bond strength of the groups (p=0.002) (Table 3). The Tamhane's test was used in paired comparisons to determine from which groups the differences between groups arised (Table 4). There was no statistically significant difference between the average bond strength of the self-adhesive RS, GCIS and PK cement groups (p>0.05). However, the bond strength average of the RMCIS group was found to be significantly lower than the self-adhesive RS, GCIS and PK cemented groups (p<0.05).

Debonded Surface Results

Debonding types are classified as adhesive, cohesive and mixed debondings. The debonding type between the luting cement and

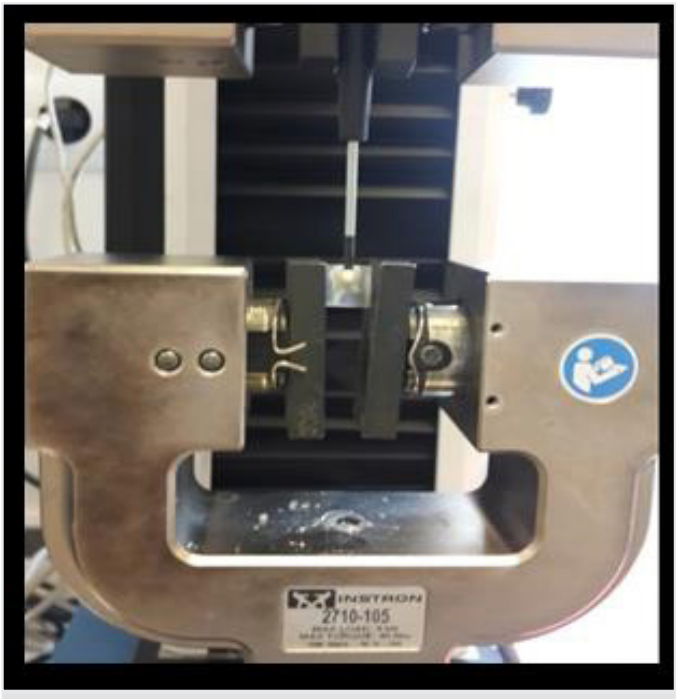

Figure 3. Instron universal test device and a sample put into the device

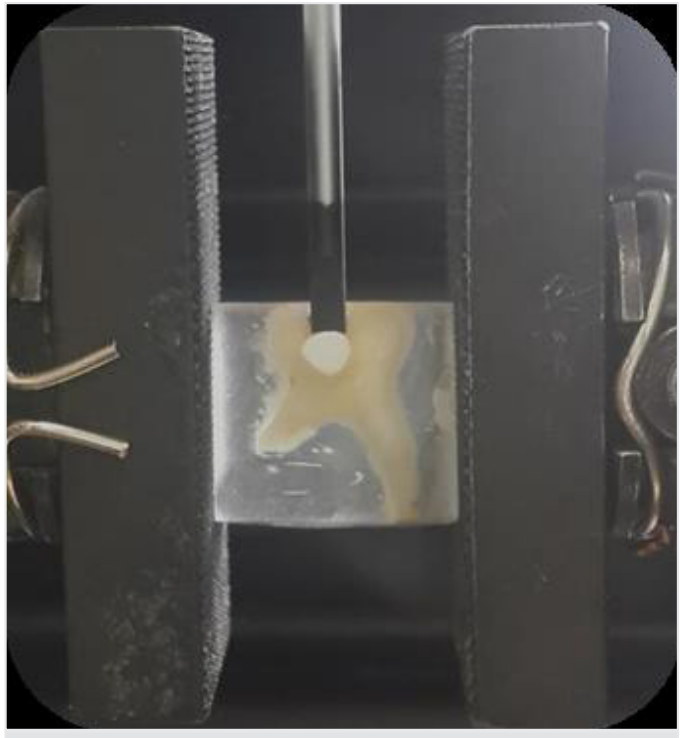

Figure 4. Close-up view of the sample placed in the test device

Table 2. Descriptive values of the bond strengths (N) of the experimental groups (n: number of samples)

Luting cements	n	Mean	Std. deviation	Minimum	Maximum
RS	20	27,222	11,864	9,00	47,18
RMCIS	20	15,011	5,319	5,65	24,00
GCIS	20	23,162	8,094	10,00	39,13
PK	20	27,540	13,521	9,00	46,82
Total	80	23,234	11,235	5,65	47,18

Std. deviation: Standard deviation, RS: Resin cement, RCIMS: Resin modified GCIS, GCIS: Glass ionomer cement, PK: Polycarboxylate cement

Table 3. Comparison of bonding strengths (N) of the experimental groups (Kruskal-Wallis test, p<0.05) (n: number of samples)

Luting cements	n	Std. deviation	x^2	p value
RS	20	2	14,868	0,002*
RMCIS	20			
GCIS	20			
PK	20			

Std. deviation: Standard deviation, RS: Resin cement, RCIMS: Resin modified GCIS, GCIS: Glass ionomer cement, PK: Polycarboxylate cement

Table 4. Paired comparison of bond strengths (N) of the experimental groups (Tamhane's test, p<0.05)

(I) Luting cement	(J) Luting cement	Mean difference (I-J)	p value
RS	**RMCIS**	12,211	0,002*
	GCIS	4,060	0,766
	PK	-0,318	1,000
RMCIS	**RS**	-12,211	0,002*
	GCIS	-8,151	0,004*
	PK	-12,529	0,004*
GCIS	**RS**	-4,060	0,766
	RMCIS	8,151	0,004*
	PK	-4,378	0,781
PK	**RS**	0,318	1,000
	RMCIS	12,529	0,004*
	GCIS	4,378	0,781

Std. deviation: Standard deviation, RS: Resin cement, RCIMS: Resin modified GCIS, GCIS: Glass ionomer cement, PK: Polycarboxylate cement

the bonding surfaces is called adhesive type, debonding inside the luting cement is called cohesive type, and the debonding type that involves adhesive and cohesive debondings is called mixed type (17). Table 5 shows the distribution rates of the debonding types according to the experimental groups. No statistically significant difference was found between the debonded surfaces of zirconia samples after cementation with different luting cements (p=0.497).

Discussion

With the increasing demand for aesthetic restoration in pediatric dentistry, the use of zirconia crowns is becoming widespread (8). However, the presence of cementation material, which positively affects the bond strength, is one of the important factors in the long-term success of crowns (13,14). In this study, the bond strength of these four widely used cementation materials to zirconia samples was investigated. As a result, no significant difference was found between the average bond strength of self-adhesive RS, GCIS and PK cements. The RMCIS group, on the other hand, showed the lowest bond strength average.

In the literature, there are studies on the bond strength of zirconia crowns applied with different bonding cements to permanent teeth (18,19). Palacios et al. (19), cemented them with RS (Panavia F 2.0), self-adhesive RS (Rely X Unicem)

Table 5. Distribution of debonding types according to experimental groups (n: sample number)

Luting cement	Debonding (n (%))		
	Adhesive	Mixed	Cohesive
RS	14 (70)	6 (30)	0 (0)
RMCIS	16 (80)	3 (15)	1 (5)
GCIS	15 (75)	5 (25)	0 (0)
PK	13 (65)	5 (25)	2 (10)

RS: Resin cement, RCIMS: Resin modified GCIS, GCIS: Glass ionomer cement, PK: Polycarboxylate cement

and RMCIS (Rely X Luting) after abrading the inner surface of zirconia crowns produced with CAD/CAM with aluminum oxide particles in their in vitro study on permanent molars. The samples were subjected to tensile testing after 5,000 cycles of thermal cycling. As a result, it was shown that there was no statistically significant difference between the average bond strength of three different adhesive cement. In our study, the average bond strength of zirconia samples cemented with self-adhesive RS was found to be significantly higher than the RMCIS group. We think that the differences between the results may be due to the use of primary teeth in our study, the fact that zirconia samples are not in the form of crowns, and the difference in cement brands. In addition, in some studies, it has been reported that RMCISs show higher bond strength on the permanent tooth dentin surface compared to primary teeth (20-22). This is explained by the fact that the peritubular area of the primary tooth dentin is thicker than the permanent tooth, that the intertubular dentin covers less volume, and that the calcium level decreases as it approaches the pulp (23).

In an in vitro study performed on primary molar teeth (10), the adapted SCT as a result of the preparations made on the teeth were cemented with RS (Panavia F), RMCIS (Rely X Luting) and GCIS (Aqua Meron). Tensile test was applied to samples that were kept in distilled water for 24 hours after cementation. According to the results of that study, no statistically significant difference was found between the bond strength averages of the RS and GCIS groups. It was reported that the RMCIS group showed the lowest bond strength average. In addition, in scanning electron microscopy examinations, it was shown that there was a good adaptation between tooth structure and SCT in samples cemented with RS. The results of that study were in agreement with the results of our study.

In another in vitro study, RMCIS (Rely X Luting), self-adhesive RS (Clearfil SA) and GCIS (Kavitan Cem) were applied to the enamel and dentin surfaces of primary teeth in standard sizes. Samples waiting in distilled water for 24 hours after the application were subjected to shear test and the debonded surfaces were evaluated. As a result of the study, it was reported that the bond strength of GCIS to primary tooth enamel was significantly lower compared to RMCIS and self-adhesive RS. In addition, there was no statistically significant difference between the average bond strength of three different bonding cements to primary tooth dentin. It was reported that the application of a surface conditioning agent such as polyacrylic acid on the dentine surface before the use of RMCIS and GCIS increased the wettability and bond strength by decreasing the surface tension (24). In the GCIS and self-adhesive RS groups, adhesive-type debonding was observed at a rate of 90-100%, and mixed-type debonding was observed at a rate of 50% in the RMCIS group (24). In our study, there was no difference between the average bond strength of the self-adhesive RS and GCIS groups, but the adhesive type debonding was higher in both groups compared to the other debonding types. However, the lowest bond strength average was found in the RMCIS group, and a higher rate of adhesive type debonding was also observed in this group. The differences between the RMCIS groups were due to the different brand and polymerization type of RMCIS used in our study, the difference in the cementation surfaces and the samples not subjected to thermal cycling in the study by Dadakoglu et al. (24).

The bond strength is affected by the polymerization type of cement (18). The polymerization type determines the degree to which the cement is affected by the moisture and saliva in the mouth in the early period after cementation. Visible light or dual-curing cements are not affected much by moisture in the early stages of cementation compared to conventional cements (16). This situation prevents the reduction of crown retention in the early period after cementation, especially in pediatric patients. The fact that GCIS is sensitive to moisture in the initial hardening and its solubility increases in the case of contamination cause a decrease in bond strength (25). RMCIS was produced in order to eliminate these disadvantages. However, due to the fact that the hydroxymethylmethacrylate (HEMA) in the structure is a hydrophilic monomer, the water absorption property of the material is increased. Although initially water absorption seems to compensate for polymerization shrinkage, it has been reported that it negatively affects the mechanical properties in the long term (26).

Study Limitations

In *in vitro* studies on the retention of cements, imitation of intraoral conditions facilitates the reflection of study results to clinical practice (18). One of the limitations of this study was that the study was performed under static conditions.

Finger pressure applied to stabilize the zirconia samples during the cementation phase did not provide a standard loading. In addition, the samples were thermally cycled in distilled water, which did not fully represent the dynamic environment of the oral cavity. Applying tensile and shear forces in conditions where samples are subjected to dynamic loading in artificial saliva will provide better imitation of intraoral conditions.

Conclusion

In the study, the bond strength of zirconia specimens cemented with different luting cements on the primary tooth dentin surface were evaluated. Considering the results within the limitations of this in vitro study, it was found that the bonding of zirconia crowns with self-adhesive RS, GCIS and PK cement did not cause a difference in bond strength. We think that bonding prefabricated zirconia crowns with self-adhesive RS may provide ease of clinical application compared to chemically cured GCIS and PK cement in children whose treatment periods are not too long and in whom moisture control is difficult. However, we are of the opinion that the issue should be supported by long-term clinical studies.

Ethics

Ethics Committee Approval: Ethics committee approval was given by Erciyes University Faculty of Medicine (date: 12.06.2018).

Informed Consent: Obtained.

Peer-review: Externally peer reviewed.

Authorship Contributions

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

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Original Article

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Impact of UCP2 -866G/A Variant on Smoking Risk

UCP2 -866G/A Varyantının Sigara İçme Durumuna Etkisi

Ayşe Feyda NURSAL[1], Mehmet Atilla UYSAL[2], Mustafa PEHLİVAN[3], Ülgen SEVER[4], Sacide PEHLİVAN[4]

[1]Hitit University Faculty of Medicine, Department of Medical Genetics, Çorum, Turkey
[2]University of Health Sciences Turkey,Yedikule Hospital For Chest Diseases and Thoracic Surgery Training and Research Hospital, Clinic of of Chest Diseases, İstanbul, Turkey
[3]Gaziantep Univesity Faculty of Medicine, Department of Hematology, Gaziantep, Turkey
[4]İstanbul University İstanbul Faculty of Medicine, Department of Medical Biology, İstanbul, Turkey

ABSTRACT

Objective: Mitochondria are multifunctional and dynamic organelles found in cells. Nicotine is a natural alkaloid found in the tobacco plant and has been well studied as a component of cigarette smoke. It has also been reported to affect mitochondrial function both *in vitro* and *in vivo*. Uncoupling protein 2 (UCP2) reduces generation of ROS by mitochondria. Our purpose in this study was to investigate whether the -866G/A variant of the *UCP2* gene is associated with smoking status.

Methods: A total of 238 individuals consisting of 138 smokers and 100 healthy controls were examined. The *UCP2*-866G/A variant was genotyped by polymerase chain reaction-restriction fragment length polymorphism method.

Results: The proportion of individuals carrying the three possible genotype was significantly different between the smoker and healthy control groups. The *UCP2*-866G/A variant GG genotype was associated significantly with an increased risk of smoking (p=0.001) while AA genotype was associated significantly with a decreased risk of smoking (p=0.001). The *UCP2*-866G/A variant G allele was found to be increased in the smoker group compared to the healthy controls (p=0.001).

Conclusion: Our data suggest that the *UCP2*-866 G/A variant GG genotype and G allele might reflect the risk of smoking status in a Turkish population.

Keywords: Smoking status, *UCP2*, variant, PCR-RFLP

ÖZ

Amaç: Mitokondriler hücrelerde bulunan çok fonksiyonlu ve dinamik organellerdir. Nikotin tütün bitkisinde bulunan doğal bir alkaloiddir ve sigara içme durumunun iyi çalışılmış bir bileşenidir. Nikotinin *in vitro* ve *in vivo* olarak mitokondriyal fonksiyonu etkilediği rapor edilmiştir. Uncoupling protein 2 (UCP2) mitokondri ile ROS üretimini azaltır. Bu çalışmadaki amacımız *UCP2*-866G/A varyantının sigara içme durumu ile ilişkili olup olmadığını araştırmaktır.

Yöntemler: Yüz otuz sekiz sigara içen ve 100 sağlıklı kontrolden oluşan toplam 238 kişi değerlendirildi. *UCP2*-866G/A varyantı polimeraz zincir reaksiyonu-sınırlayıcı enzim parça uzunluk çeşitliliği metodu ile genotiplendi.

Bulgular: Üç olası genotipi taşıyan kişileri oranı sigara içenler ve sağlıklı kontrol grupları arasında belirgin şekilde farklıydı. *UCP2*-866G/A varyantı AA genotipi belirgin şekilde azalmış sigara içme riski ile ilişkiyken (p=0,001), GG genotipi belirgin şekilde artmış sigara içme durumu ile ilişkiliydi (p=0,001). *UCP2*-866G/A varyant G alelinin sigara içen grupta sağlıklı kontrollere göre artmış olduğu bulundu (p=0,001).

Sonuç: Verilerimiz *UCP2*-866 G/A varyant GG genotip ve G alelin Türk popülasyonundaki sigara içme riskini yansıtabileceğini desteklemektedir.

Anahtar Sözcükler: Sigara içme durumu, *UCP2*, varyant, PCR-RFLP

Address for Correspondence: Ayşe Feyda NURSAL, Hitit University Faculty of Medicine, Department of Medical Genetics, Çorum, Turkey
E-mail: feyda.nursal@gmail.com **ORCID ID:** orcid.org/0000-0001-7639-1122

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Introduction

Mitochondria, which are found nearly in all eukaryotic cells, play an important role in many cellular functions. These organelles are often referred to as the "powerhouse" of cells because they provide approximately 90% of the energy needed for survival (1). The mitochondria is essential for adenosine triphosphate (ATP) production through oxidative phosphorylation (OXPHOS). In addition, they are involved in several other cell processes such as cell differentiation, calcium signaling, and apoptosis, as well as cell cycle control and cellular growth (2). Mitochondrial function impairment is related to the pathogenesis of several serious diseases due to the complexity of mitochondrial physiology. Decrease in mitochondrial function manifested by the following: lower mitochondrial membrane potential, alterations in electron transport chain complex activities, reduced ATP synthesis, inefficient Ca^{2+} buffering, enhanced reactive oxygen species (ROS) synthesis, modified mitochondrial dynamics, or release of pro-apoptotic factors found during aging and in many chronic diseases such as cardiovascular diseases, diabetes, cancer, and numerous metabolic syndromes (3).

Nicotine is the main active component of cigarettes that is involved in physical dependence, affecting nicotine receptors in the central nervous system that leads to the release of neurotransmitters [including dopamine (DA)]. Nicotine activates dopaminergic neurons in the mesolimbic reward system and facilitates DA release (4). Some studies reported inhibitory effects of nicotine on the OXPHOS machinery. Cormier et al. (5) showed that nicotine inhibits oxygen consumption of isolated rat brain mitochondria depending on its concentration. Smoking lowers arterial oxygen carrying capacity via increased serum carboxyhemoglobin levels that leads to OXPHOS dysfunction in cardiac cells (6). Rats exposed to passive cigarette smoke from two cigarettes per day for two months manifest seriously impaired myocardial OXPHOS function during reperfusion injury (7). This impact of nicotine on mitochondrial oxygen consumption indicates its direct effect on respiratory chain complexes.

Mitochondrial uncoupling proteins (UCPs) constitute a group of mitochondrial anion carriers that seem to be involved in proton transmission across the inner membrane, which can be related to thermogenesis, and play a role in regulating mitochondrial membrane potential, hence, generating mitochondrial ROS (8). *UCP2* gene belongs to this superfamily and covers a 6.3 kb region on chromosome 11 (region 11q13) and has eight exons and seven introns (9). *UCP2* is universally expressed at different levels in various tissues such as the skeletal muscle, heart, lung, spleen, thymus, and brain (10). The -866G/A (rs659366) variant of UCP2 is found in the *cis* regulatory site of the promoter region. It has been found to be a functional variant on gene expression, and the variant could alter *UCP2* function. Thus, this study aimed to investigate whether the *-866G/A* gene variant (rs659366) of the *UCP2* gene is associated with smoking status in the Turkish population.

Methods

Study Population

This cross-sectional association study included 138 smokers and 100 healthy controls. Participants were selected among the individuals from Yedikule Chest Diseases and Thoracic Surgery Training and Research Hospital, İstanbul, Turkey. Data on the average amount of tobacco consumed per day was recorded from all participants. The degree of smoking status was evaluated by the scores on Heaviness of Smoking Index and the Fagerström Test for Nicotine Dependence (11). Healthy volunteers (control group) were selected from the voluntaries who were non-smokers. All participants were of Turkish origin, and a written informed consent was obtained from each. The experimental study protocol and process was assessed and approved by the ethics committee (İstanbul Medical Faculty, 2015/17).

Genotyping

Blood samples were obtained from participants. Genomic deoxyribonucleic acid was isolated from whole blood by salting out method (12). Genotyping of the -866G/A variant in the promoter region of the *UCP2* gene was performed by polymerase chain reaction (PCR)-restriction fragment length polymorphism method as previously described (13). Primer sequence 5′-CAC GCT GCT TCT GCC AGG AC-3′ and 5′-AGG CGT CAG GAG ATG GAC CG-3′ were used to amplify -866G/A variant of *UCP2* gene. After amplification, PCR products were subjected to restrict digestion using *Mlu*1 (Invitrogen) for all night at 37 C. Fragment amplification and digestion results were revealed by 2% agarose gel electrophoresis and visualized on a ultraviolet transilluminator after ethidium bromide staining.

Statistical Analysis

All data were analyzed by using the Statistical Package for the Social Sciences (SPSS) for Windows (version 16.0; SPSS Inc, Chicago, IL, USA). The χ2-test was used for statistical analysis of genotype and allele frequencies in case and control groups (GraphpadInstat version 3, Graphpad Software, San Diego, CA, USA). Results are given as mean ± standard deviation, whereas allele frequencies and distribution of genotypes are given as percentages (%). The Hardy-Weinberg equilibrium (HWE) was calculated using the De-finetti program (Online HWE and Association Testing- InstitutfürHumangenetik, Munich, Germany). Statistical significance was considered at $p<0.05$.

Results

Our study includes 238 individuals, 138 smokers and 100 ethnicity-matched healthy controls. The allele and genotype frequencies of *UCP2* -866G/A variant for smoking participants versus controls are shown in Table 1. The proportion of individuals carrying three possible genotypes (-866G/G, -866G/A, and -866A/A) was significantly different between controls and smoker groups. The *UCP2* -866G/A variant G/G genotype was more common in smoker groups compared to the control group [p=0.001, odds ratio (OR): 0.213, 95% confidence interval (Cl): 0.116-0.392], whereas the A/A genotype was lower in smoker

group compared to the control (p=0.001, OR: 3.664, 95% Cl: 2.000-6.713). The *UCP2* -866G/A variant G allele was increased in smoker group than the control group (p=0.001, OR: 3.301, 95% Cl: 2.257-4.828). The observed genotype counts deviated significantly from those expected in smoker group according to the HWE for *UCP2* -866G /A variant.

Discussion

Mitochondria are indispensable organelles in all eukaryotic cells, modulating many crucial vital processes for cell viability and function, such as energy generation, redox control, calcium homeostasis, and several metabolic and biosynthetic pathways. The mitochondrial respiratory chain is the main source of intracellular ROS production, as well as a significant target for the harmful effects of ROS (14). Causing various physiologic and biochemical consequences generated by cigarette smoke components, tobacco use is a serious health issue. Cigarette smoking initiates oxidative stress in several organs such as the brain and plays a role in numerous diseases, such as cognitive or neurodegenerative pathological changes (15). Nicotine can pass through the blood-brain barrier and end up in high levels in the brain tissue within 10-20 s following inhalation (16). Nicotine in similar amounts to those in cigarette smoke can lead to oxidative stress, as shown *in vitro* and *in vivo* (17). Changes in brain energy metabolism indicated the role of mitochondria, the key players in cellular energy production. Cigarette smoking can result in mitochondrial dysfunction (18), as shown by elevated cholesterol, lipid peroxides, and increased cholesterol/phospholipid ratio, along with reduced mitochondrial enzymes in those exposed to cigarette smoke (19).

UCPs can dissociate mitochondrial oxidation from phosphorylation, decreasing mitochondrial energy generation and augment cell thermogenesis. So, far, UCPs has been given attention for their role in peripheral energy metabolism, where their energy-dissipating function has been uncovered primarily with regard to metabolic disorders. In the central nervous system, mammalian UCP2 messenger ribonucleic acid and protein expression is most prominent in regions that could be depicted as high risk for stress (20). High risk areas have direct access to the blood stream, axons, and axon terminals abundant in the hypothalamus, neural circuits crucial for the endocrine and homeostatic regulation, and those related to neurodegenerative disease like the substantia nigra and locus coeruleus (20). The hippocampus is a significant brain structure in both cognitive and emotional functions (21). UCP2-knockout mice were significantly more anxious in the elevated plus-maze than wild-type mice in a behavioral study with an anxiety setting (22). In addition, UCP2 seems to be involved in neuronal plasticity and regeneration (23). Most of these studies suggest an important role of UCP2 in the maintenance of cognitive functions and anxiety resistance. Thus, lack of UCP2 may impair the endocrine, mitochondrial bioenergetics, and thermoregulation of the neurons, which are related to cognition, mood, and behavior. In the brain, the DA turnover is modified in UCP2-deficient (UCP2-/-) mice, and the number of mitochondria in neurons of the substantia nigra and the ventral tegmental area is decreased in UCP2-/- mice, whereas ROS generation is increased (24). Brain DA is known to play a central role in addictive disorders including nicotine addiction. Besides, Li et al. (25) demonstrated that chronic nicotine use significantly lowered UCP2 in the cerebral cortex and cerebral arteries in rats.

Polymorphisms within the *UCP2* gene have been reported to be related with several metabolic traits including obesity and type 2 diabetes. A functional -866G/A variant has been identified in the *UCP2* promoter and is found in a multifunctional cis regulatory site with functional consequences. The -866A allele has been found to elevate *UCP2* transcriptional activity in transfected cultured cells (26); however, data in human tissues have been conflicting (27). In this study, we hypothesized that the -866G/A variant of the *UCP2* gene is associated with risk of smoking status in the Turkish population. To our knowledge, for the first time in the literature, we have demonstrated a significant association of the *UCP2* functional variant with smoking status in the Turkish cohort. We found that participants carrying *UCP2* -866G/A variant GG genotype showed a higher risk for smoking (Table 1). The participants carrying *UCP2* -866G/A variant AA genotype and A allele may have protective effects in smoking for the Turkish population (p<0.05) (Table 1). The observed overall genotype distribution in smoker group was not consistent with HWE.

Study Limitations

Several limitations were encountered in this analysis. Firstly, only one variant of *UCP2* gene was evaluated. Other variants of this

Table 1. Genotype and allele distribution of UCP2 -866G/A variant in case and control groups

UCP2	Smoker group	Control group	OR*	95% CI*	p
G genotypes	n=138 (%)	n=100 (%)			
G/G	70 (50.8)	18 (18)	0.213	0.116-0.392	0.001
G/A	46 (33.3)	41 (41)	1.390	0.816-2.368	0.275
A/A	22 (15.9)	41 (41)	3.664	2.000-6.713	0.001
Alleles					
G	186 (67.4)	77 (38.5)	3.301	2.257-4.828	0.001
A	90 (32.6)	123 (61.5)		1.933-4.357	
HWE	0.004	0.179			

HWE: Hardy-Weinberg equilibrium; results that are statistically significant are typed in bold, OR: Odds ratio, CI: Confidence interval

gene may also susceptibly play a role. Additionally, the gene-gene and gene-environment interactions were not discussed for the *UCP2* variant due to lack of original information.

Conclusion

In conclusion, our observation suggests that the *UCP2* -866G/A variant GG genotype and G allele may create a predisposition to smoking. Therefore, *UCP2* gene should be considered as candidate gene for both smoking status in the Turkish population. However, further long-term and large-scale studies are required to confirm issues regarding the mechanisms underlying the observed association.

Ethics

Ethics Committee Approval: The experimental study protocol and process was assessed and approved by the ethics committee (İstanbul Medical Faculty, 2015/17).

Informed Consent: All participants were of Turkish origin, and a written informed consent was obtained from each.

Peer-review: Externally peer reviewed.

Authorship Contributions

Data Collection or Processing: M.A.U., Ü.S., S.P., Analysis or Interpretation: A.F.N., M.A.U., Ü.S., S.P., Literature Search: A.F.N., M.P., Writing: A.F.N., M.P.

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

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Original Article

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Comparison of Two Different Applications of Proprioceptive Neuromuscular Facilitation Techniques to Increase Upper-Extremity Muscle Strength

Üst Ekstremite Kas Kuvvetinin Artırılmasında Proprioseptif Nöromusküler Fasilitasyon Tekniklerinin İki Farklı Şekilde Uygulanmasının Karşılaştırılması

Hasan Atacan TONAK[1], Nihal BÜKER[2], Ali KİTİŞ[2], Erdoğan KAVLAK[2]

[1]Akdeniz University Faculty of Health Sciences, Department of Physiotherapy and Rehabilitation, Antalya, Turkey

[2]Pamukkale University School of Physical Therapy and Rehabilitation, Denizli, Turkey

ABSTRACT

Objective: The aim of this study to investigate the efficacy of bilateral symmetrical proprioceptive neuromuscular facilitation (PNF) patterns in increasing the muscle strength in the upper extremity by comparing their combination with elastic resistance band (ERB) use versus physiotherapist-administered programs.

Methods: Participants were randomly divided into two groups (PNF, elastic resistance band). The strength increasing exercise program was administered to the participants of both groups 3 days a week for 6 weeks. All participants were evaluated before and after exercise program. The circumferences of the upper extremities and hand grip strengths were evaluated. The push-up and dip-strength tests were performed to evaluate the endurance of the arm and the shoulder girdle.

Results: A total of 40 individuals in the age range from 20 to 25 years [(PNF group: $\overline{X}$ ± standard deviation (SD) =21.80±1.05 years; ERB group: $\overline{X}$ ± SD =21.40±1.66 years] were included in this study. The comparison of before and after the exercise revealed that there was a statistically significant increase in the arm and forearm circumferences of both extremities in the both groups after the training ($p<0.05$). The groups comparisons demonstrated that there were no statistically significant change differences in

ÖZ

Amaç: Çalışmanın amacı bilateral simetrik proprioseptif nöromüsküler fasilitasyon (PNF) paternlerinin üst ekstremite kas kuvvetinin artırılmasında fizyoterapist tarafından uygulanan programlara karşı elastik dirençli bant (EDB) ile uygulanan kombinasyonunun karşılaştırılmasıdır.

Yöntemler: Katılımcılar randomize olarak iki gruba ayrıldı (PNF, elastik dirençli bant). Kuvvetlendirme egzersizleri her iki gruptaki katılımcılara 6 hafta boyunca haftada 3 gün uygulandı. Bütün katılımcılar egzersiz programının öncesinde ve sonrasında değerlendirildi. Üst ekstremite çevre ölçümleri ve kavrama kuvvetleri değerlendirildi. Kol ve omuz kuşağının enduransı değerlendirmek için push-up ve dip-strength testleri uygulandı.

Bulgular: Çalışmaya yaşları 20 ile 25 arasında değişen (PNF grubu: $\overline{X}$ ± standart sapma (SS) =21,80±1,05 yıl; EDB grubu: $\overline{X}$ ± SS =21,40±1,66 yıl] 40 birey dahil edildi. Egzersiz öncesi ve sonrası karşılaştırmalarda, her iki grupta da iki üst ekstremitesinin kol ve önkol çevre ölçümlerinde egzersiz sonrasında istatistiksel olarak bir artışın olduğunu gösterdi ($p<0,05$). Grupların karşılaştırmalarında, kol, önkol ve el bileği çevre ölçümlerinde ve push-up ve dip-strength tekrar sayılarında istatistiksel olarak değişiklik olmadığını gösterdi ($p>0,05$).

Address for Correspondence: Hasan Atacan TONAK, Akdeniz University Faculty of Health Sciences, Department of Physiotherapy and Rehabilitation, Antalya, Turkey
E-mail: atacantonak@akdeniz.edu.tr **ORCID ID:** orcid.org/0000-0002-3545-936X

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the circumferences of the arms, forearms, and the wrists of both extremities; and the number of push-ups and dip-strength repeats ($p>0.05$).

Conclusion: This study demonstrated that PNF patterns can be used in these two different modes of administration (with ERB and manually by the physiotherapist) to increase the muscle strength and improve endurance in the upper extremity and that none of them was superior to the other.

Keywords: Strength, upper extremity, exercise

Sonuç: Bu çalışma üst ekstremiteye yönelik kuvvetlendirme ve endurans eğitiminde PNF paternlerinin iki farklı şekilde (elastik dirençli bant ile ve fizyoterapist tarafından manuel olarak) kullanılabileceğini ve birbirlerine üstünlüğü olmadığı gösterdi.

Anahtar Sözcükler: Kuvvet, üst ekstremite, egzersiz

Introduction

Proprioceptive neuromuscular facilitation (PNF) techniques were developed by Margaret Knott, Dorothy Voss, and Dr. Herman Kabat in the 1940s for rehabilitation treatment (1). PNF techniques are mainly used to improve functional ability and existing muscle strength (1). PNF is mainly based on the principle that physiological human motion is characterized by rotational and oblique patterns, which can be improved by resistance exercises performed against a maximum level of resistance (2). PNF aims to improve posture and range of motion by utilizing tactile, visual, and verbal stimuli (1-3).

PNF exercises are characterized by rhythmic motions of the joints and the contraction of muscles against maximum resistance. PNF's theoretical principles are based on the stimulation of proprioceptors to improve the neuromuscular system's responses (4). PNF is generally an effective method to increase joint range of motion, muscle strength, endurance, and stabilization. It also improves joint position sense, coordinated movements, and flexibility (3,5). PNF is particularly used in athletic training programs to increase flexibility, coordination, muscle strength, and performance (2,6). Studies have shown that PNF is efficacious in increasing muscle strength (7,8). Surburg and Schrader (9) reported that the repeated contractions technique is the most preferred and the most effective method, especially to increase upper extremity muscle strength.

Studies have shown that elastic resistance band (ERB) use in PNF increased muscle strength, range of motion, flexibility, and the patient's participation in daily living activities when used for rehabilitation purposes (10,11). Studies have been published using PNF exercises to increase muscle strength (12,13). PNF exercises applied manually by a physiotherapist cause a significant increase in muscle strength (6,12). Moreover, studies suggest that PNF exercises with an ERB increase muscle strength (10,14). ERBs are commonly used in clinics because of their affordable price, ease of application, and safety (10). Nevertheless, the most common technique used in clinics is PNF exercises applied manually by a physiotherapist (2,13). However, no study investigated and compared the effects of these two techniques, commonly used in clinics to strengthen the upper-extremity muscles. Our study was designed to investigate ERB use's efficacy versus a physiotherapist-administered program in applying bilateral symmetrical PNF patterns to increase upper-extremity muscle strength, considering that the resistance would be adjusted manually by the physiotherapist and thereby the sensitive inputs would be more effective in the latter mode of application.

Methods

This study was approved by the non-interventional ethics committee (B.30.2.PAÜ.0.20.05.09/126). The informed consent process was completed after the eligible individuals were informed about the study, and then they signed the informed consent documents.

Study Population

The study included 40 university students, whose ages ranged from 20 to 25 years old, who agreed to participate in the study, and who had no disease or disability diagnoses that would impair upper-extremity performance as confirmed by a specialist physician. Patients who had a limited range of motion in the upper extremities, who had undergone upper-extremity surgery in the last six months, had any chronic neurological, musculoskeletal, metabolic, rheumatologic, or psychiatric disorders that would affect upper-extremity performance were excluded from the study.

Assessment Methods

Forty participants were randomly divided into two groups, namely the PNF and ERB groups, using gender-based block randomization. All participants were evaluated before and after the 6-week exercise program by the same physiotherapist, who was experienced in orthopedic rehabilitation and was not informed of the study details.

Descriptive information was collected from the participants and documented in a form developed for the study. The anthropometric measurements of the circumferences of the arms, forearms, and wrists were taken from both of the participants' upper extremities using a standard tape measure (15). The wrist circumference was measured while the participant was standing with both palms rotated toward each other, the arms facing the body, and the elbow flexed at 90°. Hence, the tape measure was in full contact with the styloid processes of the radius and ulna. While measuring the mid-forearm circumference, the forearm length was first measured by measuring the distance between the olecranon and the styloid process of the radius and calculating

the midpoint of the forearm. The circumference was then measured from this point with the arm in the same position as in the wrist circumference measurement. For the mid-arm circumference measurement, the distance between the acromion and the olecranon was first measured in the same position as in the wrist and forearm circumference measurements. The arm length measurement was recorded, and the midpoint of the arm was calculated. The circumference was then measured from this point, and all anthropometric circumference measurement results were recorded (15). A Baseline® hydraulic hand dynamometer was used to evaluate handgrip strength, measured according to the standard measurement method described by the American Society of Hand Therapists. Each measurement was repeated three times, and the mean of these three measurements was recorded as the handgrip strength in kilograms (16).

The push-up test was performed to evaluate arm and shoulder girdle endurance. The test started when the participant was in a prone position with his/her arms and elbows flexed. Then, the participant was asked to push up his/her head, shoulders, and trunk by bringing their elbows into full extension. The test position was applied in men and women differently; men were asked to have their trunk and lower extremities in full extension, whereas women were asked to have their trunk in extension and knees in flexion. The test continued until the participant felt fatigued. The number of repetitions was recorded as the score (17). The dip-strength test was used for measuring the shoulder girdle and the upper-extremity endurance during vertical elevation movement and the push up. The participant was asked to push up until his/her elbows were fully extended while they supported themselves with their hands using the parallel bar. The test continued until the participant felt fatigued, and the correct number of repetitions was recorded as the score (18). The participants took three-minute rest intervals between the tests to control for the potential confounding effects of fatigue.

Training

A physiotherapist performed participants' assessments in both groups after the assessments were over, PNF exercises were taught to all participants. The exercises were carried out by the physiotherapist for 20 participants in the PNF group, three days a week for 6 weeks. The participants in the ERB group performed their 6-week exercise programs under the physiotherapist's supervision. They were verbally warned by the physiotherapist to avoid any wrong exercises and correct wrong exercises. Participants in both groups were trained on the bilateral symmetrical PNF patterns of the upper extremities in two different diagonals (flexion-adduction-external rotation, extension-abduction-internal rotation; flexion-abduction-external rotation, extension-adduction-internal rotation) in the sitting position on a chair with their backs supported. The exercise programs to increase strength were administered to participants of both groups three days a week for six weeks, for 18 sessions per participant. Exercises started with four repetitions that were increased until ten repetitions were achieved progressively in time (14).

Proprioceptive Neuromuscular Facilitation (PNF) Group

PNF group participants were administered the muscle strength increase program while sitting on a chair with back support. Bilateral symmetrical PNF patterns of the upper extremity were applied using the repeated contractions technique. PNF group participants performed their exercises with a physiotherapist. The repeated contraction technique was used to increase upper-extremity muscle strength. When starting the exercise, the muscles were first put in their longest positions, and isotonic contractions were stimulated with stretching. With verbal commands, the exercise was performed with isotonic and isometric contractions after stretching. Isometric contractions were performed by commanding "hold" at every point where the movement was weakened. Isotonic and isometric contractions were repeated and applied until the endpoint of the pattern, where the agonist muscles were in the shortest position. During the exercise, the physiotherapist acted diagonally with the participant without interrupting the hand contact to prevent the movement's completion (2,19). A resting period of three minutes was allowed in the interval between each training session.

Elastic Resistance Band (ERB) Group

Red, blue, green, and black colored bands (Thera-Band®, Hygenic Corporation, Akron, OH, USA) were used during ERB technique application. The study of Areas et al. (14) was the reference to select the bands. The appropriate band was selected for each participant based on their performance after a baseline assessment with one maximum repeat test using a red Thera-Band (14). The Thera-Band® website was used to comply with the elastic resistance bands' user guidelines (20).

After selecting the appropriate ERB color, completing the training program, and confirming proper band use, the participant performed the upper-extremity bilateral symmetrical patterns in the same sitting position on a chair with back support under the physiotherapist's supervision. A resting period of three minutes was applied in the interval between each training session.

Statistical Analysis

Windows-based SPSS (IBM SPSS Statistics, Version 23.0, Armonk, NY, USA) package program was used to analyze the study data. The number of participants to be included in the study was determined to be 12 based on the power analysis results at α=0.05 and β=0.20 (for 80% power) (14). Considering that potentially 25% of data could be lost, 15 individuals were included in each group to participate in the 6-week exercise program. Analytical (Kolmogorov-Smirnov/Shapiro-Wilks tests) and visual (histograms and probability graphs) methods were used to test data conformity to a normal distribution. Descriptive statistics are presented as mean ± standard deviation ($\overline{X}$ ± SD) and percentages. The Mann-Whitney U test was used for testing intergroup differences. The Wilcoxon test was used for determining intragroup differences. The level of statistical significance was accepted to be $p<0.05$ in this study.

Results

A total of 40 individuals ranging in age from 20 to 25 years old (PNF group: $\overline{X}$ ± SD =21.80 ± 1.05 years; ERB group: $\overline{X}$ ± SD =21.40±1.66 years) were included in this study. Other participant demographic information is presented in Table 1. Before the exercises, there were no statistically significant intergroup differences in the circumferences of the arms, forearms, and wrists of the dominant and nondominant extremities and the number of push-ups and dip-strength repeats (p>0.05) (Table 2).

The intragroup comparison of the data collected before and after the exercises revealed a statistically significant increase in both extremities' arm and forearm circumferences in the PNF group after the exercise (p<0.001). Also, there was a statistically significant increase in the PNF group in the number of dip-strength repetitions than pre-exercise values (p<0.001). No statistically significant differences were observed in the wrist circumference, grip strength, and the number of push-up repeats of both upper extremities compared with pre-exercise values (p>0.05) (Table 3). In the ERB group, there was a statistically significant difference in the arm and forearm circumferences of both extremities and the dominant extremity's wrist circumference compared with pre-exercise values (p<0.05). Also, there was a statistically significant increase in the number of push-ups and dip-strength repetitions compared with pre-exercise data (p<0.05). There were no statistically significant differences in the wrist circumference of the nondominant arm and the grip strength of both upper extremities after exercise (p>0.05) (Table 3).

The intergroup comparisons demonstrated that there were no statistically significant differences in the change in the circumferences of the arms, forearms, and wrists of both

Table 1. Participant demographic data

Variables	Groups				p[a]
	PNF group (n = 20)		ERB group (n = 20)		
	min-max	$\overline{X}$ ± SD	min-max	$\overline{X}$ ± SD	
Age (years)	20-23	21.80±1.05	20-24	21.40±1.66	0.568
Height (cm)	156-187	170.40±9.68	155-183	167.65±7.43	0.621
Weight (kg)	50-84	65.15±12.62	45-80	58.90±10.42	0.209
BMI (kg/m²)	17.72-26.22	22.19±2.40	16.52-26.73	20.83±2.64	0.172
	n	**%**	**n**	**%**	
Hand dominance					
Right	20	100	20	100	
Left	0	0	0	0	

p<0.05, p[a]: Mann-Whitney U test, PNF: Proprioceptive neuromuscular facilitation, ERB: Elastic resistance band, cm: Centimeter, kg: Kilogram, BMI: Body mass index, m: Meter, min: Minimum, max: Maximum, SD: Standard deviation

Table 2. Comparison of anthropometric measurements, grip strengths and endurances of groups before training

Variables	PNF group	ERB group	p[a]
	$\overline{X}$ ± SD	$\overline{X}$ ± SD	
Arm circumference (cm)			
Dominant	25.84±2.98	24.27±3.16	0.104
Non-dominant	25.39±2.96	24.27±3.18	0.110
Forearm circumference (cm)			
Dominant	21.63±1.97	21.35±2.32	0.399
Non-dominant	21.07±2.98	21.27±2.26	0.447
Wrist circumference (cm)			
Dominant	15.84±1.40	15.47±1.41	0.383
Non-dominant	15.76±1.35	15.47±1.41	0.556
Grip strength (kg)			
Dominant	35.77±11.45	32.17±10.31	0.582
Non-dominant	32.07±10.61	29.10±10.09	0.666
Push up (rp)	13.31±4.44	10.85±6.70	0.162
Dip-strength (rp)	7.47±6.99	4.65±3.36	0.113

p<0.05, p[a]: Mann-Whitney U test, PNF: Proprioceptive neuromuscular facilitation, ERB: Elastic resistance band, cm: Centimeter, kg: Kilogram, rp: Number of repetitions, min: Minimum, max: Maximum, SD: Standard deviation

extremities; and in the change in the number of push-ups and dip-strength repeats (p>0.05) (Table 4).

Discussion

This study was designed to reveal the difference between the two different modes of PNF administration to increase the strength in the upper-extremity muscles, namely the repeated contractions method applied by a physiotherapist and PNF patterns with ERB under the supervision of a physiotherapist. It showed that the extremity circumferences and endurance were increased in both groups with no intergroup differences. Neither of the two PNF administration modes was superior in improving upper-extremity muscle strength and performance when the PNF manual repeated contractions technique applied by the physiotherapist was compared with ERB use in PNF under the supervision of the physiotherapist.

Table 3. Comparison of anthropometric measurements, grip strengths and endurances before and after training

	Before training		After training		p[a]
	min-max	$\overline{X}$ ± SD	min-max	$\overline{X}$ ± SD	
PNF group					
Arm circumference (cm)					
Dominant	21.00-32.50	25.84±2.98	21.00-33.00	26.07±3.04	**0.001**
Non-dominant	21.00-32.00	25.39±2.96	21.00-32.50	25.81±3.03	**0.001**
Forearm circumference (cm)					
Dominant	18.50-26.00	21.63±1.97	19.00-26.50	21.84±2.02	**0.007**
Non-dominant	18.00-27.50	21.07±2.18	18.50-27.50	21.31±2.10	**0.008**
Wrist circumference (cm)	14.00-18.50	15.84±1.40	14.00-18.50	15.94±1.42	0.104
Dominant	14.00-18.50	15.76±1.35	14.00-18.00	15.81±1.31	0.494
Non-dominant					
Grip strength (kg)					
Dominant	21.30-54.00	35.77±11.45	18.00-55.30	36.04±12.64	0.649
Non-dominant	16.00-52.30	32.07±10.61	17.30-51.00	33.14±12.16	0.223
Push up (rp)	6.00-22.00	13.31±4.44	6.00-30.00	14.94±6.34	0.121
Dip strength (rp)	1.00-25.00	7.47±6.99	1.00-27.00	9.89±8.19	**0.001**
ERB group					
Arm circumference (cm)					
Dominant	20.00-32.50	24.27±3.16	20.50-34.00	24.77±3.27	**0.006**
Non-dominant	20.00-32.50	24.27±3.18	20.00-34.00	24.70±3.34	**0.015**
Forearm circumference (cm)	17.00-26.00	21.35±2.32	17.00-27.00	21.80±2.59	**0.005**
Dominant	17.00-26.00	21.27±2.26	17.00-27.00	21.67±2.57	**0.014**
Non-dominant					
	13.00-19.00	15.47±1.41	13.00-19.00	15.60±1.42	**0.021**
Wrist circumference (cm)	13.50-19.00	15.47±1.41	13.50-19.00	15.62±1.36	0.083
Dominant					
Non-dominant					
Grip strength (kg)					
Dominant	15.00-51.30	32.17±10.31	14.00-52.70	32.04±10.88	0.852
Non-dominant	13.00-51.30	29.10±10.09	12.30-52.30	29.95±10.50	0.174
Push up (rp)	0.00-25.00	10.85±6.70	5.00-35.00	13.65±7.26	**0.011**
Dip strength (rp)	1.00-11.00	4.65±3.36	1.00-17.00	6.50±4.95	**0.001**

p<0.05, p[a]: Wilcoxon test, PNF: Proprioceptive neuromuscular facilitation, ERB: Elastic resistance band, cm: Centimeter, kg: Kilogram, rp: Number of repetitions, SD: Standart deviation, min: Minimum, max: Maximum

Although no consensus has been achieved on how PNF techniques act on muscle strength and performance, these techniques are utilized in practice to increase muscle strength and performance, as reported in the literature (2,6,7). However, some studies have reported that PNF techniques cause a decrease in muscle strength during the acute period (13,21). These studies suggest that this effect might be caused by an acute inhibitory response in the neural pathways due to PNF's facilitatory or inhibitory effects on muscle spindle receptors and the Golgi tendon organ (22). Furthermore, the reduction in transferred forces to the musculoskeletal system in the acute period after PNF exercises may be another reason leading to a decrease in muscle strength and performance (23). Studies investigating the chronic effects of PNF exercises on muscle strength provided evidence that PNF techniques increased muscle strength compared with the data reported on acute effects (8,10).

In different studies using PNF patterns and techniques for lower extremity strengthening, it was demonstrated that PNF exercises combined with ERB increased muscle strength in the lower extremities (10) and improved the ankle flexor muscles' strength, improving the walking speed (11). In another study on patients with bilateral scapular winging, PNF exercises were applied to the upper extremity; it was reported that muscle strength and scapular endurance were increased (24). These data demonstrate that PNF exercises are used less commonly to increase muscle strength in the upper extremities compared with the lower extremities. Therefore, there is no sufficient information in the literature about PNF to increase muscle strength in the upper extremity. In this study, we found that PNF patterns did not affect grip strength in two different ways but increased the forearm and arm circumferences in both groups. Studies in the literature report that grip strength varies depending on anthropometric parameters (the arm length, arm circumference, forearm length, and forearm circumference), hand dominance, gender, height, body mass index, age, and nutrition) (15,25). Considering this published information, we thought that the absence of differences in the handgrip strength before and after the exercises might have been due to the differences in the measured values of the participants' anthropometric parameters. Furthermore, it is reported that muscle strength is affected by the mass, physiological cross-sectional areas, and the regulation of the muscles' leverage mechanisms rinvolved in performing the movement (26).

Endurance is essential to achieve functionality and performance in the upper extremity. Therefore, it is an important component of physical fitness (27). Muscle strength plays an important role in improving endurance (28). Muscle strength depends on the type of muscle, its contraction rate, the contraction type, and the joint position sense regulated by the proprioceptive system (3). Improvements in muscle strength may also be reflected in endurance. Studies are available in the literature investigating the effects of upper-extremity PNF exercises on muscle strength (6,24). In this regard, the present study differs from other similar studies in the literature because we also investigated the effects of PNF exercises on endurance, using the push-up and dip-strength tests. We observed a statistical increase in endurance after a 6-week exercise program in both the PNF and the ERB groups. However, there was not an intragroup difference. The studies in the literature are parallel with our study results since they reported improvements after evaluating PNF exercises' effects on endurance and performance (2,17).

Studies revealed that muscle-tendon length and the muscle's physiological cross-sectional area were increased with PNF

Table 4. Comparison of change differences in anthropometric measurements, grip strength, and endurance before and after training

Variables	PNF group Δ ± SD	ERB group Δ ± SD	p[a]
Arm circumference (cm)			
Dominant	0.23±0.25	0.50±0.72	0.114
Non-dominant	0.42±0.44	0.43±0.71	0.416
Forearm circumference (cm)			
Dominant	0.21±0.30	0.45±0.62	0.109
Non-dominant	0.24±0.34	0.40±0.66	0.061
Wrist circumference (cm)			
Dominant	0.10±0.26	0.13±0.22	0.154
Non-dominant	0.05±0.32	0.15±0.36	0.530
Grip Strength (kgs)			
Dominant	0.27±2.53	-0.13±3.08	0.790
Non-dominant	1.07±3.67	0.85±2.70	0.411
Push up (rp)	1.63±4.37	2.80±4.43	0.541
Dip strength (rp)	2.42±2.52	1.85±2.23	0.487

p<0.05, p[a]: Mann-Whitney U test, PNF: proprioceptive neuromuscular facilitation, ERB: elastic resistance band, cm: centimeter, kgs: kilogram, rp: number of repetitions, SD: Standard deviation, min: Minimum, max: Maximum

techniques (4,8). After a 6-week PNF exercise program, the observed increases in the anthropometric measurements of participants' arms and forearms in both study groups support the conclusion that PNF increases muscles' physiological cross-sectional area. This cross-sectional area and the length-strain relationship generate a larger force and improve endurance (14). The resistance applied during PNF exercises provides sensory input about the motion's direction, thereby increasing awareness. Consequently, motor control and motor learning are facilitated. Afferent pathways and receptor stimulation facilitate muscle contraction mechanisms. It is known that improved neuromuscular adaptation increases muscle strength (2,4,6). This study found out that neither method was superior regarding the results of anthropometric parameters, increases in muscle strength, and improvements in endurance in the upper extremities when the outcomes of the two methods were compared at the end of a 6-week PNF exercise program.

Study Limitations

This study has strengths and limitations. One of this study's strengths is that the data were collected using a safe, objective, and standardized methodology. The participants were evaluated before and after the training by the same physiotherapist, who was uninformed of the study details. Another study strength was that the exercises in the ERB group were adjunctively supervised by the physiotherapist. However, the lack of information about the long-term results can be listed as this study's limitation.

Conclusion

This study has demonstrated that PNF techniques could be used in the abovementioned two different administration modes to increase muscle strength and endurance in the upper extremity. Neither mode is superior to the other. We think that the clinical environment's characteristics, the physiotherapist's working environment, schedule, and patient factors need to be considered for selecting the appropriate exercise type.

Ethics

Ethics Committee Approval: Pamukkale University Non-Interventional Clinical Research Ethics Committee approval was obtained for the study (B.30.2.PAÜ.0.20.05.09/126).

Informed Consent: Signed informed consent form was obtained from all participants.

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Authorship Contributions

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

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Original Article

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Turkish Version of Bruininks-Oseretsky Test of Motor Proficiency 2 Brief Form: Its Validity and Reliability in Children with Specific Learning Disability

Bruininks-Oseretsky Motor Yeterlik Testi 2 Kısa Formunun Türkçe Uyarlaması ve Özgül Öğrenme Güçlüğü Olan Çocuklarda Geçerlilik ve Güvenilirliği

Barkın KÖSE[1], Sedef ŞAHİN[1], Erdem KARABULUT[2], Hülya KAYIHAN[3]

[1]Hacettepe University Faculty of Health Sciences, Division of Occupational Therapy, Ankara, Turkey
[2]Hacettepe University Faculty of Medicine, Department of Biostatistics, Ankara, Turkey
[3]Biruni University Faculty of Health Sciences, Division of Occupational Therapy, İstanbul, Turkey

ABSTRACT

Objective: Specialists often assess the motor proficiency of children presented with suspected developmental delays. It is essential that the tools used to assess children have highly powerful measurement properties including psychometric properties. The aim of our study, analyze the reliability and construct validity of the Turkish version of the Bruininks Oseretsky Test of Motor Proficiency 2-Brief Form (BOT2-BF) in children with a specific learning disability (SLD).

Methods: Participants, which aged between 6 and 14, were included 137 children with SLD and 50 children without any diagnosis. The Turkish version of the BOT2-BF was administered by the researcher in one session to children with SLD twice with a seven days interval. In addition, BOT2-BF was re-apply to 25 children with SLD, which were randomly selected, after 10 days.

Results: The validity results of BOT2-BF were found to be significantly better in all subtests in the control group than in the study group, having higher performance levels ($p<0.05$). The BOT2-BF demonstrated high test-retest reliability (ICC >0.8) (except Fine Motor Precision (ICC=0.57) and Manual Dexterity Subtest (ICC=0.74) and good internal consistency (Cronbach's alpha =0.78) for all domains. The inter-rater reliability results were found to be perfectly reliable (ICC >0.9).

ÖZ

Amaç: Uzmanlar genellikle gelişimsel gecikmelerinden şüphelendikleri çocukların motor yeterliliklerini değerlendirirler. Çocukları değerlendirmek için kullanılan testlerin psikometrik özellikler de dahil olmak üzere kanıt değerliliği açısından güçlü ölçüm özelliklerine sahip olmaları önemlidir. Çalışmamızın amacı, özgül öğrenme güçlüğü (ÖÖG) olan çocuklarda Bruininks Oseretsky Motor Yeterlilik Testi 2-Kısa Formunun (BOT2-KF) Türkçe versiyonunun güvenilirliğini ve yapı geçerliliğini analiz etmektir.

Yöntemler: Çalışmamıza 6-14 yaş arasındaki, 137 ÖÖG tanısı almış ve 50 herhangi bir tanı almamış (sağlıklı), çocuk dahil edildi. BOT2-KF'nin Türkçe versiyonu, ÖÖG'li çocuklara bir araştırmacı tarafından tek oturumda mola verilmeden, yedi gün arayla iki kez uygulandı. Ek olarak, BOT2-KF 10 gün sonra rastgele seçilen ÖÖG'li 25 çocuğa tekrar uygulandı.

Bulgular: BOT2-KF'nin geçerlilik sonuçlarının, kontrol grubundaki tüm alt testlerde çalışma grubuna göre daha yüksek olduğu ve daha yüksek performans düzeylerine sahip olduğu bulundu ($p<0,05$). BOT2-KF, tüm etki alanları için yüksek test-tekrar)ince motor hassasiyeti (ICC=0,57) ve el becerisi alt testi (ICC=0,74) hariç test güvenilirliği (ICC >0,8) ve iyi düzeyde iç tutarlılık (Cronbach's alpha=0,78) göstermiştir. Değerlendiriciler

Address for Correspondence: Barkın KÖSE, Hacettepe University Faculty of Health Sciences, Division of Occupational Therapy, Ankara, Turkey
E-mail: barkinkose@gmail.com **ORCID ID:** orcid.org/0000-0002-2840-3309

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Conclusion: The Turkish version of BOT2-BF was found to be a valid and reliable assessment to evaluate motor proficiency in children with SLD.

Keywords: Specific learning disability, motor proficiency, validity, reliability

arası güvenilirlik sonuçlarının mükemmel derecede güvenilir olduğu bulunmuştur (ICC >0,9).

Sonuç: BOT2-KF'nin Türkçe versiyonunun SLD'li çocuklarda motor yeterliliği değerlendirmek için geçerli ve güvenilir bir değerlendirme olduğu bulunmuştur.

Anahtar Sözcükler: Özgül öğrenme güçlüğü, motor yeterlilik, geçerlilik, güvenilirlik

Introduction

Specific Learning Disorder (SLD) is a developmental disorder with a neurological origin seen in individuals who actually have normal or above normal intelligence (IQ >85) but no primary psychological disorder, obvious brain damage, or sensory disability, experiencing certain difficulties in the acquisition. Children with SLD encounter many problems in activities, such as listening, speaking, reading, writing, reasoning, math skills, self-governance, understanding social events, and establishing social communication (1-5). Son and Meisels (6) grouped under two main headings and revealed that children with SLD had problems in self-care and academic activities performance.

Children with SLD was observed to face difficulties in performing activities such as shoe lacing, tooth brushing, dressing, buttoning up of clothes, and falling down while climbing up or down the stairs especially in daily living (6,7). In academic life, children have problems in holding a pencil, writing legibly, doing mathematical operations, and simultaneously writing down a text dictated by the teacher (8,9). Given these problems encountered by children with SLD in activities of daily life, weak muscle strength, weakness in fine and gross motor abilities, insufficiency of balance-coordination, visual perception problems, and failure to perform sequential skills come to the forefront as problems of motor proficiency (10-12).

Motor proficiency was defined as the level of realization of motor abilities such as balance, coordination, and orientation using a combination with sensory and perceptual skills (13). Bruininks-Oseretsky Motor Proficiency Test 2 (BOT2), Bruininks-Oseretsky Motor Proficiency Test 2 Brief form (BOT2-BF), Fundamental Movement Pattern Assessment Instrument, and *Developmental Sequence* of Fundamental *Motor Skills Inventory* and Gross Motor Development Test are used commonly to assess motor proficiency levels in the children (14-18). Considering the features of these tests used in this field, BOT2-BF comes to the fore for its features like short duration, understandable and easy-to-conduct, and providing holistic evaluation features.

Bruininks-Oseretsky Motor Proficiency Test (BOMPT) was developed in 1972 in order to measure the motor abilities of children aged between 4.5 and 14.5 years (15). BOMPT was updated in 2005 and became BOT2. Thus, BOT2 is applicable to children and youth 4-21 years of age. Administration of the BOT2 contains eight sub-test with 42 items and takes approximately 45-60 min (15). The validity and reliability of the BOT2 was determined by Balli with a study conducted on a group of healthy children aged 5 years in Turkey in 2012 (19).

BOMPT Short Form, which is the old version of BOT2-BF, contains eight sub-test and 14 items. It takes 20-25 min. BOMPT Short Form was updated in 2010 and became BOT 2-BF, reducing the total number of items from 14 to 12. This version takes 15-20 min (13). It is important to show version in different languages due to BOT2-BF could be completed in a short time practically to assess motor abilities in children. Therefore, this study aimed to assess the reliability and validity of the Turkish version of the BOT2-BF among children with SLD.

Methods

Our study was evaluated by University Non-invasive Clinical Research Ethics Committee and found to be ethically appropriate on 21.11.2017 with the decision GO17 /892-26. The permission required for the application of the Turkish version of BOT2-BF was obtained at the beginning of the study from the licensee. All participants and their parents agreed to participate in the study and were informed about the study and their consent was obtained.

Participants

The study was conducted at the pediatric clinic of the department of Occupational Therapy at the University. Inclusion criteria for the study group were the following: (1) diagnosed with SLD according to Diagnostic and Statistical Manual of Mental Disorders V criteria, (2) between 4-21 years of age, (3) volunteer to participate in the study, (4) absence of other diagnosis (e.g.,., attention deficit hyper-activity, autism, etc.), and (5) not professionally involved in any sportive activity (gym, volleyball, basketball, etc.). Inclusion criteria for the control group were as follows: (1) between 4-21 years of age, (2) voluntary participation to the study, (3) not professionally involved in any sportive activity (gym, volleyball, basketball, etc.), and (4) not born as preterm (20).

In the calculation of minimum sample size, which is 120 children with SLD and 40 children without any diagnosis, the model developed by Tabachnik and Fidell was used (21-23). During the study period, children who applied at the occupational therapy department for treatment were screened as potential participants. In this process, who could not be included or does not continue the study due to various reasons was noted. As a result, 137 healthy children were included in our study as the study group

and 50 children without any diagnosis as the control group (Figure1-Flow Chart).

Measures

Demographic Information Form

A demographic information form was completed by participants. Information included participants' age, gender, class, and dominant hand information.

Bruininks-Oseretsky Test of Motor Proficiency-Brief Form (BOT 2-BF)

BOT2-BF consists of eight sub-tests with 12 items. Test duration takes nearly 15-20 min. Sub-tests and items of BOT2-BF are as follows: (1) Fine Motor Accuracy: Completing the inside of a star and drawing a line along a path; (2) Fine Motor Integration: Copying nested circles and copying a diamond shape; (3) Manual Dexterity: Tying blocks on to a string; (4) Bilateral Coordination: Touching the tip of the nose with the index finger (eyes closed) and drawing a square with the thumb and index finger; (5) Balance: Walking forward heel-to-toe on a line (6) Speed and Agility: Jumping on one foot; (7) Coordination of Upper Extremities: Catching a thrown ball (with one hand) and dribbling; and (8) Endurance: Full push-ups and push-ups on knees (13).

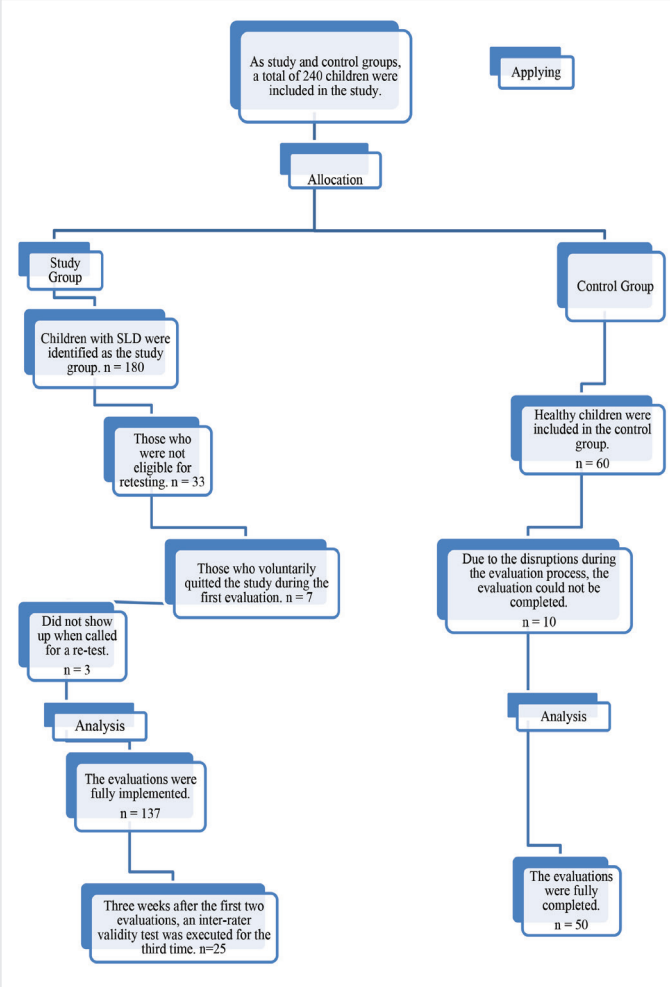

Figure 1. Flow chart

Item point scores for each task are converted to processed points using the Likert scale in each task. The Likert score values for each task are different. The conversion of the item point scores to the processed score is made by finding the processed score on the Likert scale that corresponds to the raw score taken from the best performance. In all items, the second trial is not performed if the child has received a full score on the first attempt. The internal consistency of the test was found to be sufficient (Cronbach's α=0.87) (13).

Procedures

Demographic data of participants were recorded. BOT2-BF was applied to the study and control groups to determine the motor capabilities of children. For the determination of test-retest reliability, BOT2-BF was re-administered to the study group a week after the initial evaluations. All evaluations were carried out by a single therapist (BK) during a single face-to-face session with children. Following the second evaluation of the study group, 25 children were randomly selected from the study group to determine the inter-rater reliability, and a third evaluation was carried out on them by another therapist (HK) 10 days after the first two evaluations (24).

Translation and Cross-Cultural Adaptation

The BOT2-BF was adapted from English version into Turkish in accordance with its standard methodology recommended by Beaton et al. (25). The adaptation was performed by two independent native speaking Turkish translators with fluent knowledge in English. The final Turkish version of the adaptation was later translated from Turkish to English by two English native speakers who can speak Turkish fluently to check compatibility. A committee with expertise in this field reviewed the translations and procedure. The comparison of the translation to the original version of the test revealed no inconsistency.

Data Analysis

International Business Machines Statistical Package for the Social Sciences Statistics for Windows version 23.00 was used for statistical analysis of data. The Kolmogorov-Smirnov Test was used to determine whether the data showed normal distribution. Categorical variables were expressed as numbers and percentages, whereas quantitative data were expressed as mean and standard deviation (26-28).

Validity Analysis

A construct validity was used to determine the validity degree of BOT 2-BF. In our study, the construct validity of BOT2-BF was evaluated through Known-Groups validity. This analysis was performed with children who were diagnosed with SLD and children without any diagnosis. The normal distribution assumption could not be obtained, thus the scale scores were analyzed with Mann-Whitney U Test to see whether they were different in two groups, and statistical significance level was accepted to be 0.05 (27,29).

Reliability Analysis

In our study, internal consistency and item analysis methods were used for the analysis of the reliability of the scale, and test-retest method and inter-rater reliability method were used for invariance reliability over time. Internal consistency has been indicated as Cronbach's alpha value. An alpha value >0.70 is indicative of a satisfactory consistency, whereas values between 0.50 and 0.70 are mean acceptable consistency (30). In our study, mixed model of intra-class correlation coefficient (ICC) was used for the inter-rater and test-retest reliability analyses of BOT2-BF. Intervals used for the interpretation of ICC values include the following: values <0.5 means poor reliability; 0.50-0.74 mean moderate reliability; 0.75-0.90 fine reliability; and >0.90 are defined as excellent degree of reliability (30).

Results

Descriptive Statistics

The average age of children included in the study are as follows: children in the study group (n=137) was 10.07±2.22 years [minimum (min) 6, maximum (max) 14 years] and in the control group (n=50) was 9.94±2.29 years (min: 7, max: 15 years). Both groups had resembled each other (Z=-1.98; p=0.4).

Validity

Construct Validity

With the exception of Fine Motor Precision and Strength Tests, validity results of BOT2-BF were found to be significantly better in all other sub-tests in the control group than in the study group, having higher performance levels (p<0.05). Sub-results of the construct validity are shown in Table 1.

Reliability

Internal Consistency Reliability

As a result of statistical analysis, the internal consistency of BOT2-BF was found to be statistically sufficient (Cronbach's α=0.78). Results of the internal consistency and item analysis of the test are shown in Table 2.

Test-Retest Reliability

Fine Motor Precision sub-test (ICC=0.57) and Manual Dexterity sub-test (ICC=0.74) of BOT2-BF were found to be moderately

Table 1. Construct validity

	Study group (n=137) X ± SD	Control group (n=50) X ± SD	Z	p
Fine motor precision	4.15±3.11	3.78±0.73	-0.95	0.92
Fine motor integration	7.10±2.18	8.20±1.19	-2.94	0.003*
Manuel dexterity	2.86±1.11	3.16±0.95	-2.03	0.041*
Bilateral coordination	3.89±1.59	5.14±1.35	-4.94	0.0001**
Balance	2.43±1.02	3.08±0.72	-4.03	0.0001**
Speed and agility	3.54±1.82	5.34±2.23	-4.71	0.0001**
Upper-limb coordination	3.54±1.82	5.34±2.23	-4.71	0.0001**
Strength	2.85±2.06	2.50±1.38	-0.96	0.33
Total score	32.51±8.97	32.92±8.06	-4.22	0.0001**

*p<0.05, **p<0.001, SD: Standard deviation

Table 2. Internal consistency reliability

Item	Mean-standard deviation (n=137) (X ± SD)	Corrected item-total correlation (r)	α if item deleted
Item 1	2.70±0.57	0.44	0.78
Item 2	1.11±1.13	0.48	0.77
Item 3	3.94±1.10	0.43	0.77
Item 4	3.21±1.35	0.49	0.76
Item 5	2.80±0.93	0.53	0.77
Item 6	2.67±0.93	0.37	0.78
Item 7	1.23±1.08	0.12	0.79
Item 8	2.38±1.02	0.50	0.77
Item 9	3.57±1.80	0.62	0.75
Item 10	2.05±1.65	0.51	0.76
Item 11	4.02±1.94	0.59	0.75
Item 12	2.83±2.03	0.31	0.79

SD: Standard deviation

reliable. Other sub-tests except those two were found to be between fine and excellent reliable. The invariance reliability sub-results of BOT2-BF over time are presented in Table 3.

Inter-Rater Reliability

All sub-tests of BOT2-BF were found to be perfectly reliable. The inter-rater reliability subscales of BOT2-BF are shown in Table 4.

Discussion

This study describes the translation and psychometric testing in terms of validity (construct) and reliability (Internal Consistency, Test-Retest, and Inter-Rater) of the Turkish version of the BOT2-BF in children with SLD. Analyzing the psychometric properties of tests in the literature, study examples investigating the features of BOT2 and BOT2-BF were found to be limited. An acceptable reliability and validity were observed only in the study carried out by Lucas et al. (31) using BOT2-BF on children who live in rural areas. As for BOT 2, psychometric features and normative data have been investigated in studies generally conducted on healthy children and children diagnosed with mental retardation (19,31-36). Therefore, studies dwelling into the validity and reliability features of BOT2 and BOT2-BF tests that evaluate motor abilities were also studied (19,34-36).

Factor analysis was used for construct validity in example studies that already exist in literature. However, in our study, construct validity was measured by means of the Known-Groups Validity. This is because the test is not suitable for factor analysis due to the number and distribution of items (26). As our results have satisfactory contract validity between children with SLD and controls matched for age and gender.

For all sub-tests of the Turkish version, the internal consistency of the BOT2-BF was found to be acceptable (coefficient alpha values were ≥0.7). The degree of internal consistency observed in the present study (α=0.78) was lower than that of the original validation study of the BOT2-BF (α=0.85) (13). Other version studies using BOT2-BFwere not found other than the original study in literature. In this respect, our study is the first version study using BOT2-BF and it is also the first study to show its usability in children with SLD.

The original version examined the reliability of BOT 2-BF via test and retest methods. As for the study results, the invariance of items in time varied from medium to good validity (13). In addition, Wuang and Su (36) used the test and retest analysis in the BOT2 version study and found the test to be reliable enough. In our study, the invariance of BOT2-BF in time was examined via test and retest methods similar to the examples in literature.

Table 3. Test-retest reliability

BOT2-BF (n=137)	Before X ± SD	After X ± SD	Test-retest reliability ICC
Fine motor precision	4.15±3.11	3.62±1.30	0.57
Fine motor integration	7.10±2.18	7.51±2.03	0.88
Manuel dexterity	2.86±1.11	2.88±0.91	0.74
Bilateral coordination	3.89±1.53	4.07±1.49	0.90
Balance	2.43±1.02	2.29±0.95	0.84
Speed and agility	3.54±1.82	3.80±2.11	0.93
Upper-limb coordination	6.06±3.07	6.05±3.18	0.95
Strength	2.85±2.06	2.67±2.03	0.86

ICC: , SD: Standard deviation

Table 4. Inter-rater reliability

	Therapist 1 (n=25) X ± SD	Therapist 2 (n=25) X ± SD	Inter-rater ICC
Fine motor precision	4.15±3.11	4.80±4.74	0.99
Fine motor integration	7.10±2.18	7.20±2.46	0.99
Manuel dexterity	2.86±1.11	2.96±1.39	1.00
Bilateral coordination	3.89±1.59	3.88±1.48	1.00
Balance	2.43±1.02	2.04±0.84	1.00
Speed and agility	3.54±1.82	3.84±1.86	1.00
Upper-limb coordination	6.06±3.07	6.40±2.51	1.00
Strength	2.85±2.06	2.24±1.92	1.00
Total score	32.51±8.97	32.28±7.44	1.00

SD: Standard deviation

Compatible with the literature, results of the reliability of time invariance ranged from medium to excellent. This point of view, unlike the original version, results of speed and agility and upper-limb coordination sub-tests were found to be higher in our study.

In the original version, Bruininks and Bruininks (13) demonstrated an excellent inter-rater reliability of BOT2-BF. We also measured the inter-rater reliability of the BOT2-BF as an assessment tool in the Turkish version that could make a difference between practitioners. In our study results similar to the original version, the reliability level of items was found to be excellent. Considering these values, our study can be considered to have an excellent degree of reliability, in contrast with results of many examples in literature. The degree of excellence in reliability means that the Turkish version of BOT 2-BF can easily be adopted and used by many experts.

When the version studies in the literature conducted using BOMYT, BOMYT Short Form, BOT2, and BOT 2-BF are considered, sample groups in most studies can be selected from children without disabilities. Studies were conducted in only healthy children, which may be led to a significant deficiency in rehabilitation clinics for children with disability. The application of these tests in the evaluation of rehabilitation programs on disability groups are unknown (13,15,34-37). Thus, we believe that our study will positively contribute both to the literature and clinical professionals, as it has included children with SLD.

Study Limitations

Due to the time limitation in our study, adequate number of participants could not be included to make a better distribution analysis according to ages, which can be regarded as a limitation of our study. Examining the methodology of other studies in the literature, it can be seen that results are introduced according to age distributions (13,36). Taking into consideration the fact that distribution by age can provide positive contributions in the interpretation of evaluations that have been made by experts working in the clinical field, examining those distributions according to ages in the following studies were thought to be crucial.

Conclusion

This has been the first study to evaluate the validity and reliability of the BOT2-BF, which measures motor competence, planning, orientation, coordination, and speed in children with SLD. Considering the fact that studies conducted in this area focus largely on healthy groups, it is thought that it will contribute positively to the development of further study fields to assist both academic and clinical professionals. It is considered essential that further studies where BOT2-BF is used should be conducted to obtain validity and reliability as well as high value of evidence in clinical practices.

Ethics

Ethics Committee Approval: Our study was evaluated by University Non-invasive Clinical Research Ethics Committee and found to be ethically appropriate on 21.11.2017 with the decision GO17 /892-26.

Informed Consent: All participants and their parents agreed to participate in the study and were informed about the study and their consent was obtained.

Peer-review: Externally peer reviewed.

Authorship Contributions

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

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Original Article

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Abdominal Paragangliomas: A Single Center Experience

Abdominal Paragangliomalar: Tek Merkez Deneyimi

Ahmet Gökhan SARITAŞ[1], Mehmet Onur GÜL[2], Orçun YALAV[1], Zafer TEKE[2], İsa Burak GÜNEY[3]

[1]Çukurova University Faculty of Medicine, Department of General Surgery, Adana, Turkey
[2]Çukurova University Faculty of Medicine, Division of Surgical Oncology, Adana, Turkey
[3]Çukurova University Faculty of Medicine, Department of Nuclear Medicine, Adana, Turkey

ABSTRACT

Objective: Paragangliomas are rare tumors arising from extra-adrenal chromaffin tissue, which are widely distributed near or within the autonomic nervous system in the retroperitoneal sites and in the sympathetic ganglia of various viscera. We present a review of our 18-year institutional experience with resected abdominal paragangliomas.

Methods: The data collected from 12 patients who underwent surgery due to abdominal paraganglioma in our clinic between 2002 and 2020 were analyzed retrospectively.

Results: There were 12 patients in our study. The median age was 44 years (range: 21-81 years). The patients had one or more of the symptoms of headache (n=2, 16,6%), palpitations, abdominal pain (n=5, 41.6%), sweating (n=2, 16.6%) and hypertension (n=5, 41.6%), which are the classic clinical symptoms. One of the cases (1/12; 8,3%) was detected incidentally. The mass location was in the retroperitoneal region in 10 cases (83.3%) and in the pelvic region in 2 cases (16.6%). Five of the patients applied to our clinic with episodes of paroxysmal hypertension, and vanillylmandelic acid and metanephrine levels were found to be high in the blood and 24-h urinary tests. After a median follow-up period of 60 months, only 1 patient (8.3%) had metastasis and required reoperation 2 years after the first operation. One patient (8.3%) died on postoperative 36th month due to cardiac problems.

Conclusion: Abdominal paragangliomas are rare tumors whose optimal management requires the surgeon to be highly attentive to

ÖZ

Amaç: Paragangliomalar, retroperitoneal bölgelerde otonom sinir sisteminin yakınında veya içinde ve çeşitli organların sempatik gangliyonlarında yaygın olarak dağılım gösteren ekstra-adrenal kromaffin dokusundan kaynaklanan nadir görülen tümörlerdir. Biz bu çalışmada 18 yıllık abdominal paraganglioma cerrahisine ait klinik deneyimimizi sunuyoruz.

Yöntemler: 2002-2020 yılları arasında kliniğimizde abdominal paragangliomaya bağlı cerrahi uygulanan 12 hastadan elde edilen veriler retrospektif olarak incelendi.

Bulgular: Çalışmamızda 12 hasta vardı. Ortalama yaş 44 (21-81) idi. Hastalarda klasik klinik semptomlar olan baş ağrısı (n=2,% 16,6), çarpıntı, karın ağrısı (n=5, 41,6), terleme (n=2, %16,6) ve hipertansiyon (n=5, %41,6) mevcuttu. Olgulardan biri (1/12; %8,3) insidental olarak tespit edildi. Kitle yerleşimi 10 olguda (%83,3) retroperitoneal bölgede, 2 olguda (%16,6) ise pelvik bölgede idi. Kliniğimize paroksismal hipertansiyon atakları ile başvuran hastaların beşinde kan ve 24 saatlik idrar testlerinde vanil mandelik asit ve metanefrin düzeyleri yüksek bulundu. Ortalama 60 aylık takip süresi boyunca sadece 1 hastada (%8,3) metastaz gelişti ve bu hasta ilk ameliyatından 2 yıl sonra tekrar ameliyat edildi. Bir hastada (%8,3) ameliyat sonrası 36. ayda kardiyak problemler nedeniyle mortalite gelişti.

Sonuç: Abdominal paragangliomalar nadir görülen tümörler olup optimal yönetiminde cerrahın hastalık seyri boyunca son derece dikkatli olmasını gerektirir. Bu süreç, fonksiyonel veya

Address for Correspondence: Ahmet Gökhan SARITAŞ, Çukurova University Faculty of Medicine, Department of General Surgery, Adana, Turkey
E-mail: drags0001@hotmail.com **ORCID ID:** orcid.org/0000-0003-2715-6390

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the disease course, from diagnosis of functioning or nonfunctioning lesions, through operative treatment that may require adjacent organ resection, to lifelong follow-up for recurrences.

Keywords: Paraganglioma, endocrine hypertension, retroperitoneal, metastasis, surgical treatment, survival

non-fonksiyonel lezyonların tanısından başlayarak komşu organ rezeksiyonu gerektirebilecek geniş cerrahi tedavilere ve de nüks açısından yaşam boyu takibe kadar uzanmaktadır.

Anahtar Sözcükler: Paraganglioma, endokrin hipertansiyon, retroperiton, metastaz, cerrahi tedavi, sağkalım

Introduction

Paragangliomas are rare neuroendocrine tumors with an incidence of 1 case per million and are similar in their clinical features to adrenal pheochromocytomas. Head and neck paragangliomas are usually of the parasympathetic type. They are not the hormone-releasing type and are generally located near the carotid bifurcation (1). The Zuckerkandl's organ, first described by Zuckerkandl in 1901, is located between the inferior mesenteric artery root and the aortic bifurcation on the right edge of the abdominal aorta. It is normally present in the fetus, localized in the para-aortic plexus; however, in the adult, it takes the form of a residue and is the most frequent location of extra-adrenal pheochromocytomas. Subramanian and Maker (2) identified only 135 abdominal paraganglioma patients in their literature review. Mediastinal paragangliomas are generally located along the aortopulmonary window. Paragangliomas developing in the mediastinum are sympathetic-type paragangliomas, such as abdominal paragangliomas (3).

The neoplastic cells found in paragangliomas are positive for the immunohistochemical marker CD56, synaptophysin and chromogranin A, and there is focal S100 protein positivity in sustentacular cells. Histopathological examination is insufficient to predict benign or malignant features. Since there are no histological or molecular markers that distinguish malignant and benign paragangliomas, malignancy can only be proven by the appearance of metastases that occur during the initial diagnosis or after diagnosis. Tumor size is also not considered as an important factor in determining malignancy (3).

Extra-adrenal paragangliomas occur in individuals of all ages but most often in the fourth or fifth decades of life. Paragangliomas releasing hormones characterized by excessive catecholamine secretion are called active paragangliomas. Although active paragangliomas are rare in the head and neck region, they are more common in the thoracic, abdominal, and retroperitoneal regions (4). The most common clinical symptoms due to catecholamine hypersecretion are headache, palpitations, and sweating (5). Cardiac arrest, brain hemorrhage, and malignant hypertension are life-threatening complications. Approximately 10% of paragangliomas are clinically silent and are detected incidentally during radiological imaging studies (6-9). Familial paragangliomas account for approximately 10% of cases, and 35%-50% of familial paraganglioma cases are multicentric tumors (9,10). Surgical resection is the most important step in the treatment of paragangliomas. Depending on the location, abdominal paragangliomas can develop hypervascular invasion to the abdominal aorta, inferior vena cava, or other adjacent tissues and even invasion leading to large vascular resections (10).

In this study, we aimed to present radiological and histological features, surgical treatment strategies, and postoperative follow-up results of these very rare abdominal paraganglioma cases in the light of the literature.

Methods

Study design and setting

In this study, the medical records of 12 patients who were operated on for an abdominal mass between January 2002 and January 2020 and diagnosed with paragangliomas on histopathological examination were analyzed retrospectively. This study was approved by the Institutional Review Board of our institute (IRB No. 10.01.2020/95/23). An informed consent was read and signed by all participants. All procedures performed in this study involving human participants were performed in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Patients' clinical features as well as biochemical, radiological, surgical, and histopathological data were collected, and a data set was created. Patients with confirmed paragangliomas as a result of clinical features, radiological imaging methods, and histopathological evaluations were included in the study. Pathologically, tumor size, immunohistochemical analyses of S100, *chromogranin A*, synaptophysin, and neuron-specific enolase, as well as the Ki-67 index were investigated. The largest tumor size in the pathological specimens was measured. The patients were followed up for at least 24 months after surgical removal of the tumor. All patients were referred to the medical genetics clinic for genetic evaluation in terms of neurofibromatosis, von Hippel-Lindau disease, and multiple endocrine neoplasia syndromes. Cases that could not be confirmed histopathologically as paragangliomas, cases with inaccessible information, and cases with adrenal pheochromocytoma were excluded from the study.

Statistical analysis

Statistical analysis was carried out using IBM SPSS Statistics ver. 24.0 (IBM Corp., Armonk, NY, USA). Continuous data were presented as mean (standard deviation) or median (range), and categorical data as frequency. Student's t-test was used for comparison of continuous variables. A Shapiro-Wilk normality test was performed for numerical variables such as age, tumor size, and follow-up time. Student's t-test was used to analyze the relationship between tumor size and mortality and recurrence. Based on the results of analyses, a p value <0.05 was considered to indicate statistical significance.

Results

There were six male (50%) and six female (50%) patients in our study. The median age was 44 years (range: 21-81 years) (Table 1). The patients had one or more of headaches (n=2, 16.6%), palpitations, abdominal pain (n=5, 41.6%), sweating (n=2, 16.6%), and hypertension (n=5, 41.6%), which are the classic clinical symptoms of paraganglioma. One of the cases (1/12; 8.3%) was detected incidentally. The mass was located in the retroperitoneal region in 10 cases (83.3%) and in the pelvic region in 2 cases (16.6%). Five of the patients applied to our clinic with episodes of paroxysmal hypertension, and vanillylmandelic acid and metanephrine levels were found to be high in the blood and 24-h urinary tests. All demographic, clinical, pathological, and radiological data for the 12 patients are presented in Table 2.

The first patient was admitted with high blood pressure attacks. A paracaval paraganglioma was detected on radiological imaging, and then she was operated on. The tumor was resected with the wall of the inferior vena cava due to suspicion of tumor invasion, and then the inferior vena cava was repaired. A tumor invading the vein was detected in the pathological examination. In the second patient, laparoscopic resection was performed for the mass at the level of the renal hilus. After the operation, the patient developed a fistula between the left renal artery and vein, and the arterio-venous fistula regressed in the first year of follow-up. The fifth patient had a lesion located lateral to the superior mesenteric vein in the posterior of the pancreas. Subtotal pancreatectomy and splenectomy with mass excision were performed due to suspected pancreatic invasion. Fibrosis was revealed by a pathological evaluation of the region considered as tumor invasion to the pancreas on preoperative radiological imaging. The patient's disease-free survival is 60 months, and follow-up continues. In the sixth patient, 2 years after tumor excision of the Zuckerkandl's organ, total omentectomy was performed due to suspicion of omental metastasis and was followed up with eight cycles of peptide receptor radionuclide therapy consisting of Lutetium 177 (Lu-177) in the postoperative period. The time to metastasis was 24 months, and disease-free survival after metastasectomy was 16 months. In the eighth patient, a renal artery injury occurred during removal of the paraganglioma mass located at the level of renal hilus. The renal artery was repaired, and no additional pathology developed during follow-up. In the ninth patient, the initial complaint was hematuria due to a paraganglioma mass located on the bladder side wall. Thickening was detected on the lateral wall of the bladder on pelvic ultrasonography (USG), and the lesion was excised using cystoscopy. The patient was followed up through routine annual check-ups, and no recurrence occurred.

Table 1. Clinicopathologic characteristics for the 12 patients under study

Characteristic	(n) (%)
Age (year) (median)	44 (range: 21-81)
Gender	
Male	6 (50%)
Female	(50%)
Clinical presentation	
Incidentaloma	1 (8.3%)
Hypertension	5 (41.6%)
Abdominal pain	5 (41.6%)
Headache	2 (16.6%)
Hematuria	1 (8.3%)
Radiological method	
CT	7 (58.3%)
MRI	10 (83.3%)
USG	10 (83.3%)
PET/CT	4 (33.3%)
Localization	
Posterior pancreatic	2 (16.6%)
Paracaval/para-aortic	2 (16.6%)
Renal hilum	2 (16.6%)
Zuckerkandl's organ	2 (16.6%)
Bladder side wall	2 (16.6%)
Sacral/sacrococcygeal	2 (16.6%)

CT: Computed tomography, MRI: Magnetic resonance imaging, USG: Ultrasonography, PET/CT: Positron emission tomography/computed tomography

Laparoscopic surgery was performed in 2 of the 12 patients in our study. The surgical, pathological, and oncological characteristics of the 12 patients are presented in Table 3. The median follow-up time was 60 months (range: 12-84 months). Metastasis developed in only one patient after the first operation. Despite the advanced age and comorbid diseases of the 11th patient, she lived for 36 months without local recurrence and/or metastasis after excision of a tumor, approximately 5 cm in size, from the sacrococcygeal region. This patient died in the 36th postoperative month from cardiac problems.

When the preoperative radiological imaging methods used on all the patients were reviewed, 10 patients had abdominal USG, and 2 patients had no pathological findings on the ultrasound examination. A paraganglioma in two cases and a lymphadenopathy in one case were reported in the abdominal USG. In one case, bladder wall thickness was reported, and cystoscopy was recommended. The remaining patients' conditions were described as abdominal mass lesions without being reported as paraganglioma. Abdominal computed tomography (CT) imaging was performed in seven cases in the preoperative period, and a mass was detected in all cases. In four patients, the differential diagnosis of paraganglioma was considered, and in three patients, only a mass lesion was reported. Magnetic resonance imaging (MRI) was performed in 10 patients, and the differential diagnosis of paraganglioma could be made in these patients. Positron emission tomography/CT (PET/CT) examination was performed in four patients, and the SUVmax value was found to be high in all patients (20 in Case 1, 21.38 in Case 4, 14.84 in Case 6, and 14.3 in Case 8). On PET/CT imaging of the case with metastasis detected in the omentum, the lesion was found to have a high SUVmax value (14.84).

The median tumor size was 5.2 cm (range: 0.5-15 cm) in our series (Table 3). There was no statistically significant relationship between tumor size and mortality (p>0.05) or local recurrence (p>0.05). Histopathological evaluations confirmed the diagnosis of paraganglioma in all 12 patients. Capsular invasion was positive in 10 patients. *Positive* immunohistochemical staining for S100 was detected in 10 patients, *chromogranin A* in 8 patients, synaptophysin in 5 patients, and neuron-specific enolase in 2 patients. The Ki-67 index was 6% in one patient and 4% in another patient.

Discussion

Paragangliomas are rare neuroendocrine tumors with similar clinical and histopathological features of pheochromocytomas originating from the adrenal medulla (1). Paragangliomas are

Table 2. Demographic, clinical, pathological and radiological data for the 12 patients under study

Case	Age/ Gender	Clinical presentation	Tumor diamater (cm)	Localization	Operational complication	Metastasis/ Local recurrence	Radiological methods	Follow-up time (month)
1	52/F	Hypertension, Abdominal pain	3x2x0.5	Paracaval	IVC repair	None	USG, MRI, PET/ CT	84
2	34/M	Hypertension	3x2x2	Renal hilum	Left renal AVF	None	USG, MRI	84
3	27/F	Hypertension	4x4x3.5	Zuckerkandl's ogan	None	None	USG, MRI	72
4	40/F	Abdominal pain	8x6x5	Posterior pancreatic	None	None	USG, CT, MRI, PET/CT	60
5	81/M	Hypertension	15x5x2.5	Posterior pancreatic	None	None	CT, MRI	60
6	44/F	Hypertension	8x5x5	Zuckerkandl's organ	None	Yes	USG, CT, MRI	40
7	45/M	Incidentaloma	4x2x2	Bladder lateral wall	None	None	CT	84
8	31/M	Weight loss, Nausea and vomiting	6x4x2	Renal hilum	None	None	USG, CT, MRI, PET/CT	24
9	29/M	Hematuria	0.5x0.5x0.3	Bladder lateral wall	None	None	USG, MRI	60
10	45/M	Abdominal pain	3x2x1.5	Para-aortic	None	None	USG, MRI	84
11	81/F	Abdominal pain	5x4x4	Sacral	Pelvic hematoma	None	USG, CT, PET/ CT	36
12	21/F	Abdominal pain	3x0.3x2	Sacrococcygeal	None	None	USG, CT, MRI	36

CT: Computed tomography, MRI: Magnetic resonance ımaging, USG: Ultrasonography, PET/CT: Positron emission tomography/computed tomography, IVC: Inferior vena cava, AVF: Arterio-venous fistula

Table 3. Surgical, pathological, and oncological characteristics of the 12 patients under study

Characteristic	(n) (%)
Tumor diameter (median) (cm)	5.2 (range: 0.5-15)
Metastasis	1 (8.3%)
Time to metastasis (month)	24
Local recurrence	0
Follow-up time (median) (month)	60 (range: 24-84)
Mortality	0
Mortality related to comorbidities	1 (8.3%)
Surgical procedure	
• Tumor resection	10 (91.6%)
• Subtotal pancreatectomy + splenectomy	1 (8.3%)
• Cystoscopic resection	1 (8.3%)
• Vascular repair (inferior vena cava/renal artery)	2 (16.6%)

usually located in the vicinity of the organ of Zuckerkandl, and they are common in this region due to the development of chromaffin tissue from extra-adrenal paraganglioma cells (1).

The benign or malignant character of a paraganglioma is related to the behavioral features of the tumor and cannot be diagnosed histopathologically (11). Abdominal paragangliomas tend to be malignant (2,12,13). Diagnosis of the disease is made by laboratory tests and radiological imaging methods performed in patients presenting with symptoms suggestive of paraganglioma. The most common symptoms are hypertension that occur as sudden attacks and abdominal pain (6-9). In our study, the patients had one or more of headaches (n=2, 16.6%), palpitations, abdominal pain (n=5, 41.6%), sweating (n=2, 16.6%), and hypertension (n=5, 41.6%), which are the classic clinical symptoms of paragangliomas.

Radiological imaging methods aid in primary tumor localization and show metastatic lesions in malignant cases. The combined use of two or more radiological imaging techniques is often required for diagnosis and staging. In these radiological imaging methods, PET/CT examination is recommended when distant metastasis is suspected (14-16). If the lesion cannot be found during the operation, intraoperative USG examination can be useful (17). Dan et al. (18) reported that a bladder paraganglioma was detected by pelvic USG preoperatively. Malthouse et al. (19) used transabdominal USG and determined paragangliomas adjacent to the pancreas. In our study, a paraganglioma located on the bladder side wall was detected by pelvic USG. The disadvantage of USG is that it does not show tumor involvement of organs such as the lung, brain, and bone and is dependent on the physician who performs it (20). Ultrasonographic examination was performed in 10 patients in our study. In two patients, the lesions could not be detected on ultrasound. Preliminary diagnosis of paraganglioma was considered in two patients, and the primary tumor and its localization were reported in the remaining six patients. It was thought that the inability to detect the lesion by ultrasound was associated with anatomical localization and the radiologist's experience. MRI can detect catecholamine-secreting tumors in 95% of cases and has a sensitivity of 93%-100% (21,22). Paragangliomas have a characteristic hyperintensity on T2-weighted images due to the tumor's hypervascularity. In pregnant women, children, and patients with an iodine-based contrast allergy, MRI is the test of choice. In our study, 10 patients had MRI, and typical characteristic findings of paraganglioma were detected in all. Despite these advantages of MRI, most of the clinicians still prefer CT scan because it provides better anatomic detail and does not aggravate claustrophobia (23). CT and MRI are considered the gold standard for radiological imaging in hereditary paraganglioma screening (24). Paraganglioma is not the first preliminary diagnosis that comes to mind during the diagnosis of masses located intraabdominally or retroperitoneally; therefore, CT is usually used before MRI. Thin-sliced CT scans have 98% sensitivity and 92% specificity with intravenous contrast enhancement in the diagnosis of paraganglioma (25-27). CT imaging was performed in seven of the patients in our study, and the preliminary diagnosis of paraganglioma was reported in four, while others were described only as a mass lesion.

^{18}F-fluoro-2-deoxy-d-glucose (F-18) FDG PET/CT scan guides the diagnosis based on the glucose uptake level of the tumor tissue. Most clinicians use F-18 FDG PET/CT scanning not as the primary localization method but when ^{123}I-labeled metaiodobenzylguanidine (^{123}I-MIBG) scintigraphy scanning is negative or suspected or when fast growing tumors with a high metabolic rate are detected (14-16). Paragangliomas overexpress somatostatin receptors (SSR), especially SSR2 (28). ^{68}Ga-labeled DOTA peptides have been shown to be far superior to ^{111}In-DTPA-octreotide (Octreoscan®) for the detection of neuroendocrine tumor (NET) lesions (29). Additionally, [^{68}Ga]-DOTATATE PET/CT gives the opportunity to evaluate these patients for their potential eligibility for peptide receptor radionuclide therapy, since DOTA peptides can also be labeled with therapeutic β-emitters such as ^{177}Lu and ^{90}Y. Jansen *et al.* reported that ^{68}Ga-DOTATATE PET/CT was superior to all other PET radiopharmaceuticals including ^{18}F-FDOPA and especially ^{18}F-FDG, suggesting that ^{68}Ga-DOTATATE has the potential to affect patient treatment plans and outcomes by identifying not only more metastatic lesions but also additional involved sites of disease as compared with all other functional imaging modalities and CT/MRI (30). In our study, PET/CT was used in four patients, and high SUVmax values were detected in all tumor tissues. In one case, it was used to confirm metastasis and to perform another focus scan. In some of our patients, USG, CT, MRI, and PET/CT scans were all performed. We thought that the reason for this was difficulties encountered in the diagnosis of this very rare disease and the fact that the patients had been admitted to more than one hospital before applying to us.

The risk of life-threatening intraoperative and postoperative complications in symptomatic patients must be reduced, and appropriate preoperative preparations must be provided by following the recommendations of the endocrinology clinic. In the treatment of paraganglioma, resection is recommended without leaving tumor tissue at the surgical margins. The difficulty of surgery in the anatomical localization of paragangliomas is the most important factor preventing negative surgical margins (31). The treatment method to be applied when tumor recurrence develops is resection. Tumor recurrence was found to be 6%-15% in the studies, and the average survival was reported to be 47-60 months (32,33). Johnston et al. (33) reported that after initial surgery, tumor development occurred at 8.6, 12, and 17.7 years. This shows that the patient's follow-up should not be disrupted, and annual follow-up should continue. Although metastases and recurrences are expected to develop in the early period, metastases occurring 41 years after primary surgery have also been reported in the literature (34). In our study, metastasis developed in the 24th month in a patient with a primary tumor diameter of 8 cm after initial surgery, but no local recurrence or metastasis occurred in other patients. Assadipour et al. (35) reported that the risk of local recurrence and distant metastasis in paraganglioma and pheochromocytoma

is higher in the presence of an *SDHB* mutation and/or when the tumor diameter is greater than 5 cm. Previously published studies on pheochromocytomas have shown that the risk factors for recurrence are young age, large tumor diameter, extra-adrenal tumor, and genetic pheochromocytomas (36,37). Cunningham et al. (38) reported that tumor diameter, whether the tumor was symptomatic or not, surgical margin, and lymph node resection did not contribute to survival, and survival was affected only if the tumor had metastasized. Due to the lack of adequate prospective studies in the literature on paragangliomas, surgical margins, recurrence, metastasis, and other factors affecting the prognosis of disease have not been revealed very clearly. In our study, one patient (8.3%) developed metastasis and the median survival of patients in our study was found to be 60 months.

Currently, there is no reliable histological, immunohistochemical, molecular, or radiological imaging criterion for determining malignancy in paragangliomas (39). Hamidi et al. (40) found that male gender, advanced age, dopamine hypersecretion, the presence of synchronous metastasis, primary tumor size, and not undergoing surgical resection for the primary tumor were associated with an aggressive disease course and high mortality. In our study, the patient who developed metastasis was a 44-year-old female who did not fit the high-risk group criteria. The small number of patients, the lack of genetic data, and the retrospective design are important limitations of this study. However, large, prospective randomized controlled studies including genetic features are needed.

Conclusion

Paragangliomas may occur anywhere paraganglia are found, from the base of the skull to the floor of the pelvis. It should be borne in mind that masses in abdominal localization with hypermetabolic activities in F-18 FDG PET/CT may be paragangliomas. ^{68}Ga-DOTATATE has the potential to affect patient treatment plans and outcomes by identifying not only more metastatic lesions but also additional involved sites of disease, as compared with all other functional imaging modalities and CT/MRI. Surgical treatment of these tumors should be performed in such a way as to obtain a negative surgical margin after preoperative examinations and preparations are conducted.

Ethics

Ethics Committee Approval: Approval was obtained from the Non-invasive Clinical Research Ethics Committee of Çukurova University Faculty of Medicine (date: 10.01.2020).

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Authorship Contributions

Surgical and Medical Practices: A.G.S., M.O.G, O.Y., Z.T., İ.B.G., Concept: A.G.S., M.O.G., O.Y., Z.T., İ.B.G., Design: A.G.S., M.O.G., O.Y., Z.T., İ.B.G., Data Collection or Processing: A.G.S., M.O.G., O.Y., Z.T., İ.B.G., Analysis or Interpretation: A.G.S., M.O.G., Z.T., İ.B.G., Literature Search: A.G.S., M.O.G., Z.T., İ.B.G., Writing: A.G.S., M.O.G., Z.T., İ.B.G.

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Original Article

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Analyzing the Odds Ratio Via Distribution Function

Odds Oranının Dağılım Fonksiyonuna Bağlı Olarak İncelenmesi

Mehmet GÜRCAN[1], Mehmet Onur KAYA[2], Yunus GÜRAL[1]

[1]Fırat Univeristy Faculty of Science, Division of Statistics, Elazığ, Turkey

[2]Fırat University Faculty of Medicine, Department of Biostatistics and Medical Informatics, Elazığ, Turkey

ABSTRACT

Objective: The use of cross tables was frequently seen in early literature research in the biostatistics. Furthermore, its importance in many clinical examinations is still evident today. The aim of this study is to investigate how the 2x2 type tables are perceived in probability literature and how some studies are applied in practice. Thus, different methods can be developed for the purposes of applications.

Methods: The method used to determine the distribution of a 2x2 type table is to consider one cell of a table as a random variable and calculate the probability that this variable can take the observed value. Hypergeometric distribution was taken into consideration in the study. This issue is explained in the methodology section of the study.

Results: Some of the important statistics obtained from 2x2 type tables are the numerical statistical values that direct the researcher in experimental studies such as odds ratio. Considering the distribution of the table, the probabilities of these values are a very important finding for the experimental study. In particular, a high probability value is a measure of how well the statistical value commonly used in biostatistics applications, such as the odds ratio, represents the experimental study performed.

Conclusion: According to the findings of the study, one of the observed results is the determination of the maximum probability ratio representing the experimental study, and the other is the weighted odds ratios that are used to combine odds ratios in the meta-analysis.

Keywords: Contingency table, probability distribution, hypergeometric distribution, odds ratio, relative risk, meta-analysis

ÖZ

Amaç: Biyoistatistik alanındaki erken literatür araştırmalarında dahi çapraz tabloların kullanımına sıkça rastlanmakla beraber günümüzde de önemini birçok klinik incelemedeki kolay kullanımı ile göstermektedir. Bu çalışmadaki amacımız 2x2 tipli tabloların olasılık literatüründe nasıl algılandığını ve bu konu ile ilgili literatürde yapılan birtakım çalışmaların pratikte nasıl uygulandığının araştırılmasıdır. Bu sayede uygulamalardaki amaçlar için farklı birtakım yöntemler rahatlıkla geliştirilebilir.

Yöntemler: 2x2 tipindeki bir tablonun dağılımın belirlenmesi için temelde kullanılan yöntem tablonun bir gözesinin tesadüfi değişken olarak kabul edilmesi ve bu değişkenin gözlenen değeri alma olasılığının hesaplanmasıdır. Genelliği bozmaksızın çalışmada hipergeometrik dağılım dikkate alınmıştır. Bunun nedeni çalışmanın yöntemler kısmında açıklanmaktadır.

Bulgular: 2x2 tipli bir tablodan elde edilen önemli istatistiklerden bazıları, relatif risk ve odds oranı gibi, deneysel çalışmalarda araştırmacıya yön gösteren, sayısal istatistik değerleridir. Tablonun dağılımı dikkate alındığında bu değerlerin olasılıkları yapılan deneysel çalışma için oldukça önemli bir bulgudur. Özellikle olasılığın yüksek olması, odds oranı gibi, biyoistatistik alanındaki uygulamalarda sıkça kullanılan bir istatistik değerinin yapılan deneysel çalışmayı ne kadar iyi temsil ettiğinin bir ölçüsüdür.

Sonuç: Yapılan çalışmada elde edilen bulgulara bakıldığında gözlemlenen sonuçlardan birisi deneysel çalışmayı temsil eden maksimum olasılıklı odds oranının belirlenmesi, diğeri ise meta-analizinde odds oranları birleştirilirken ağırlık olarak odds oranlarının olasılıklarının kullanılabilmesidir.

Anahtar Sözcükler: Kontenjans tablosu, olasılık dağılımı, hipergeometrik dağılım, odds oranı, relatif risk, meta-analizi

Address for Correspondence: Mehmet Onur KAYA, Fırat University Faculty of Medicine, Department of Biostatistics and Medical Informatics, Elazığ, Turkey
E-mail: monurkaya@gmail.com **ORCID ID:** orcid.org/0000-0001-8052-0484

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Introduction

One of the problems encountered in scientific research is the inadequacy of data. This can be due to the rarity of data, as well as the lack of time and cost or the lack of specialized personnel. For this reason, especially in health researches, clinical trials and studies are undertaken on a limited number of units. Sometimes, it is necessary to work with small samples for ethical purposes. In such a case, combining studies with similar characteristics by different researchers may make the study findings more meaningful. For these reasons, developing suitable combination methods is necessary.

The most striking example of this is the combination of odds ratios (ψ). Odds ratio combining methods in the literature are Mantel-Haenszel, Peto, General Variance, and DerSimonian-Laird methods. Detailed information on these methods can be found in Katz et al. (1) and Morris and Gardner (2). In these studies, important information is given about establishing confidence intervals of odds ratio. The normal distribution was used to establish the confidence interval. However, the condition of normal distribution may not always be possible. In this case, it is important to determine the distribution of odds ratio. No study in the literature has reported the distribution of odds ratio. However, a distribution that can be used in contingency tables has been examined by Patnaik (3) and Stevens (4). Studies of these researchers will be given with examples in the following sections. These examples were very useful in calculating the distribution of odds ratio. The distribution of odds ratio will be shown in the example in the Results section of our study. In addition, the distribution of combined odds ratios will be calculated in real data application.

Some Probabilistic Notes on Contingency Tables

In biostatistics, the statistical methods which are frequently used in both retrospective and prospective studies are based on statistics such as relative risk and odds ratio obtained from the information in Table 1. Therefore, it is very important to examine the probabilistic features of this table.

As retrospective study is limited to observed data, experimental values are fixed. However, it does not mean that it cannot vary depending on the retrospective follow-up period or other reasons. Thus, the value of a in the table is the observation value of X_1. The same applies to the control group. The probability $\Pr\{X_1 = a\}$ can be calculated by the ratio of desired states to all possible states, as in the hypergeometric probability. The number of possible states is written as follows,

$$\frac{N!}{a!b!c!d!}$$

The number of desired states can be calculated as follows,

$$\binom{m}{a}\binom{n}{d}\binom{r}{c}\binom{s}{b} = \frac{m!n!r!s!}{a!(m-a)!d!(n-d)!c!(r-c)!b!(s-b)!}$$

We have

$$Pr\{X_1 = a\} = \frac{m!n!r!s!}{N!a!b!c!d!}$$

where $max\{0, m + r - N\} \leq X_1 \leq min\{m, r\}$.

Sample 1. Consider the data in Table 2

In this example, the variable X_1 takes values between $0 \leq X_1 \leq 10$. Let us show the possible states and probabilities of variable X_1 in Table 3.

According to Table 3, the probability that X_1 takes the value of 5 is the highest probability. The graph of the probability values in Table 3 is as follows,

Methodology

In literature, the first study about this probability belongs to P. B. Patnaik in 1948. In the study, the common cell of the case and the positive effect was accepted as a random variable, and it was shown by P. B. Patnaik that it has a hypergeometric distribution. This makes it easier to obtain the term representing the odds ratio from the conditional probability of the hypergeometric distribution. Therefore, hypergeometric distribution was taken into consideration in the study. Patnaik calculated the mean and variance of the distribution with the help of the hypergeometric distribution as $EX_1 = mr/N$ and $VarX_1 = mnrs/N^2(N-1)$ [3]. The mean EX_1 calculated by Patnaik is used as the expected value of the cells in the chi-square relationship test. This was

Table 1. Structure of contingency table

	Positive effect	Negative effect	Total
Case	$X_1 = a$	b	m
Control	$X_2 = c$	d	n
Total	r	s	N

Table 2. Sample data

	Positive effect	Negative effect	Total
Case	$X_1 = a = 8$	$b = 2$	$m = 10$
Control	$X_2 = c = 10$	$d = 15$	$n = 25$
total	$r = 18$	$s = 17$	$N = 35$

followed by W. L. Stevens. Stevens assumed the conditional probability of the variable X_1 as a function of a under the condition that all marginal totals are known. As follows [4],

$$Pr\{X_1 = a|X_1 + X_2 = r, m\} = C\binom{m}{a}\binom{n}{r-a}\psi^a$$

where $\psi = ad/bc$ is odds ratio. The conditional probability mentioned above can be obtained as the multiplication of two binomial probabilities,

$$Pr\{X_1 = a|X_1 + X_2 = r, m\} = Pr\{X_1 = a\}Pr\{X_2 = c\}$$

$$= \binom{m}{a} p_1{}^a q_1{}^{m-a}\binom{n}{c} p_2{}^c q_2{}^{n-c}$$

$$= \frac{q_1^m q_2^n p_2^r}{q_2^r}\binom{m}{a}\binom{n}{r-a}\psi^a$$

where p_1 and p_2 are the probability of success in case and control groups, respectively. In addition, the ratio p_1/p_2 is called relative risk. As a result, this equation ensures that conditional probability can be written as a function of a. This is an important result for 2x2 tables. If the observation value of variable X_1 is smaller than some values in the possible order, ψ will remain smaller than odds ratios of these values, otherwise vice versa. Using this feature, Jerome Cornfield formed confidence interval with $1 - \alpha$ probability for odds ratio in his study done in 1956 (5). Cornfield obtained the lower limit ψ_1 for ψ from the solution of the following equation,

$$\sum_{y=X_1}^{m} C\binom{m}{a}\binom{n}{r-a}\psi^a = \frac{\alpha}{2}$$

Similarly, he obtained the upper limit ψ_2 for ψ from the solution of the following equation,

$$\sum_{y=0}^{X_1} C\binom{m}{a}\binom{n}{r-a}\psi^a = \frac{\alpha}{2}$$

Thus, the confidence interval can be written as $Pr\{\psi_1 \leq \psi \leq \psi_2\} = 1 - \alpha$.

Results and Discussion

Here, conditional probability is obtained as the multiplication of two binomial distributions by independent variables X_1 and X_2. Then normal distribution test procedures can be used in hypothesis tests since the limit distributions X_1 and X_2 approach normal distribution. However, this may be the case if the marginal totals are large enough. Otherwise, it may cause incorrect interpretations. It is more accurate to obtain the exact distribution and to test with nonparametric method when an exact test statistic for ψ is desired to be created. In order for the mean and variance of ψ to be real, it is sufficient for the cells to satisfy the conditions of $a < m$ and $c > 0$. In this case, it is necessary to obtain the conditional distribution of ψ depend on these conditions. Therefore, many researchers use the normal distribution approach. The conditional distribution can be obtained by dividing binomial probabilities to the probability of $Pr\{X_1 < m\}$ for X_1 and to the probability of $Pr\{X_2 > 0\}$ for X_2. In the following example, we show the possible values and possibilities of ψ.

Sample 2. Let be the sample data as follows,

The multiplication probability table and the probability table of ψ can be formed with the data in Table 4 using the conditional probabilities of variables X_1 and X_2,

The graph of multiplication probabilities in Table 5 is as follows,

When the probability in Table 6 is taken into consideration, the variable is seen to have the highest probability at $\psi = 4$. It is seen that odds ratio in the experimental data in Table 4 would take high probability value between 2 and 5 ($Pr\{2 \leq \psi \leq 5\} = 0.443$). Such probabilistic information can also be supported in

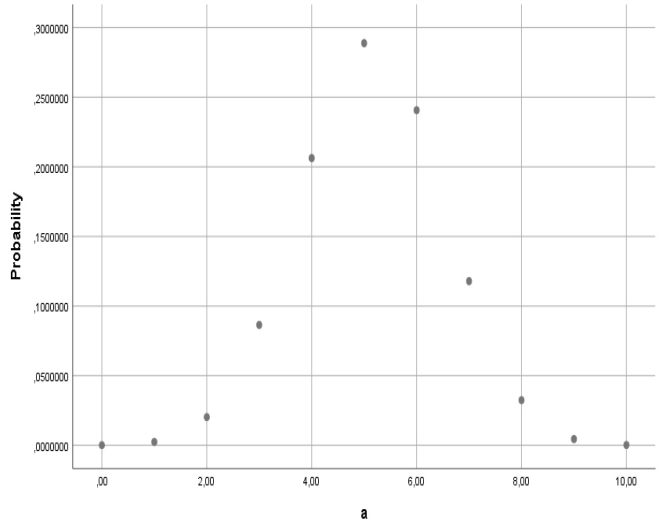

Figure 1. Graph of the probability values in Table 3

Table 3. Probability table

a	$Pr\{X_1 = a\}$	a	$Pr\{X_1 = a\}$
0	0.0001059	6	0.2406714
1	0.0023836	7	0.1178798
2	0.0202606	8	0.0324169
3	0.0864452	9	0.0045023
4	0.2062898	10	0.00023836
5	0.2888057	Total	1

statistical terms by creating rejection and acceptance zones from the distribution obtained at α significance level. Moreover, the mean $E\psi = 6.7133$ obtained from the distribution is an important statistic for ψ. The graph of probabilities in Table 6 is as follows.

Table 6 shows the distribution of ψ. The distribution of ψ can be easily obtained when multiple tables are for the same X_1. Let's assume that there are k tables of X_1. In this case, probabilities for each table are shown as follows,

$$Pr\{\psi_j = u\} = p_j(u), j = 1, \cdots, k.$$

The distribution of all tables will be as follows,

$$Pr\{\psi = u\} = \frac{1}{k}\sum_u p_j(u), j = 1, \cdots, k.$$

Since the mean E is derived from the k sample selected from the mass, it will be able to represent the mass ideally. Finally, the following sample about combined odds ratio are presented.

The following example table was taken from Afshari et al.(6). This meta-analysis study by Mahdi investigates the effect of opium and smoking on bladder cancer. Table 7 was created by considering only opium use. The distribution and expected value of odds ratio were obtained for each study. At the end of the table is the expected value of the combined odds ratio. The matlab program used in the calculation is attached.

Conclusion

In general, when we look at the studies in the field of biostatistics, a comprehensive and technically rich literature is emerging. This is due to the fact that many scientific techniques are combined with medical data gathered under biostatistics. A scientific technique needs not only an opinion, but also an interpretation. The interpretation to be made is usually attributed to the data. However, this interpretation is the common point of data and technique, which increases the scientific value of results

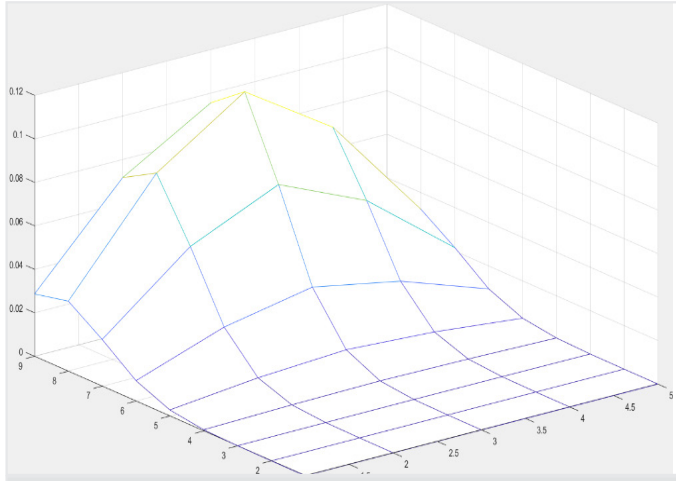

Figure 2. Graph of the multiplication probabilities in Table 5

Table 4. Sample data

	Positive effect	Negative effect	Total
Case	$X_1 = a = 8$	b = 2	m = 10
Control	$X_2 = c = 3$	d = 3	n = 6
Total	r = 11	s = 5	N = 16

Table 5. Multiplication probabilities

	$Pr\{X_2 = k \mid X_2 > 0\}$					
$Pr\{X_1 = k \mid X_1 < 10\}$	k	1	2	3	4	5	6
	0	1.09×10^{-8}	2.73×10^{-8}	3.64×10^{-8}	2.73×10^{-8}	1.09×10^{-8}	1.82×10^{-9}
	1	4.37×10^{-7}	1.09×10^{-6}	1.45×10^{-6}	1.09×10^{-6}	4.37×10^{-7}	7.28×10^{-8}
	2	7.86×10^{-6}	1.96×10^{-5}	2.62×10^{-5}	1.96×10^{-5}	7.86×10^{-6}	1.31×10^{-6}
	3	8.39×10^{-5}	2.09×10^{-4}	2.79×10^{-4}	2.09×10^{-4}	8.39×10^{-5}	1.39×10^{-5}
	4	5.87×10^{-4}	1.46×10^{-3}	1.95×10^{-3}	1.46×10^{-3}	5.87×10^{-4}	9.78×10^{-5}
	5	2.81×10^{-3}	6.04×10^{-3}	9.39×10^{-3}	7.04×10^{-3}	2.81×10^{-3}	4.69×10^{-4}
	6	9.39×10^{-3}	2.35×10^{-2}	3.13×10^{-2}	2.34×10^{-2}	9.39×10^{-3}	1.56×10^{-3}
	7	2.14×10^{-2}	5.37×10^{-2}	7.16×10^{-2}	5.37×10^{-2}	2.14×10^{-2}	3.58×10^{-3}
	8	3.22×10^{-2}	8.05×10^{-2}	0.1074	8.05×10^{-2}	3.22×10^{-2}	5.37×10^{-3}
	9	2.86×10^{-2}	7.16×10^{-2}	9.54×10^{-2}	7.16×10^{-2}	2.86×10^{-2}	4.77×10^{-3}

obtained from data and importance of the technique used. Therefore, biostatistics studies are important studies that bring data and technique together. If the odds ratio value obtained from a data in Table 1 is smaller than 1, the factor decreases the risk of disease. If the odds ratio is equal to 1, the factor has no effect on the disease. If the odds ratio is bigger than 1, the factor increases the risk of the disease. Thus, X_1 is the most important variable to ensure a high odds ratio. Considering the coincidence of the value of X_1, it is more important to know the maximum probability value. For this, the distribution of X_1 and its interpretation should be made. In the study, a data table of 2 × 2 type has been shown to have hypergeometric distribution when considered unconditionally. Depending on this distribution, the variable X_1 takes the maximum probability with value $(m + 1)(n + 1)/(N + 2)$. In addition, this value is the maximum probability value of the odds ratio. This result is very important in terms of both data and theory. If data were not interpreted with the theoretical structure, then a conclusion will never be obtained. Similarly, obtaining the distribution of ψ is also important in terms of interpretation. When values obtained from different tables are combined in a probability distribution, the distribution of a single variable ψ can be obtained for all tables. This result is also very important for meta-analysis. The mean $E\psi$ for a single table is so important for combined tables. Many methods have been presented to combine odds ratios in literature; however, no such method has been presented. The reason for this is that presented methods have the ease of calculation in terms of researchers. However, using probabilistic methods is more important for more optimal results. Finally, one point that should be taken into consideration is that if the number of case and control is sufficient in a 2x2 table, parametric methods can be used easily. An example would be the Mantel-Haenszel, Peto, General Variance, and DerSimonian-Laird methods. If the number of data is quite low, it is more appropriate to use probabilistic methods.

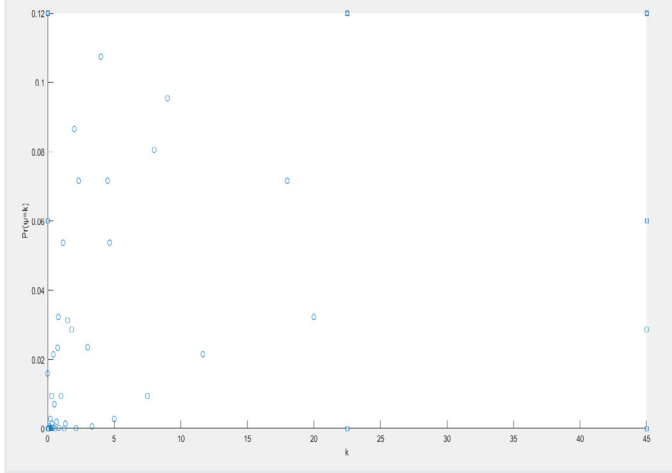

Figure 3. Graph of the probabilities in Table 6

Table 6. Probability values of ψ

k	$Pr\{\psi = k\}$	k	$Pr\{\psi = k\}$
0	0.015862197	1	9.39×10^{-3}
0.022	4.37×10^{-7}	1.16	5.37×10^{-2}
0.031	1.96×10^{-5}	1.25	7.86×10^{-6}
0.05	7.86×10^{-6}	1.333	1.46×10^{-3}
0.055	1.09×10^{-6}	1.5	3.13×10^{-2}
0.085	8.39×10^{-5}	1.8	2.86×10^{-2}
0.111	1.45×10^{-6}	2	0.08654
0.133	5.87×10^{-4}	2.142	8.39×10^{-5}
0.2	2.81×10^{-3}	2.33	7.16×10^{-2}
0.214	2.09×10^{-4}	3	2.35×10^{-2}
0.222	1.09×10^{-6}	3.333	5.87×10^{-4}
0.25	2.62×10^{-5}	4	0.1074
0.3	9.39×10^{-3}	4.5	7.16×10^{-2}
0.333	1.46×10^{-3}	4.66	5.37×10^{-2}
0.428	2.79×10^{-4}	5	2.81×10^{-3}
0.466	2.14×10^{-2}	7.5	9.39×10^{-3}
0.5	7.06×10^{-3}	8	8.05×10^{-2}
0.555	4.37×10^{-7}	9	9.54×10^{-2}
0.666	1.95×10^{-3}	11.66	2.14×10^{-2}
0.75	2.34×10^{-2}	18	7.16×10^{-2}
0.8	3.22×10^{-2}	20	3.22×10^{-2}
0.857	2.09×10^{-4}	45	2.86×10^{-2}

Table 7. Opium exposure among cases and controls in the primary studies entered into meta-analysis

First author		+	–	*Eψ*
1. Akbari	Case	43	155	6.2324
	Control	18	378	
2. Aliramaji	Case	58	117	
	Control	27	148	2.8501
3. Asgari	Case	13	39	
	Control	5	103	9.0933
4. Ghadimi	Case	16	136	
	Control	2	150	10.2698
5. Hosseini	Case	60	119	
	Control	7	172	5.0727
6. Ketabchi	Case	80	32	
	Control	31	99	8.5306
7. Lotfi	Case	52	147	
	Control	21	179	3.1957
8. Nourbakhsh	Case	41	214	
	Control	12	243	4.2959
9. Sadeghi	Case	44	44	
	Control	7	81	14.2746
10. Tootonchi	Case	16	126	
	Control	7	135	2.9773
Combined *Eψ* = 6.6792				

Ethics

Ethics Committee Approval: There is no approval of the Ethics Committee, since there is no "animal or human element" in our study, and the study was completely conducted on hypothetical theoretical data.

Peer-review: Externally peer reviewed.

Authorship Contributions

Concept: M.G., M.O.K., Y.G., Design: M.G., M.O.K., Analysis or Interpretation: M.G., M.O.K., Y.G., Literature Search: M.G., Writing: M.G., Y.G.

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

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Appendix

```
A = input ("a="); b = input("b="); c = input("c="); d = input("d=");
M = a + b; n = c + d; pv = a/m; qv = b/m; pk = c/n; qk = d/n;
V = [0:1:m]; k =[0:1:n]; A = zeros (m, 1); B = zeros (n, 1);
p1v = zeros (m + 1, 1); p2k = zeros (n + 1, 1); phi = zeros (m – 1, n – 1); Pc = zeros (m – 1, n – 1); C = zeros (m – 1, n – 1);
SC = zeros (1, n – 1); SSC = 0; for i = 1: m + 1
        p1v (i) = nchoosek (m, v[i]) × (pv^v[i]) × (qv^[m – v(i)]); end for I = 1: n + 1
        p2k(i) = nchoosek (n, k [i]) × (pk^k[i]) × (qk^[n – k(i)]);
end
tv = 1 – p1v (m + 1); tk = 1 – p2k (1);
for i = 1: m
        A(i) = p1v(i)/tv;
end
for i = 1:n
        B (i) = p2k (i + 1)/tk;
end
Phi0 = A (1) × (sum [B]) + B(n) × (sum [A] – A [1])
a1 = (1:1:m – 1);c1 = (1:1:n – 1);
for i = 1:m – 1
for j = 1:n – 1
b1 = m – a1(i);
 d1 = n – c1(j);
 phi (i, j) = a1(i) × d1/(b1 × c1[j]);

for i = 2:m
for j = 1:n – 1
Pc(i – 1, j) = A (i) × B (j);
end
end
for i = 1:m – 1
for j = 1:n – 1
C(i, j) = phi (i, j) × Pc (i, j);
end
end
phi %values of phi
Pc %multiplication table
SC = sum(C);
SSC = sum (SC)    %expected value of phi
```

Original Article

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Validation of Turkish Version of Newest Vital Sign Scale to Assess Health Literacy

Sağlık Okuryazarlığını Değerlendirmede NVS Ölçeği Türkçe Geçerliliği

Fatih ÇİFTÇİ[1], Hakan DEMİRCİ[1], Hilal Nemli ÇİFTÇİ[2], Gökhan OCAKOĞLU[3]

[1]Bursa Yüksek İhtisas Training and Research Hospital, Clinic of Family Medicine, Bursa, Turkey
[2]Bursa Yüksek İhtisas Training and Research Hospital, Clinic of Internal Diseases, Bursa, Turkey
[3]Uludağ University Faculty of Medicine, Department of Biostatistics, Bursa, Turkey

ABSTRACT

Objective: The aim of the study was to check Turkish validation of the Newest Vital Sign (NVS) scale.

Methods: The participants were composed of the patients who were registered in a Family Health Center located in Bursa between September 2016 and February 2017. The subjects selected by random sampling method and they were invited to the study. The research was performed by applying survey with the face to face method to the participants.

Results: Turkish version of the NVS scale is a valid tool to assess health literacy. Cronbach alpha coefficient was calculated as 0.720 for NVS scale. Receiver Operating Characteristic analysis revealed a cut-off point for the NVS scale which was 4. According to the NVS scale, 57.9% of the participants had an adequate health literacy level.

Conclusion: This sample was found to be adequate for validation analysis of NVS scale. NVS is a reliable and valid tool to evaluate health literacy among Turkish population and the cut-off point for the scale is 4. Health literacy is inadequate for less than half of the people living in our region.

Keywords: Health literacy, newest vital sign, reliability, validation

ÖZ

Amaç: Bu çalışmanın amacı En Yeni Yaşamsal Bulgu (EYYB) ölçeğinin Türkçe geçerliliğinin araştırılmasıdır.

Yöntemler: Katılımcılar, Eylül 2016-Şubat 2017 tarihleri arasında Bursa'da bulunan bir aile sağlığı merkezine kayıtlı hastalardan oluşuyordu. Rastgele örnekleme yöntemi ile seçilen kişiler çalışmaya davet edildi. Araştırma, katılımcılara yüz yüze yöntemi ile anket uygulanarak gerçekleştirildi.

Bulgular: EYYB ölçeğinin Türkçe versiyonu, sağlık okuryazarlığını değerlendirmek için geçerli bir araçtır. Cronbach alfa katsayısı EYYB için 0,720 olarak hesaplandı. Alıcı işletim karakteristiği analizi, EYYB ölçeği için 4 olan bir kesme noktası ortaya çıkardı. EYYB ölçeğine göre, katılımcıların %57,9'u yeterli sağlık okuryazarlığı seviyesine sahipti.

Sonuç: Bu örneklem EYYB ölçeğinin geçerliliğini araştırmak için yeterlidir. EYYB, Türk nüfusu arasında sağlık okuryazarlığını değerlendirmek için güvenilir ve geçerli bir araçtır ve ölçeğin kesme noktası 4'tür. Sağlık okuryazarlığı bölgemizde yaşayan insanların yarısından fazlası için yeterlidir.

Anahtar Sözcükler: Sağlık okuryazarlığı, En Yeni Yaşamsal Bulgu ölçeği, güvenirlik, geçerlilik

Address for Correspondence: Hakan DEMİRCİ, Bursa Yüksek İhtisas Training and Research Hospital, Clinic of Family Medicine, Bursa, Turkey
E-mail: drhakandemirci@hotmail.com **ORCID ID:** orcid.org/0000-0003-0434-4807

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Introduction

World Health Organization defines health literacy (HL) as "The cognitive and social skills that determine the motivation and ability of individuals to gain access to understand and use information in ways that promote and maintain good health" (1). According to The European HL Survey Consortium definition, HL has a scope that evaluates the knowledge, motivation, and sufficiency of individuals about benefiting from health services, preventing diseases, and improving health in order to improve the quality of life (2). HL can also be defined as the individual's capacity of acquisition, interpretation, and comprehension of basic health information and services in terms of protecting, developing, and curing the individual's health (3,4).

HL has gained a significant importance with respect to the past. Recently, reasons such as increasing importance of preventive health services, consideration of individual health service, generally low level of HL, insufficient reflection of health-related issues to the society, and increase in health expenses, have raised the importance of HL (5-7). Individuals with low level of HL cause some unintended consequences, such as high hospital charges, long duration of hospital stay, inappropriate use of emergency services, and inappropriate increase in health expenses (8-10).

Assessing HL is of particular importance for public health. Newest Vital Sign (NVS) scale, consisting of 8 questions, is an easy-to-implement and easy-to-interpret instrument to assess HL. NVS was studied by Ozdemir et al. (11); however, the validity of the study could not be performed because of the absence of equivalent Turkish questionnaire that could match at that time. This study aimed to check the Turkish validation of the NVS scale.

Methods

Target population of the study is composed of patients between ages 18-65 years, who are registered to the Family Health Center in Bursa City between September 2016 and February 2017. During the period when the research was conducted, 12,468 individuals were registered to the Family Health Center and 8,164 of them were between ages 18-65 years. A total of 400 volunteers were selected from the patients' list by random sampling method and were invited to the study. The research was performed using a face-to-face survey method to all participants. This study was approved by the Bursa Yüksek İhtisas Training and Research Hospital, Ethics Committee for Clinical Investigations. A written informed consent was obtained from all participants.

A survey form interrogating the socio-demographic features and NVS scale and the Turkish translation of the European HL Survey Questionnaire (HLS-EU-Q) were used to measure the HL level of patients.

The Turkish version of the HLS-EU-Q was validated by Abacigil et al. (12). The scale is composed of 47 questions and each of these 47 questions is graded in a scale of 1-4 points (1: very difficult, 2: difficult, 3: easy, 4: very easy). HL about the health care service, prevention from diseases, and promotion of health is analyzed within the matrix of the following issues: access to the information, comprehension, evaluation, and implementation of the information.

NVS scale was studied by Ozdemir et al. (11) in 2010. Its validation could not be achieved at that time due to the absence of a scale to compare results. In NVS scale, patients are given a copy of a food label and asked six questions related to the label. The first four questions among them require calculation capability and the last two questions do not require numerical skills. Each correct answer given is graded as 1 point and the HL level of the individual is determined according to the total score he/she gets. Retest for NVS scale was performed 15 days after the first interview.

Statistical Analysis

Normal distribution suitability of variables was analyzed with Shapiro-Wilk test. Variables were expressed in mean, standard deviation, or median (minimum-maximum) values. Mann-Whitney U test between two groups was used accordingly to test the normality of results, and Kruskal-Wallis test was used in case with more than two groups. Following Kruskal-Wallis test, Dunn-Bonferroni approach was used in order to determine the group or groups that were different, and multiple comparison procedures were applied. Receiver Operating Characteristic (ROC) analysis was made in order for the NVS scale to predict HL, and related cut-off point was calculated. The construct validity of NVS scale was investigated using explanatory factor analysis. While internal consistency of scales was analyzed with Cronbach's alpha and Kuder-Richardson 20 (KR-20) coefficients, relationships between scores of the scales were analyzed with correlation analysis, and Spearman correlation coefficient was calculated. The Statistical Package for the Social Sciences (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.) and MedCalc Statistical Software trialversion 16.4.3 (MedCalc Software BVBA, Ostend, Belgium; https://www.medcalc.org; 2016) programs were used for statistical analysis, and $p < 0.05$ was accepted as statistically significant.

Results

A total of 376 participants agreed to enroll in the study (response rate was 94%). The mean age of participants was 37.17±11.60 years (range between 18-65 years). General characteristics of participants are shown in Table 1.

The distribution of mean points for HLS-EU-Q and NVS scale are presented in Table 2. The mean HLS-EU-Q score was 33.24±7.45, whereas the mean NVS scale score was 3.76±1.83.

The distribution of HL status classified by HLS-EU-Q and NVS is shown in Table 3. According to HLS-EU-Q, 45.2% of participants had adequate HL level, whereas 57.9% according to the NVS scale. Comparisons among the gender groups revealed that NVS scale scores of male participants were higher than those

Table 1. General characteristics of participants

	The number of participants (n)	Percent (%)
Gender		
Female	202	(53.72%)
Male	174	(46.28%)
Education		
Literate	91	(24.20%)
Secondary school	51	(13.56%)
High school	100	(26.50%)
University	134	(35.64%)
Economic status		
Low	17	(4.52%)
Moderate	238	(63.30%)
High	121	(32.18%)
Occupation		
House wife	122	(32.45%)
Student	28	(7.45%)
Worker	76	(20.21%)
Self-employee	8	(2.13%)
Professional	67	(17.82%)
Retired	32	(8.51%)
Others	43	(11.44%)
Marital status		
Married	284	(75.50%)
Single	92	(24.5%)

of the female (p=0.023). Period of study of men was statistically significantly better than that of women (p<0.001).

Same directional significant relationship was found between NVS scale score and Health service, Disease prevention HL, Health protection HL, General HL scale scores (p<0.001, p<0.001, p<0.001, p<0.001, respectively) (Table 4).

HLS-EU-Q scores were achieved by classifying the answers of participants to each question as "very difficult or difficult" or "very easy and easy," as shown in Table 5. Participants of the current study are found to be more successful in terms of health care and information comprehension compared to other studies reflecting Europe and countrywide of Turkey. On the contrary, our participants express their difficulties in periodic examinations and adult vaccination issues.

In order to predict the sufficient and perfect HL for NVS scale, cut-off point value is found as "4," and the field below the ROC curve including this cut-off point is found as 0.688 (p value: p<0.001) (Figure 1).

HLS-EU-Q Reliability Analysis

In the reliability analysis of scales, Cronbach's alpha coefficient is used for general HLS-EU-Q and its sub-scales, and Kuder-Richardson 20 (KR-20) coefficient is used for NVS scale. When coefficients are analyzed, reliability of the HLS-EU-Q is found perfect for health service HL (α=0.904), health protection

Table 2. Distribution of mean points for HLS-EU-Q and NVS scale

	Mean ± SD	Minimum: Maximum
Health Care (Questions 1-16)	35.72±7.45	0:50
Disease prevention (Questions 17-31)	31.11±9.18	7.78:50
Health promotion (Questions 32-47)	32.75±8.75	4.17:50
Total HLS-EU-Q (Questions 1-47)	33.24±7.45	15.60:50
NVS	3.76±1.83	0:6

HLS-EU-Q: The European Health Literacy Survey Questionnaire; NVS: Newest Vital Sign, SD: Standard deviation

Table 3. Distribution of Health Literacy Status classified by HLS-EU-Q and NVS scale

		NVS			
		0-1 (Limited HL)	2-3 (Possibly limited HL)	4-6 (Adequate HL)	Total
HLS-EU-Q	Inadequate HL (0-25)	14	18	15	47
	Problematic HL (>25-33)	30	47	82	159
	Sufficient HL (>33-42)	7	31	75	113
	Excellent HL (>42-50)	4	7	46	57
	Total	55	103	218	376

HLS-EU-Q: The European Health Literacy Survey Questionnaire; NVS: Newest Vital SignHL: Health Literacy

(α=0.905), and general (α=0.953); good for disease prevention sub-index (α=0.895).

NVS Scale Internal Consistency

The internal consistency of the NVS scale for an individual was examined using Cronbach's alpha coefficient. From the overall assessment of the scale, Cronbach's alpha coefficient was calculated as 0.720. When Cronbach's alpha coefficient is considered, it is seen that the NVS scale is an acceptable measurement tool in terms of internal consistency.

NVS Scale Construct Validity

Explanatory factor analysis (EFA) has been applied to determine the structure validity of the NVS scale. Prior to EFA, the Kaiser Meyer-Olkin (KMO) sample proficiency test and Bartlett's globality test were performed to assess sample adequacy and factor correlation matrix appropriateness. The KMO value was 0.76 and the Bartlett test result was $\chi 2$=4284.7 and statistically significant (p<0.001). The factor analysis is applicable if the KMO test result is above 0.50, and the Bartlett test is significant so that the correlation matrix of the substances found in the scale is suitable for factor analysis (13). Polychoric correlation matrix was used in factor analysis due to the fact that responses to substances forming the NVS scale are binary valued (true/false). Parallel analysis has been used to determine the number of factors in EFA (14). Principle Component Analysis was used as a factor extraction method and the Varimax rotation technique, which is one of the most commonly used upright rotation techniques, was used to provide independence and clarity in interpretation during the analysis. The Eigen values of 6 items was found to be above 1 and grouped under factor 1, which explained 55% of the total variance (Table 6).

Table 4. Relationship between HLS-EU-Q and NVS scale

	NVS	
	r_s	P
Health Care (Questions 1-16)	0.385	<0.001
Disease Prevention (Questions 17-31)	0.328	<0.001
Health Promotion (Questions 32-47)	0.322	<0.001
Total HLS-EU-Q (Questions 1-47)	0.388	<0.001

HLS-EU-Q: The European Health Literacy Survey Questionnaire; NVS: Newest Vital Sign; Spearman correlation coefficients were given as (rs) and p values

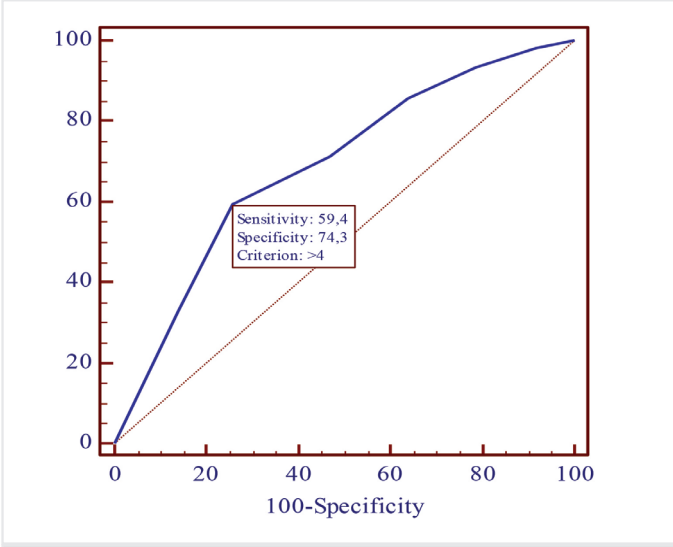

Figure 1. ROC curve for Newest vital sign scale

NVS Scale Test-retest Reliability

The consistency of the scale and the correlation of the test-retest values were assessed. When the test-retest reliability of the NVS scale was examined, a correlation between test and retest scores (r_s=0.36; p=0.031) was found.

NVS Scale Criterion/Construct Validity

The relationship between the NVS scale and equivalent scale (HLS-EU) was investigated in this study. A significant relationship was found between the calculated NVS scale scores and HLS-EU-Q scores (p<0.001).

Discussion

This sample was found to be adequate for validation analysis of the NVS scale. We found that the NVS scale was a reliable and valid tool to evaluate HL among the Turkish population, with the cut-off point of 4. According to the NVS scale, 57.9% of participants had adequate HL level. Participants express their difficulties in judging the information on health risks in the media as reliable and issues related to adult vaccination.

According to the research results, a significant positive relationship was seen between the results achieved from NVS scale and HLS-EU-Q and its sub-groups. At the same time, internal validity of these two scales is provided in our study group. NVS scale cut-off point for the Turkish society was determined as 2 in a previous study by Ozdemir et al. (11); however, according to the present study, suggested cut-off point for NVS scale to diagnose adequate HL was 4.

Studies show that the level of HL varies between communities. A survey of eight European countries found sufficient HL in 36% of the population and excellent HL in 16.5%. In this study, Netherlands was found to be the best country with sufficient/excellent HL in total (2). A study held in primary care in the United States found that 1/3 of the population belongs to the limited HL group (15). In a review, limited HL in five countries in the Southeast Asian region was reported as 55.3% on average (16). Values we identified in our study are similar to these values. Comparisons with non-European countries are open to debate as different scales are used.

According to the HLS-EU scale results, participants had the most difficulty with the subject "to judge if the information on health risks in the media is reliable." Media in our country and

Table 5. Comparisons of answers of the Health Literacy European Union Questionnaire with the existing literature

Questions	Difficult and Fairly Difficult (%)		
	Europe	Turkey	Results of the present study
Q.1 Finding information about symptoms of illnesses that concern you.	22.8	32.2	15.16
Q.2 Finding information on treatments for illnesses that concern you.	26.9	32.3	15.43
Q.3 Finding out what to do in case of a medical emergency.	21.8	39.1	13.30
Q.4 Finding out where to get professional help when you are ill.	11.9	19.4	5.32
Q.5 Understanding what your doctor says to you.	15.3	22.5	7.18
Q.6 Understanding the leaflets that come with your medicine.	28.0	42.9	11.70
Q.7 Understanding what to do in a medical emergency.	21.7	42.0	21.54
Q.8 Understanding your doctor's or pharmacist's instruction on how to take a prescribed medicine.	6.5	11.9	4.26
Q.9 Judging how information from your doctor applies to you.	18.0	19.6	9.31
Q.10 Judging the advantages and disadvantages of different treatment options.	42.6	43.1	34.04
Q.11 Judging when you may need to get a second opinion from another doctor.	38.6	32.3	30.32
Q.12 Judging if illness information from the media is reliable.	49.7	48.2	47.87
Q.13 Using information the doctor gives you to make decisions about your illness.	23.1	28.4	16.49
Q.14 Following medication instructions.	6.8	11.9	8.24
Q.15 Calling an ambulance in an emergency.	8.8	13.0	3.46
Q.16 Following instructions from your doctor or pharmacist.	5.6	8.7	2.39
Q.17 Finding information about how to manage unhealthy behavior such as smoking, low physical activity, and excessive drinking.	14.7	30.3	13.30
Q.18 Finding information on how to manage mental health problems like stress or depression.	33.5	41.1	21.28
Q.19 Finding information about vaccinations and health screenings that you should have.	24.0	38.4	22.61
Q.20 Finding information on how to prevent or manage conditions like being overweight, high blood pressure, or high cholesterol.	18.1	34.1	20.21
Q.21 Finding health warnings about behavior such as smoking, low physical activity, and excessive drinking.	10.3	21.0	14.36
Q.22 Understanding the need for vaccinations.	16.6	22.6	16.22
Q.23 Understanding the need for health screenings.	10.4	20.5	13.30
Q.24 Judging the reliability of health warnings, such as smoking, low physical activity, and excessive drinking.	14.4	31.2	16.49
Q.25 Understanding the need to go to a doctor for a check-up.	16.3	27.8	37.50
Q.26 Understanding the necessary vaccinations.	32.7	47.2	49.73
Q.27 Understanding the necessary health screenings.	25.1	45.9	44.41
Q.28 Understanding the reliability of information on health risks from the media.	42.1	49.2	51.33
Q.29 Deciding to have a flu vaccination?	26.2	33.4	48.67
Q.30 Deciding how you can protect yourself from illness based on advice from family and friends.	22.2	28.9	38.30
Q.31 Deciding how you can protect yourself from illness based on information from the media.	36.9	36.0	44.68
Q.32 Finding information on healthy activities such as exercise, healthy food, and nutrition.	14.3	27.8	14.89
Q.33 Finding out about activities that are good for your mental wellbeing.	22.6	34.0	24.47
Q.34 Finding information on how your neighborhood could be more health-friendly.	40.3	33.6	19.68
Q.35 Finding out about political changes that may affect health.	53.2	50.9	37.50
Q.36 Finding out about efforts to promote your health at work.	34.8	36.5	28.99
Q.37 Understanding advice on health from family members or friends.	13.0	16.7	14.63
Q.38 Understanding information in food packaging.	36.2	32.9	25.00
Q.39 Understanding information from the media on how to get healthier.	23.3	30.8	27.39
Q.40 Understanding information on how to keep your mind healthy.	26.1	31.7	24.73
Q.41 Judging on how your place or residence affects your health and wellbeing.	24.6	27.5	17.55
Q.42 Judging how your housing conditions help you to stay healthy.	19.5	19.7	13.30
Q.43 Judging which everyday behavior is related to your health.	12.6	21.3	14.89
Q.44 Making decisions to improve your health.	21.7	21.7	20.21
Q.45 Joining sports club or exercise class if you want to.	24.1	44.3	40.96
Q.46 Influencing your living conditions that affect your health and wellbeing.	25.5	38.6	34.84
Q.47 Taking part in activities that improve health and wellbeing in your community.	38.9	44.7	41.49

Table 6. NVS scale construct validity

Factor 1	Question	1	2	3	4	5	6
	Factor load	0.53	0.66	0.77	0.73	0.84	0.87
Eigen value	3.29						
%Variance	55%						
	Kaiser-Meyer-Olkin (KMO) test: 0.76 Bartlett Test: $x^2_{(15)}$ =4284.7; p<0.001						

across the world may be at odds with evidence-based medicine (17-19). For example, parents who are misguided about neonatal vaccinations in Turkey do not vaccinate their children, and as a result, the incidence of preventable infectious diseases has recently increased (20,21). In another example, the media inculcates pregnant women not to have oral glucose tolerance test, thus placing the mother and children at risk (22,23). The fact that the reliability of health information in the media is the most challenging topic in the HL survey suggests that the scale can be used to identify health-related issues. Another issue that participants feel themselves insufficient is adult vaccinations. Adult vaccinations protect the individual's health as well prevention of labor loss. Pneumonia and influenza vaccines are recommended for patients with asthma, diabetes mellitus, and coronary failure, and in individuals over 65 years old, thus the social security institution covers some part of these vaccinations (24,25). Family physicians should inform their patients about vaccination in case of the existence of these diseases and other indications.

Study Limitations

One of the limitations of the study is that this is a mono-center study. This study that is performed in Bursa Yuksek İhtisas Training and Research Hospital Baglaralti Family Health Center reflects the results of this region and these results cannot be generalized. Only participants who could visit the Family Health Center are included in our study. The situation may be different in advanced patients who get home care services. HL status of patients who cannot visit the polyclinic may affect the total scores.

Conclusion

NVS scale is a reliable and valid tool to evaluate HL among the Turkish population, having the cut-off point of 4. HL is inadequate for less than half of the people living in our region. Patients should be more informed about health risks and issues related to adult vaccination through the media.

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Ethics

Ethics Committee Approval: Bursa Yüksek İhtisas Training and Research Hospital, Ethics Committee for Clinical Investigations approved the study (10.08.2016 2011-KAEK-25 2016/14-11).

Informed Consent: A written informed consent was obtained from the participants.

Peer-review: Externally peer reviewed.

Authorship Contributions

Concept: H.D., Design: F.Ç., H.D., Data Collection or Processing: F.Ç., H.N.Ç., Analysis or Interpretation: G.O., F.Ç., H.D., Literature Search: F.Ç., H.D., H.N.Ç., Writing: F.Ç., H.D., H.N.Ç. G.O.

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Original Article

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Effectiveness of Virtual Bronchoscopy in Evaluation of Centrally Located Lung Cancer

Santral Akciğer Kanserinin Değerlendirilmesinde Sanal Bronkoskopinin Etkinliği

Dilek Hacer ÇEŞME

Bezmialem Vakıf University Faculty of Medicine, Department of Radiology, İstanbul, Turkey

ABSTRACT

Objective: The aim of this study was to compare virtual bronchoscopy (VB) with multi-slice CT (MSCT) in the diagnosis of centrally located lung cancer and to investigate the effectiveness of VB in the diagnosis of the radiologist.

Methods: These patients had central lung mass detected during thorax tomography and were diagnosed as having centrally located lung cancer by histopathological examination. Detection and localization of lesions were determined by MSCT and VB. In addition, the evaluation time was determined in seconds with an electronic stopwatch.

Results: The mean age of 32 patients included in the study was 58.14±15.09. Centrally located squamous cell carcinoma (n=19) and small cell carcinoma (n=13) were included. Eighteen (54.8%) patients were male and 14 (47.6%) were female. All patients diagnosed as having centrally located lung cancer detected by MSCT were also detected by VB (100%). In the evaluation of patients with MSCT and VB; obstruction and/or lesion and their localization completely overlapped. The mean duration of MSCT evaluation of the patients was determined as 58.7 sec, and the mean duration of VB assessment was 549.9 sec. The mean VB evaluation duration was found to be statistically significantly longer than the mean MSCT evaluation duration.

Conclusion: Virtual bronchoscopy does not provide a significant additional contribution to MSCT in the diagnosis of centrally located malignant lung tumor. We think that using VB for diagnosis and navigation will be important in terms of efficiency especially in selected patients due to its low specificity and high sensitivity.

Keywords: Virtual bronchoscopy, multislice CT, lung cancer

ÖZ

Amaç: Santral yerleşimli akciğer kanseri tanısında sanal bronkoskopi uygulamasının çok kesitli BT (ÇKBT) ile karşılaştırmak ve radyoloğun tanıya ulaşmasında sanal bronkoskopinin (SB) etkinliğini araştırmak.

Yöntemler: Otuz iki hasta çalışmaya dahil edildi. Bu hastalarda toraks tomografisi sırasında santral akciğer kitlesi saptanmıştı ve histopatolojik inceleme ile santral akciğer kanseri tanısı konmuştu. ÇKBT ve SB ile lezyonların tespiti ve lokalizasyonları sağlandı. Ayrıca elektronik kronometre ile değerlendirme süresi saniye olarak tespit edildi.

Bulgular: Çalışmaya alınan 32 hastanın yaş ortalaması 58,14±15,09 idi. Santral yerleşimli skuamöz hücreli karsinom (n=19) ve küçük hücreli karsinom (n=13) çalışma kapsamına alındı. Hastaların 18'i (%54,8) erkek, 14'ü (%47,6) kadın idi. ÇKBT ile tespit edilen santral yerleşimli akciğer kanseri tanısı alan hastaların tamamında kanser SB ile de tespit edildi (%100). Hastaların ÇKBT ve SB değerlendirmelerinde obstrüksiyon ve/veya lezyon varlığı ve lokalizasyonu tamamen örtüşmektedir. Hastaların ÇKBT değerlendirme sürelerinin ortalaması 58,7 sn, SB değerlendirme süresi ise ortalama 549,9 sn olarak belirlendi. SB değerlendirme sürelerinin ortalaması, ÇKBT değerlendirme sürelerinin ortalamalarından istatistiksel olarak anlamlı derecede uzun bulundu (p=0,0001).

Sonuç: Sanal bronkoskopi, santral malign akciğer tümörü tanısında ÇKBT'ye belirgin ek katkı sağlamamaktadır. SB'nin duyarlılığının yüksek olmasına rağmen özgüllüğünün düşük olması nedeniyle özellikle seçilmiş hastalarda tanı ve navigasyon amacıyla kullanılmasının verimlilik açısından önemli olacağını düşünmekteyiz.

Anahtar Sözcükler: Sanal bronkoskopi, çok kesitli BT, akciğer kanseri

Address for Correspondence: Dilek Hacer ÇEŞME, Bezmialem Vakıf University Faculty of Medicine, Department of Radiology, İstanbul, Turkey
E-mail: dhcesme@bezmialem.edu.tr **ORCID ID:** orcid.org/0000-0002-3869-0524

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Introduction

Lung cancer ranks first among cancer types that cause death in men and second in women. The 5-year survival in the early stage of the disease ranges from 38% to 67% and 1% to 8% in the late stage (1).

The first diagnostic method used in the diagnosis of lung cancer is postero anterior (PA) X-ray and Thorax computed tomography (CT). Centrally located lung cancers are usually diagnosed by direct bronchoscopy and biopsy. With advances in CT technology and three-dimensional imaging, obtaining sagittal and coronal reformat images from axial sections with the help of thin sections increases the diagnostic accuracy. Multiplanar imaging-guided evaluation and three-dimensional perception make it easier to master anatomical structures. With the development of gantry systems in slip-ring geometry in CT devices, it is possible to obtain information from a certain volume. Volume information can be transformed into three-dimensional images with special software and thus virtual bronchoscopic (VB) examinations can be performed. It is possible to provide an anatomically realistic view of the tracheobronchial tree lumen. Although VB can evaluate the wall and lumen of the respiratory tract, it can guide the lesion with its navigation feature (2-5).

Our aim in our study was to compare virtual bronchoscopy application with multi-slice CT (MSCT) in the diagnosis of centrally located lung cancer and to investigate the effectiveness of virtual bronchoscopy in the diagnosis

Method

Patient Selection

Among the patients who underwent thoracic tomography, 32 patients diagnosed as having central lung cancer by histopathological examination were evaluated. Centrally located squamous cell carcinoma (n=19) and small cell carcinoma (n=13) were included in the study. Patients with peripheral lung cancer were not included in the study.

Thorax Multi-Slice CT (MSCT) Imaging

Somatom Sensation 40 (Siemens MedicalSystems, Germany) MSCT device was used. The imaging was performed in supine position at axial plane with a section thickness of 0.6 mm, and with parameters of 100 mAs on average and 120 kV, using contrast agent. In the "lungcare" program used, the whole thorax was scanned in approximately 10 seconds with a single breath, with the pitch factor (table speed/cross section collimation x number of detectors) being 1.4. CT images were reconstructed with a thickness of 2 mm and a reconstruction interval of 1 mm. All image data were sent electronically to a separate workstation (Leonardo, Siemens, Erlanger, Germany) via the network for interpretation. Axial CT images and coronal-sagittal multiplanar reformatted images were evaluated with standard lung window settings (level, -600 HU; width, 1200 HU) and mediastinal window settings (level, 40 HU; width, 400 HU).

Tracheobronchial tree was evaluated by imaging the following structures: The trachea, right main bronchus, right upper lobe bronchus, right upper lobe antheriopical and posterior segment entrances, intermediary bronchus, right middle lobe bronchus, right middle lobe medial and lateral segment entrances, right lower lobe bronchus, right lower lobe superior, anterobasal, mediobasal, laterobasal and posterobasal segment entrances, left main bronchus, left upper lobe bronchus, left upper lobe apicoposterior and anterior segment entrances, lingula bronchus, lingula superior and inferior segment entrances, left lower lobe bronchus, and left lower lobe superior, posterobasal, laterobasal and anteromediobasal segment entrances. The region and/or regions where obstruction or lesions were detected were determined. Additionally, the evaluation time in seconds was noted with the electronic stopwatch.

Virtual Bronchoscopy

For reconstruction of the images, 512x512 matrix gray-scale images were obtained using standard and wide reconstruction algorithms (360° linear interpolation). Image segmentation was done based on the threshold value. For threshold voxel values, the lower limit was accepted as -500 and the upper limit was accepted as 800 Hounsfield units (HU). These values were chosen in such a way that they could distinguish the mucosal structure and soft tissues within the airways. VB images obtained with a computer program called "Shaded Surface Display (SSD) b" were simultaneously evaluated with axial CT, coronal and sagittal multiplanar reformation (MPR) images on a computer screen divided into four equal quadrants in multi-view mode. Tracheobronchial tree navigation was carried out in "flythrough" mode involving the trachea, right main bronchus, right upper lobe bronchus, right upper lobe anteriorapical and posterior segment entrances, intermediary bronchus, right middle lobe bronchus, right middle lobe medial and lateral segment entrances, right lower lobe bronchus, right lower lobe superior, anterobasal, mediobasal, laterobasal and posterobasal segment entrances, left main bronchus, left upper lobe bronchus, left upper lobe apicoposterior and anterior segment entrances, lingula bronchus, lingula superior and inferior segment entrances, left lower lobe bronchus, and left lower lobe superior, posterobasal, laterobasal and anteromediobasal segment entrances. The regions and/or regions where obstruction or lesions were detected were determined. In addition, the evaluation time in seconds was noted with the electronic stopwatch.

Statistical Analysis

Statistical analyzes in this study were performed using the NCSS (NumberCruncher Statistical System) Statistical Software (Utah, USA) package program. In addition to descriptive statistical methods (mean, standard deviation) in the evaluation of the data, independent t-test was used for the comparison of paired groups. The results were evaluated at the significance level of $p<0.05$.

Results

The mean age of 32 patients included in the study was 58.14±15.09. Eighteen (54.8%) patients were male and 14

(47.6%) were female. All of the patients diagnosed as having centrally located lung cancer detected by MSCT were detected by VB (100%) (Figure 1). Presence and localization of obstruction and/or lesion in MSCT and VB evaluations of the patients completely overlapped (Figures 2, 3). The mean MSCT evaluation time of the patients was 58.7 seconds, and the mean time for VB evaluation was 549.9 seconds. The mean of the VB evaluation times was found to be statistically significantly longer than the mean of MSCT evaluation times (p=0.0001).

Discussion

Studies in the field of CT virtual endoscopy were first reported by Vining et al. (6,7). Researchers presented the first studies on both the tracheobronchial system, the colon, and the bladder (6,7). With the developments in software programs and detector technologies, diagnostic use has become routine today.

Virtual bronchoscopy, colonoscopy and cystoscopy methods are frequently used in our routine practices today. Virtual bronchoscopy has high sensitivity in evaluating stenoses in the tracheobronchial tree. However, the extrinsic-intrinsic differentiation of stenoses cannot be made with VB alone. For this, axial, coronal, and sagittal reformatted images should also be carefully examined (8-10). Accuracy rates in detecting stenoses were found to be 98% for VB, 96% for sagittal reformatted images and 96% for coronal reformed images in a study conducted with MSCT. However, the sensitivity was found to be 95% in axial images, while it was found to be 90% in VB (8,10).

Pathological events affecting the central airways consist of a heterogeneous group of diseases. Focal changes can be classified as malignant (non-small cell carcinoma, small cell carcinoma), benign (papilloma, hamartoma, carcinoid) and nonneoplastic diseases. Diffuse changes are enlargement, narrowing and tracheobronchomalacia in the airways (3-5). Mucosal layer can be seen directly with direct bronchoscopy. VB provides important information in evaluating the walls of the airways (3-5).

In our study, a retrospective evaluation was made to reveal whether the VB facilitated the radiologist's work in daily practice in the diagnosis of centrally located lung cancer compared with the MSCT. In the MSCT and VB evaluations of the patients, it was found that the presence of obstruction and/or lesion and the determination of localization completely overlapped. The addition of VB to MSCT did not add any additional contribution to diagnostic accuracy of centrally located lung cancer. However, the mean MSCT evaluation time of the subjects included in the study were 58.7 seconds, and the mean VB evaluation time was 549.9 seconds, and the mean VB evaluation time was considerably longer than the mean MSCT evaluation time. Multiplanar evaluation of malignant lesions in MSCT takes less time than VB. The imaging duration can be seen as a disadvantage for VB. In the study conducted by Hoppe et al. (8) to evaluate the stenoses in the tracheobronchial tree, it was emphasized that the sensitivity in axial MSCT was higher than VB, and that the VB did not provide significant superiority to MPR images in terms of diagnostic accuracy. However, Hoppe et al. (8) did not consider the evaluation duration in their study.

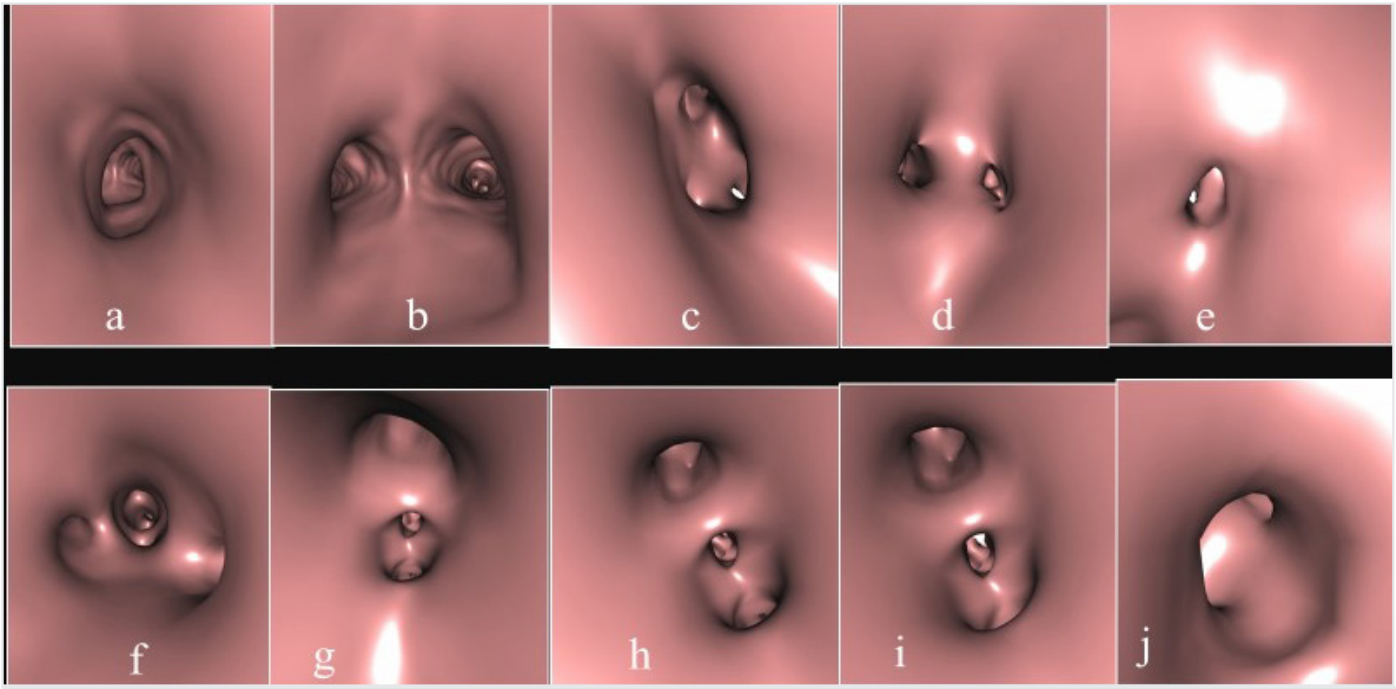

Figure 1. VB showing the airways. Trachea (a), Right and left main bronchus entrances (b), Right upper lobe anterior, posterior and apical segment entrances (c), Right lung middle lobe medial and lateral segment entrances (d), Right lung lower lobe superior segment entrance (e), Right lung lower lobe basal segment entrance (f), Left lung upper lobe bronchus, view of second carina (g), Left lung upper lobe anterior and apicoposterior segment entrances (h), Left lung lingula superior and inferior segment entrances (i), Left lung lower lobe superior segment entrance (j)

VB: Virtual bronchoscopy

In the evaluation of endobronchial lesions, measurements such as bronchial diameter, lesion size and stenosis length can be made with MPR images. But specificity of VB is low. Differentiation of bronchial cancer, mucous plaque, benign tumor, clot, foreign body cannot be made, so its selectivity is low. In a study, it was reported that the sensitivity of VB in the evaluation of endobronchial tumors was high (88%) and its specificity was low (50%) (11).

VB navigation (VBN) system is commonly used today. It consists of two phases; prebroncoscopic planning phase in which VB images of the bronchial tree navigating to the target lesion are created and guidance phase in which the navigation of

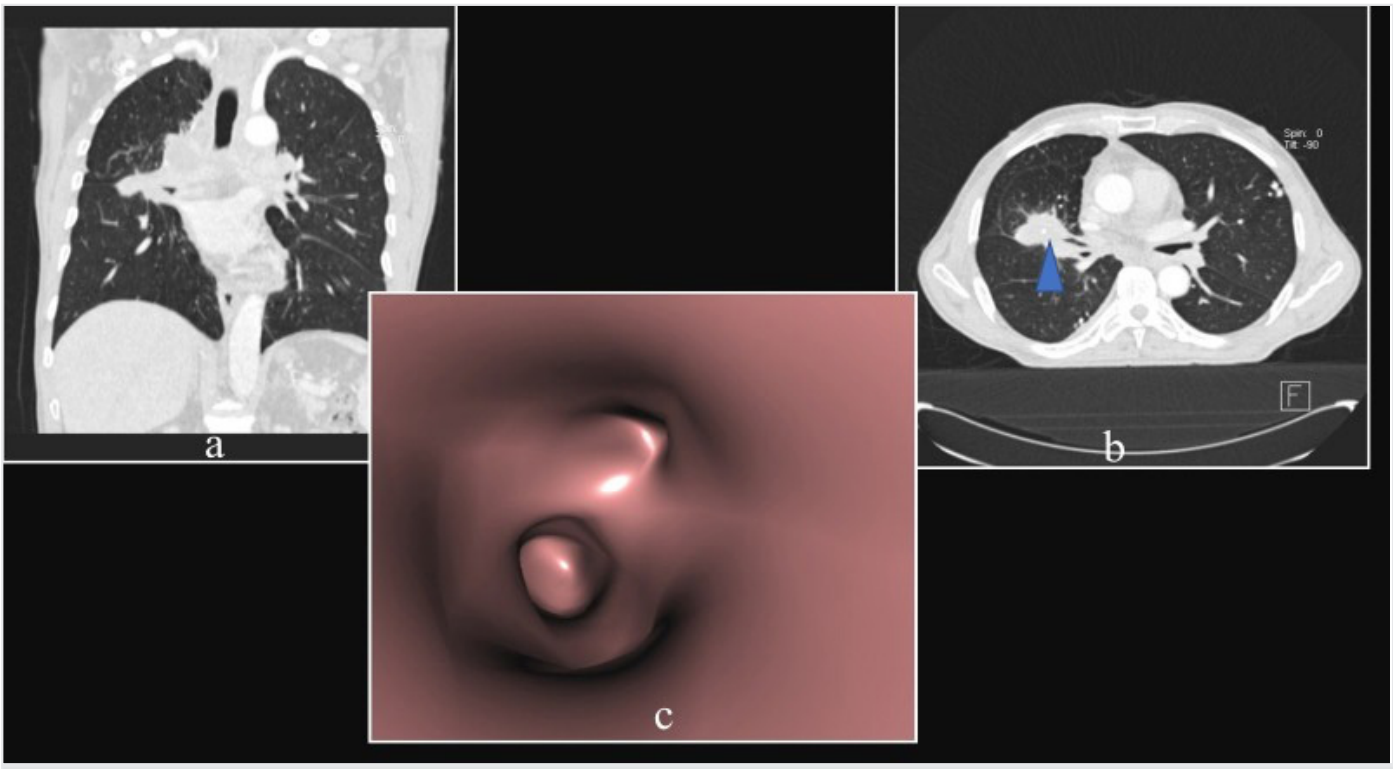

Figure 2. A 58-year-old male patient diagnosed as having centrally located squamous cell carcinoma. Coronal MPR (a), axial MSCT (arrowhead) (b) and VB images (c) showing centrally located mass lesion obstructing the right middle lobe lateral segment

MPR: Multiplanar reformation, MSCT: Multi-slice CT, VB: Virtual bronchoscopy

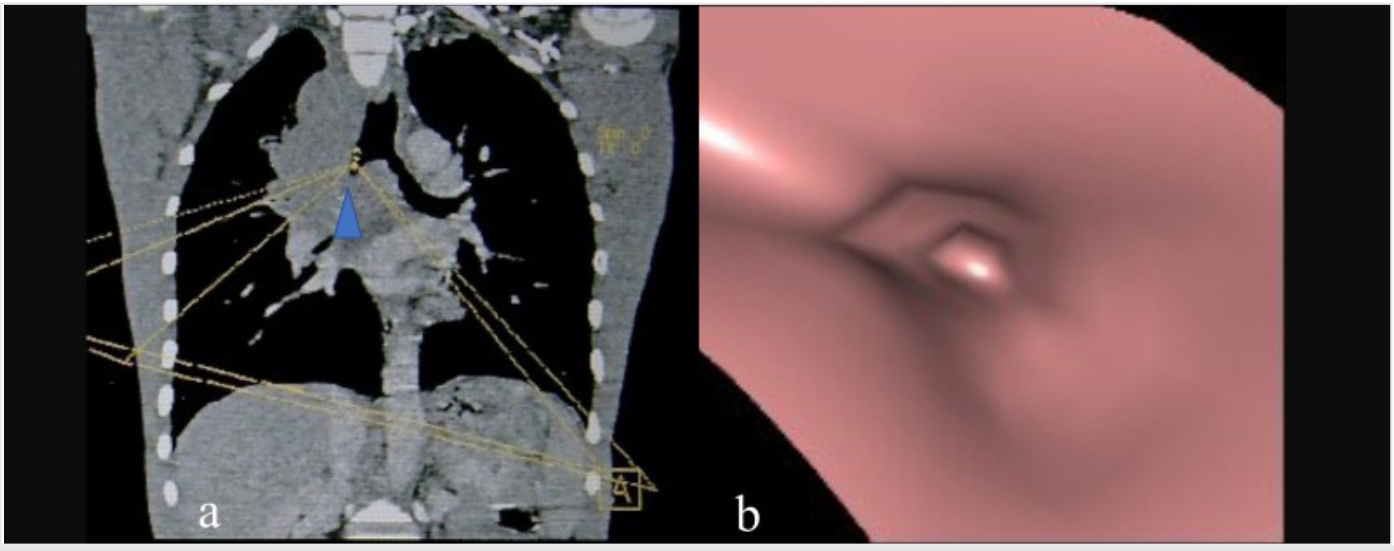

Figure 3. A 63-year-old male patient diagnosed as having small cell carcinoma. Coronal MPR (a), MSCT and SB images (b) showing a mass lesion (arrowhead) obstructing the right main bronchus completely

MPR: Multiplanar reformation, MSCT: Multi-slice CT, VB: Virtual bronchoscopy

bronchoscope to the target lesion is performed using the VBN system (12-14). VB is very important, especially in terms of preoperative mapping and navigation (15, 16). In addition, the most reliable CT finding in foreign body aspiration in children is the visualization of the foreign body in the tracheobronchial tree. The location of the foreign body can be easily demonstrated noninvasively with VB (17). It was reported that VB had high sensitivity (90%) and specificity (100%) in foreign body aspiration (17).

In our study, no difference was found in terms of detection and localization of lesions with VB and MSCT. However, we think that there is a significant difference in terms of loss of time in reaching the diagnosis between VB and MSCT and therefore, the use of VB in intensive radiology practice will be beneficial in selected patients. The use of VB as a guide in some invasive procedures, use in the evaluation of patients after surgical interventions such as transplantation, lung resection or repair of post-traumatic airway anastomoses, use in the evaluation of stenosis in pediatric patients with esophageal atresia, distal tracheoesophageal fistula, H-type fistula and bronchial anastomosis, use in the evaluation of lobar or segmental bronchial abnormalities and use in the evaluation of the distal part of the obstruction in the patient population with high rates of obstruction would be appropriate for efficiency.

Study Limitations

Among the limitations of the study, the small number of patients seemed to be the most important limitation. The second limitation was that benign lesions and peripheral lung lesions were not included in the study. The third limitation was that thorax CT and VB were not evaluated by different observers, so whether there was any difference between observers was not investigated.

Conclusion

VB does not significantly contribute to MSCT in the diagnosis of centrally located malignant lung tumor. We think that it will be important in terms of efficiency to use VB for diagnosis and navigation, especially in selected patients due to its low specificity despite its high sensitivity.

Ethics

Ethics Committee Approval: 2021/0226

Informed Consent: Informed consent was not obtained as the study design was retrospective.

Peer-review: Externally peer reviewed.

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

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Case Report

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Bezmialem Science 2021;9(2):231-3

Acute Appendicitis Caused by Enterobius Vermicularis: Case Report

Enterobius Vermicularis'in Neden Olduğu Akut Apandisit: Olgu Sunumu

Murat ABA[1], Ahmet Onur DEMİREL[1], Ahmet Gökhan SARITAŞ[1], Büşranur ATEŞ[2]

[1]Çukurova University Faculty of Medicine, Department of General Surgery, Adana, Turkey
[2]Çukurova University Faculty of Medicine, Department of Patology, Adana, Turkey

ABSTRACT

Enterobius vermicularis is one of the most common bowel parasites worldwide. It can be located in the lumen of the ceacum and appendix and rarely causes acute appendicitis disease. In this case, we present a 36 years old female patient who underwent appendectomy with the preliminary diagnosis of acute appendicitis. *Enterobius vermicularis* was detected by histopathological examination.

Keywords: Apppendectomy, acute appendicitis, *Enterobius vermicularis*

ÖZ

Enterobius vermicularis tüm dünyada en sık görülen barsak parazitlerindendir. Çekum ve apendiks lümeninde yerleşim göstererek nadir de olsa akut apandisit tablosuna sebep olabilmektedir. Bu olguda akut apandisit ön tanısıyla apendektomi uygulanan ve histopatolojik incelemesinde *Enterobius vermicularis* saptanan 36 yaşında kadın hasta sunulmaktadır.

Anahtar Sözcükler: Apendektomi, akut apandisit, *Enterobius vermicularis*

Introduction

Acute appendicitis (AA) is the most common cause of acute abdomen requiring surgical treatment (1). Appendectomy is one of the most common surgeries performed in general surgery practice, and the indication is often AA (2). While the lifetime probability of getting the diagnosis of AA is 8.6% in men and 6.7% in women, this probability is 12% in men and 25% in women for undergoing appendectomy (3). AA often occurs as a result of obstruction of the lumen of the appendix due to fecalitis or lymphoid tissue hypertrophy; however, parasitic infestations such as *Enterobius vermicularis* may rarely cause AA (4). *Enterobius vermicularis* is known as the most common helminthic infestation among gastrointestinal infections worldwide (5). The relationship between *Enterobius vermicularis* infestation and appendicitis was first described in 1899 (6). In our patient, we aimed to present the *Enterobius vermicularis* infestation in a 36-year-old female patient who was operated with a pre-diagnosis of AA.

Case Report

A 36-year-old female patient presented to the emergency department with complaints of abdominal pain, nausea and loss of appetite for the last two days. On physical examination, there was defense, rebound and tenderness in the right lower quadrant of her abdomen. In laboratory tests; white blood cell count was 11,800 10^3/mL, hemoglobin level was 14.5 g/dL, hematocrit was 41.9%, and other biochemical parameters were evaluated in the normal range. Appendix could not be visualized in ultrasonography. In abdominal computed tomography, it was

Address for Correspondence: Murat ABA, Çukurova University Faculty of Medicine, Department of General Surgery, Adana, Turkey
E-mail: Mdmurataba@gmail.com **ORCID ID:** orcid.org/0000-0003-0167-4923

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evaluated that there was a blunt-ending tubular structure with an increased wall thickness and an inflamed appearance in the right paracecal region, which was separating from the cecum and extending to the subhepatic area (findings compatible with AA) (Figure 1).

A laparoscopic appendectomy decision was made for the patient. Consent was obtained from the patient for the operation. In the intraoperative exploration, it was observed that the wall thickness increased at the level of the cecum. The appendix with extensive mesenteric inflammation and serous free fluid around it and with increased wall thickness, extending retrocecally to the subhepatic area, was detected. Laparoscopic appendectomy was performed. There were no intraoperative complications. On the first postoperative day, oral regimen was started after the passing of gas and stool, and the patient could tolerate the oral regimen.

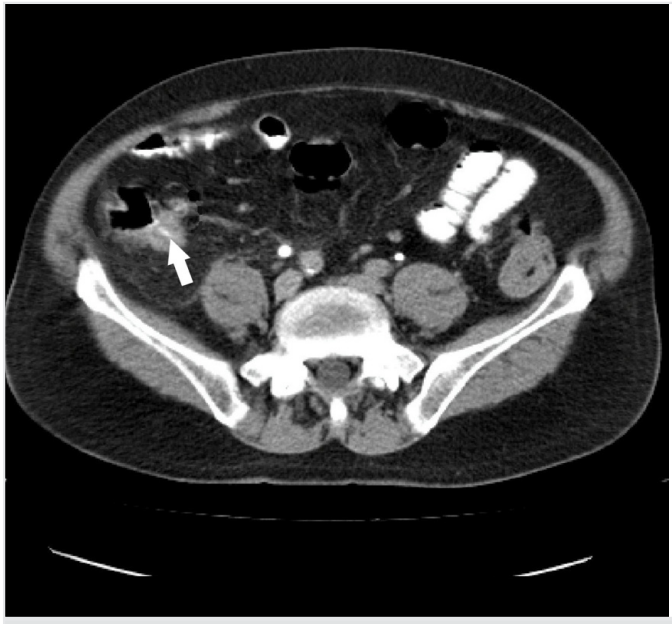

Figure 1. Computed tomography image of the appendix

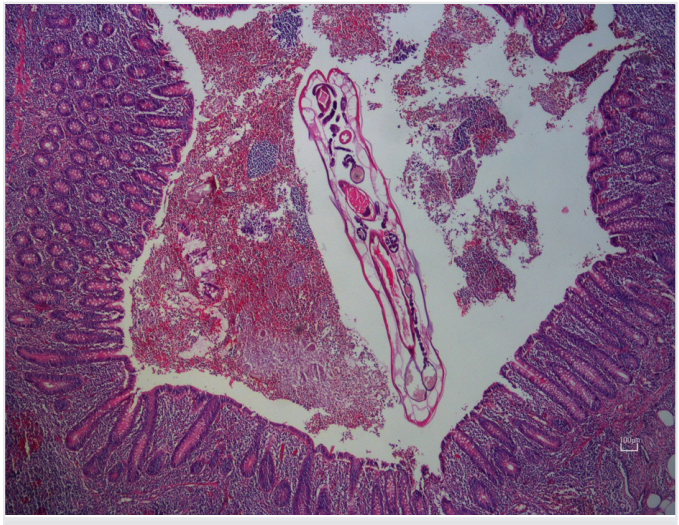

Figure 2. Image of Enterobius Vermicularis in the lumen of the appendix

The patient was discharged on the third postoperative day. Postoperative pathology specimen was found to have *Enterobius vermicularis* in the lumen of the appendix, and findings compatible with AA were found (Figure 2). The patient and her family relatives were referred to the clinic of infectious diseases for anthelmintic treatment.

Discussion

AA is one of the most common causes of emergency surgery today (7). The gold standard method in its treatment is appendectomy. Although *Enterobius vermicularis* infestation is seen in all age groups, its prevalence is higher in childhood (8). Its spread in humans is most common through the fecal-oral route (9). The most common locations of the infestation are appendix and cecum lumen (10). It is more common in women than in men (11). The overall incidence of *Enterobius vermicularis* in patients with AA is between 0.2% and 4% (12). It is generally thought to cause mechanical obstruction in the lumen or cause a colic condition secondary to hypersensitivity (13).

It should be kept in mind that *Enterobius vermicularis* infestation may be present in the patient's history in the preoperative period. A careful pathological examination to be made in the post-operative period will have important contributions to the treatment process of the patient and his/her relatives.

Anthelmintic treatment and laboratory examinations should be performed in all family members in a patient with *Enterobius vermicularis* infestation, because there is a risk of transmission among the family members of the patient.

Enterobius vermicularis is a rare cause in the etiopathogenesis of AA, and antiparasitic treatment should be planned for the patients and their relatives living in the same house, who have positive parasitic examinations in the postoperative period.

Informed Consent: Obtained.

Peer-review: Externally peer reviewed.

Authorship Contributions

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

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

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

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Original Image

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Bezmialem Science 2021;9(2):234-5

Spontanous Rupture of Renal Pelvis Due to Bladder Globe

Renal Pelvisin Mesane Globuna Bağlı Spontan Rüptürü

Ayşe Serra ARTAN[1], Meltem GÜRSU[1], Rasul SHARIFOV[2]

[1]Bezmialem Vakıf Univeristy Faculty of Medicine, Department of Nefrology, İstanbul, Turkey
[2]Bezmialem Vakıf University Faculty of Medicine, Department of Radyology, İstanbul, Turkey

Keywords: Bladder globe, postrenal acute kidney injury, renal pelvic rupture
Anahtar Sözcükler: Mesane globu, postrenal akut böbrek yetersizliği, renal pelvik rüptür

A 55-year-old man presented to the emergency clinic due to difficult urination and confusion. A suprapubic mass was palpated during examination. Urea (452 mg/dL) and creatinine (21.3 mg/dL) levels were elevated. Non-contrast enhanced computed tomography (CT) scan revealed bilateral hydronephrosis and dilated ureters. Within a few hours after a Foley catheter insertion, 4,500 mL of urine was drained. At the 24th hour of admission, the postrenal acute kidney injury improved rapidly with serum creatinine and urea levels declining to 1 mg/dL and 58 mg/dL, respectively. On CT-urography, free passage of contrast media from the pelvis renalis to around the left kidney was detected. It is interpreted as rupture of the renal pelvis (Figure1 A and B).

Figure 1. Free fluid around the kidney seen at CT-urography scan on coronal and axial views
CT: Computed tomography

Address for Correspondence: Ayşe Serra ARTAN, Bezmialem Vakıf Univeristy Faculty of Medicine, Department of Nefrology, İstanbul, Turkey
E-mail: ayseserra@gmail.com **ORCID ID:** orcid.org/0000-0002-6461-3178

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Spontaneous rupture of the renal pelvis is a rare condition, usually caused by ureteral calculi, ureteral instrumentation, tumors, and trauma (1-3). In this patient, the cause was assumed to be a bladder globe, thus, a double J-stent was inserted into the left ureter. The urodynamic examination revealed a hyposensitive and hypocompliant bladder with normal capacity. Prostate volume was 38 mL. The patient performed intermittent urinary self-catheterization and has a stable serum creatinine value.

Informed Consent: Obtained.

Peer-review: Externally peer reviewed.

Authorship Contributions

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

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

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Review

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Current Terminology of Pelvic Floor Dysfunctions

Kadınlarda Pelvik Taban Bozukluklarının Güncel Terminolojisi

Büşra YILMAZ, Ergül ASLAN

İstanbul University-Cerrahpaşa Florence Nightingale Faculty of Nursing, Department of Women's Health and Gynecologic Nursing, İstanbul, Turkey

ABSTRACT

Pelvic floor disorders (PFD) affect the quality of life of millions of people worldwide. PFD, which increase in frequency with advancing age, affect not only the individual with the symptoms but also the family, caregivers and the society. Increased diagnostic methods and applications in treatment cause complexity in symptom terminology. For this reason, international organizations dealing with PFD have been working for updating definitions and/or adding new definitions for many years. Accordingly, the symptoms of PFD are; urinary, pelvic organ prolapse, sexual dysfunction, lower urinary tract pain and/or other pelvic pain and anorectal dysfunction. In the diagnosis and treatment stages of PFD and in studies, expressing the symptoms in a common language is very important for achieving standardization. Standardization in terminology will provide consensus and increase the success of treatment and prevention of PFD symptoms. The aim of this review is to examine the standardized current terminology of the symptoms of PFD in the light of the literature.

Keywords: Pelvic floor disorder, symptom, standardized terminology, female

ÖZ

Pelvik taban bozuklukları (PTB) dünya genelinde milyonlarca kişinin yaşam kalitesini etkiler. İlerleyen yaşla sıklığı artan PTB yalnızca semptomu olan bireyi değil ailesini, bakım verenleri ve toplumu etkiler. Tanılama yöntemlerinin ve tedavide uygulamaların artması semptom terminolojisinde de karmaşıklığa sebep olmaktadır. Bu sebeple PTB ile ilgilenen uluslararası kuruluşlar uzun yıllardır tanımların güncellenmesi ve/veya yeni tanımların eklenmesi amacıyla çalışmalar yürütmektedir. Bu doğrultuda PTB semptomları; üriner, pelvik organ prolapsusu, cinsel işlev bozukluğu, alt üriner sistem ağrısı ve/veya diğer pelvik ağrı ve anorektal disfonksiyon olarak gruplandırılmıştır. PTB'nin tanı ve tedavi aşamalarında ve araştırmalarda semptomların ortak bir dille ifade edilmesi standardizasyon sağlanabilmesi açısından oldukça önemlidir. Terminolojide standardizasyon fikir birliğini sağlayarak pelvik taban bozukluğu semptomlarının tedavisi ve önlenmesinde başarıyı artıracaktır. Bu derlemenin amacı PTB semptomlarının standardize güncel terminolojisini literatür doğrultusunda incelemektir.

Anahtar Sözcükler: Pelvik taban bozukluğu, semptom, standardize terminoloji, kadın

Address for Correspondence: Büşra YILMAZ, İstanbul University-Cerrahpaşa Florence Nightingale Faculty of Nursing, Department of Women's Health and Gynecologic Nursing, İstanbul, Turkey
E-mail: ylmzbsr678@gmail.com **ORCID ID:** orcid.org/0000-0001-6937-5351

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Introduction

Urinary, genital and intestinal systems located in the pelvic floor are intricately linked with each other in terms of anatomical structure and function. The striated muscles of the pelvic floor together with their fascial connections prevent the displacement of the pelvic organs, maintain urinary and fecal control, provide dynamic support, increase satisfaction in sexual life, and create a driving force in the expulsion phase of normal birth (1). The pelvic floor, which is desired to continue its life-long functions without any problems, may be insufficient to maintain its function for some reasons. Female gender, Caucasian race, low education level, excessive caffeine and cigarette consumption, obesity, difficult and traumatic birth, advanced age and postmenopausal period are among the main factors that disrupt the pelvic floor structure. Other factors that disrupt the pelvic floor muscle structure are chronic cough, chronic constipation, multiple sclerosis, diabetes, joint problems, connective tissue weakness, previous pelvic surgery, hysterectomy and urinary tract infection. The most important features of pelvic floor disorders (PFD) are the pelvic floor muscles being loose or tense than normal, pelvic pain and increased or decreased sensitivity. In the clinic, symptoms are grouped by associating them with complaints such as urinary/fecal incontinence, defecation problems, pelvic organ prolapse (POP), sexual dysfunction, chronic pelvic pain, and vulvodynia (2). Mortality rates associated with PFD are not high, but problems such as POP, pelvic organ dysfunction, dyspareunia, back pain, sexual dysfunction and decreased sexual satisfaction cause a decrease in the quality of life of women (3). In addition, PFD treatment brings a significant financial burden for individuals and public resources. It is estimated that more than one billion dollars are spent annually on the treatment of PFDs in the United States of America (4). Although PFDs are common all over the world, they cannot be adequately addressed due to both the delay in the admission of the patient and the lack of standard criteria for diagnosis (5). Increasing and complexity of definitions, diagnosis and treatment methods related to the symptoms of PFDs increased the need for new terminologies. Lack of standardization in the diagnostic criteria of PFDs and the use of their own definitions by centers may cause ambiguity in terminology, confusion and interdisciplinary communication gaps (6). It is very important to standardize the definitions of PFD symptoms in the diagnosis and treatment process of PFD and in studies conducted. This standardization is beneficial in providing language and notion consensus, and increases the success in diagnosis, treatment, and prevention of symptoms in PFD (5). The International Continence Society has pioneered the standardization of the definitions of PFD symptoms for many years. The terminology reports of the Standardized Terminology Committee published in 1988 and 2002 are among the first examples in this field (7,8). As a result of the joint studies of the International Urogynecological Association and the International Continence Association, a joint report on the terminology for female pelvic floor dysfunction was published in 2010 (9). As a result of the joint efforts of the International Consultation on Urological Diseases and the International Continence Association, the terminology of symptoms, diagnosis and treatment processes related to urinary incontinence and fecal incontinence were updated with the title of "incontinence" in 2017 (10).

The purpose of this review is to examine the current standardized terminology of PFD symptoms in accordance with the literature.

Standardized Terminology for Symptom Groups of Pelvic Floor Disorder

Symptom is a sign of a health problem or disease perceived by the woman as a deviation from normal in structure, function, perception or experience. Symptoms either occur spontaneously or are subjective data reported by the individual and/or the caregiver. Symptom groups are urinary symptoms, lower urinary tract pain and/or other pelvic pain, urinary tract infection, POP, sexual dysfunction, and symptoms of anorectal dysfunction (9,11). While some definitions remained the same in the updating phase of the terminology of PFD symptoms in the literature, some definitions were highlighted with the expressions "new", "updated" or "changed". Table 1 includes definitions regarding the current terminology of PFDs in women.

Conclusion

Increasing diagnosis and treatment methods in PFDs have caused the symptom terminology to become complex. For this reason, organizations such as the International Continence Association, the International Urogynecology Association, the European Association of Urology, the American Urology Association and the Canadian Urology Association carry out studies and publish reports on updating and standardizing the terminology of pelvic dysfunction symptoms. The use of common terminology in expressing the symptoms of PFDs is very important in terms of standardization. In this review, which deals with the current terminology of pelvic dysfunction symptoms, existing, newly added, amended and updated definitions of symptoms are included. These definitions will be added in future studies and will play a facilitating role in the understanding of the terminology to be updated.

Table 1. Definitions for current terminology of pelvic floor disorders in women

1. Urinary symptoms: Urinary symptoms are divided into three groups as those related to urine storage, those related to urinate, and post-micturition symptoms (11).	
1.1. Storage symptoms: They are the lower urinary tract symptoms that occur during the storage phase of the bladder (New) (11).	
1.1.1. Increased urinary frequency: It is the occurrence of urinary sensation more frequently than is considered normal by the individual/caregivers. Time of day and number of urination are not specified (New) (11).	**Increased daytime urinary frequency:** It is the expression that urination occurs more frequently during waking hours than is considered normal by the individual/caregivers (Changed) (11).
	Nocturia: It is one or more waking complaints for the need to urinate. The patient continues to sleep after each urination. This symptom should be measured using the bladder diary (Same) (11).
Polyuria: It is defined as a significantly higher amount of urine within 24 hours than its previous state (New) (12).	**Diurnal polyuria:** It is markedly higher amount of daytime urine compared to its previous state (New) (12).
	Nocturnal polyuria: It is significantly higher amount of nighttime urine compared to the previous situation (New) (12).
Sensory symptoms **1.1.2. Bladder filling/sensory symptoms:** They are abnormal findings during bladder filling (New) (13).	**Increased bladder filling sensation:** It is the complaint of bladder filling sensation being more intense and long-lasting than before. This is different from urgency in that it can be delayed in a busy state despite the feeling of bladder filling sensation (Modified) (13).
	Urgency: It is a difficult, sudden and compelling urge to urinate (Same) (13).
	Reduced bladder filling sensation: The feeling of bladder filling is less intense and longer in duration than before (Changed) (11).
	Absent bladder filling sensation: It is the coexistence of both the lack of bladder filling sensation and the inability to empty the bladder completely (Same) (7).
	Non-specific (atypical) bladder filling sensation (bladder dysesthesia): It is a complaint of abdominal distension, vegetative (involuntary) nausea, vomiting, pallor, or abnormal bladder filling sensation such as spasticity (7).
	Urinary incontinence symptoms
	Urinary incontinence: It is involuntary urinary incontinence (Same) (11).
	Urgency urinary incontinence: It is involuntary urinary incontinence associated with sudden urgency (Same) (11).
	Stress urinary incontinence is involuntary incontinence while coughing, sneezing, including sportive activities. In some languages, the expression "activity-related incontinence" may be preferred in order to prevent confusion with mental stress (Same) (11).
	Mixed urinary incontinence: It is the coexistence of urinary incontinence complaints due to stress and sudden urgency (Same) (11).
	Enuresis: It is an intermittent complaint of urinary incontinence while asleep (Changed) (12).
	Continuous urinary incontinence: It is involuntary continuous incontinence (Changed) (12).
	Insensible urinary incontinence: It is the situation in which the individual knows that urinary incontinence has occurred but does not realize how and when it has occured (Changed) (12).
	Postural urinary incontinence: The complaint of urinary incontinence during posture or position change, for example, urinary incontinence in the lying or sitting position (New) (12).
	Disability associated incontinence: It is urinary incontinence caused by inability to reach the toilet in time due to orthopedic, neurological and/or mental disorders (New) (14).
	Overflow incontinence: It is urinary incontinence associated with excessive filling of the bladder, although it does not have a defined cause (New) (14).
	Sexual arousal incontinence: It is involuntary incontinence during sexual arousal, foreplay, and/or masturbation (New) (14).
	Climacturia: It is involuntary incontinence during orgasm (New) (14).
	Overactive bladder syndrome: It is a sudden sensation of urgency, frequent urination during the day, nocturia at night, without urinary tract infection or other detectable pathologies. Complaints are not necessarily accompanied by urinary incontinence (Changed) (11).
1.2. Voiding symptoms: The presence of lower urinary tract symptoms during the voiding phase (Experienced during urination) (New) (15).	

Table 1. Continued

Hesitancy: It is a complaint of delay in initiating urination (Modified) (9).
Paruresis "bashful" or "shybladder": It is a problem in initiating urination when next to other people, although there is no difficulty in urinating while alone (New) (16).
Episodic inability to void: It is occasional difficulty in initiating urination despite attempts such as increasing abdominal pressure, valsalva maneuver and/or suprapubic compression (New) (9).
Slow urinary stream: It is when the flow of urine becomes slower than before or the realization that the flow of urine is slow compared to other people (Same) (9).
Intermittency: It is the stopping of urine flow one or more times and starting again during urination (Same) (11).
Terminal dribbling: It is the transformation of urine flow to drip in the last part of urination (Modified) (11).
Spraying (splitting) of urinary stream: It is the spraying of urine rather than direct full flow while urinating (11).
Position-dependent voiding: It is the individual's adoption of unique positions such as a sitting position to urinate (New) (11).
D**ysuria:** It is sensation of pain, burning and other symptoms during urination (Changed) (11).
Stranguria: It is generally characterized by pain-related, slow, difficult and spasmodic (drop by drop) urination (New) (11).
Hematuria: It is the appearance of blood in the urine when starting to urinate, during or at the end of urination.
Pneumaturia: It is the sensation of gas/air presence in the urethra during or after urination (New) (11).
Fecaluria: It is the presence of feces in the urine (New) (11).
A**lbiduria (Chyluria):** It is the presence of chilus (pale/white, milky liquid) in the urine (New) (11).
Urinary retention: It is the inability to empty the bladder completely (New) (11).
Acute urinary retention: It is generally characterized by a painful bladder, and the inability to urinate despite intense effort even though the bladder is full (New) (11).
Chronic urinary retention: It is a chronic/recurrent feeling of inability to empty the bladder. This situation may cause urinary incontinence and bladder globe (New) (11).
1.3. Post-voiding symptoms: They are lower urinary tract symptoms experienced after urinating (New) (16).
Feeling of incomplete bladder emptying: It is the feeling that the bladder cannot be emptied completely after urinating (Changed) (11).
Need to immediately re-void "encore or double voiding": It is the need to urinate again immediately after urinating due to interruption of urine flow (Changed) (11).
Post-voiding incontinence: It is involuntary urinary incontinence after the completion of urination (New) (11).
Post-micturition urgency: It is the feeling of urgency after urinating (New) (11).
2. Lower urinary tract pain and/or other pelvic pain
Pain: It is a very uncomfortable feeling defined by the patient as pressure or discomfort. Pain is defined according to region, type, frequency, duration, and factors that increase or decrease pain (Changed) (17).
Bladder pain: It is generally characterized by suprapubic or retropubic pain as a result of pressure caused by bladder filling. Pain may persist after the bladder is emptied or relief can be achieved (Modified) (7).
Urethral pain: It is a complaint of pain, pressure or discomfort in the urethra before, during and/or after urination (Modified) (7).
Pelvic pain: It is a complaint of pain, pressure, or discomfort related to the pelvis. Pain has no direct connection with the bladder, urethra or perineal region (Same) (7).
Anorectal pain symptoms: They are complaints of pain, pressure, or discomfort, especially when defecating or straining. Pain can also occur at any time (New) (18).
Pain during straining/defecation: It is feeling pain during defecation or straining (Same) (18).
Inflammatory anorectal pain: It is pain in the anorectal region characterized by burning (New) (18).
Non-inflammatory anorectal pain: It is blunt anorectal pain (due to pain in the rectal area, levator ani syndrome, pudendal nerve compression) (New) (18).
Coccygeal pain (coccydynia): It is a complaint of pain, pressure or discomfort in the coccygeal region (New) (18).
Pudendal pain (neuralgia): It is a complaint of pain, pressure or discomfort caused by infection of the pudendal nerve (Changed) (18).
Chronic pelvic pain syndrome: It is characterized by persistent pain for more than six months or recurrent attacks of abdominal / pelvic pain. There is a feeling of hypersensitivity and discomfort in the elimination changes and may be accompanied by sexual dysfunction (Same) (18).

Table 1. Continued

3. Urinary tract infection	
Symptoms of acute urinary tract infection: Symptoms of increased bladder sensitivity, urgency, increased urination frequency and dysuria accompanied or not accompanied by sudden urinary incontinence may cause urinary tract infection. The presence of some microorganisms in the urine and evidence of pyuria are required for the confirmation of urinary tract infection (Modified) (11).	
Recurrent urinary tract infections: Diagnosis of at least 2 urinary tract infection in the last 12 months in the presence of symptoms in the history. In order to diagnose a new urinary tract infection, previous urinary tract infections must be treated (Modified) (11).	
Urethral discharge: It is pus, mucus or bloody discharge coming from the urethral meat (New) (11).	
4. Pelvic organ prolapse (POP) Symptoms	
Findings expressed differently from normal in terms of feeling, structure or function in the area where the pelvic organs are located by the woman. Symptoms are aggravated by prolonged standing or after exercise, such as when gravity is effective, and decreases when lying on the back. Prolapse is more pronounced in conditions where abdominal pressure increases, such as defecation (9).	
Vaginal bulging: It is a feeling of "something coming down" or bloating in the vagina. The woman may state that she can realize the swelling by feeling it directly with her hand or see it with the help of a mirror (Same) (9).	
Pelvic pressure: It is the feeling of increased weight or pulling downward in the suprapubic region and/or pelvis (Same) (9).	
Bleeding, discharge, infection: Presence of vaginal bleeding, discharge or infection due to prolapse ulceration (Same) (9).	
Low backache: It is the complaint of temporary pain in the sacral or back region associated with pelvic organ prolapse (Same) (9).	
5. Symptoms of anorectal dysfunction: They are grouped as incontinence, storage, sensory, during or after defecation, prolapse, pain, sexual dysfunction and other anorectal symptoms in the anorectal region (Same) (14).	
5.1. Anorectal incontinence symptoms	**Anal incontinence:** It is involuntary stool or gas leakage (Same) (14).
	Fecal incontinence: It is involuntary incontinence of solid or liquid fecal matter (Same) (14).
	Flatus Incontinence: It is involuntary gas leak (Same) (14).
	Double incontinence: Coexistence of anal and urinary incontinence complaints (New) (14).
	Coital fecal incontinence: It is fecal incontinence during vaginal penetration (New) (14).
	Passive fecal leakage: It is involuntary incontinence of solid or liquid stool without warning/feeling of contamination (New) (14).
	Overflow fecal incontinence: It is leakage of stool in the intestine (New) (14).
5.2.Anorectal storage symptoms	**Increased daytime defecation:** It is the statement that defecation occurs more frequently during waking hours than is considered normal by the woman (New) (14).
	Nocturnal defecation: It is the interruption of sleep one or more times with the need to defecate (New) (14).
	Fecal urgency: It is a sudden and compulsive urge to defecate and difficulty in delaying defecation (Same) (14).
	Fecal urgency warning time: It is the time from the first feeling of urgency to voluntary defecation or fecal incontinence (Same) (14).
	Fecal/flatal urgency incontinence: It is fecal urgency and involuntary stool/gas leakage (Same) (14).
	Tenesmus: It is the desire to empty the bowels with frequent pain, cramping, and a feeling of tension in the rectum without stool (New) (14).
	Coital fecal urgency: It is the sensation of bowel movement during vaginal intercourse (New) (14).

Table 1. Continued

5.3. Anorectal sensory symptoms	**Diminished rectal sensation:** It is the decrease in sensation in the rectum (Same) (14).
	Increased rectal sensation: It is the increase in the desire to defecate during rectal filling compared to the previous (New) (14).
5.4. Symptoms during or after defecation	**Constipation:** It is infrequent bowel movements and/or absence of them. It is the need for frequent straining for defecation or digitization for defecation (Updated) (14).
	Slow transit: It is the decrease in bowel movements due to the delay in the arrival of stool to the rectum (Same) (14).
	Obstructed defecation: It is difficulty during defecation (Same) (14).
	Feeling of incomplete bowel evacuation: It is the feeling that the bowels are not emptied completely after defecation and the desire to defecate again (Same) (14).
	Straining to defecate: It is exerting intense effort with abdominal straining or valsalva to initiate, maintain or improve defecation (Same) (14).
	Sensation of blockage: It is the presence of complaints suggesting anorectal occlusion (New) (14).
	Digitation: It is the finger manipulation of the rectum or vagina to assist in the evacuation of stool contents (New) (14).
	Rectal digitation: It is to support the rectum with a finger to help defecation (Same) (14).
	Vaginal digitation: It is the use of thumbs or other fingers through the vaginal route to aid defecation (Same) (14).
	Splinting: It is to manually support the perineum or buttocks, usually with thumbs or fingers, to aid defecation (New) (14).
	Post defecatory soiling: It is contamination/spotting that occurs after defecation (New) (14).
5.5. Symptoms of anorectal prolapse	**Anorectal prolapse:** It is a complaint of a "bulge" or feeling of "something descending" through the anus/rectum. The woman can either feel the swelling directly by hand or see it with the help of a mirror (Updated) (10).
5.6. Anorectal pain symptoms	**Pain during straining/defecation:** It is the feeling of pain during defecation or straining (Same) (10).
	Inflammatory anorectal pain: It is a burning and/or itching sensation in the anorectal region caused by fissure, inflammation or sepsis (Same) (10).
	Non-inflammatory anorectal pain: It is a complaint of blunt anorectal pain due to reasons such as proctalgia fugax, levator ani syndrome, pudendal neuralgia (Same) (10).
5.7. Symptoms of anorectal sexual dysfunction	**Symptoms of sexual dysfunction:** They are the different feelings and/or functions of the woman felt during sexual activity (Same) (10).
	Female sexual dysfunction: It is pain or dysfunction during sexual desire, arousal, orgasm or penetration (Same) (10).
	Dyspareunia: It is a continuous or recurrent complaint of pain/discomfort associated with vaginal penetration attempt or full penetration (Same) (9).
5.8. Superficial (introital) dyspareunia: It is the feeling of pain or discomfort at the vaginal entrance during sexual intercourse (Same) (9).	
5.9. Deep dyspareunia: In severe penetration, it is the complaint of pain/discomfort in the middle or upper vagina (Same) (9).	

Table 1. Continued

5.10. Obstructed intercourse: It is a situation in which vaginal penetration is prevented due to any obstruction (Same) (9).	
5. 11. Vaginal laxity: Complaints caused by the laxity of the vagina (Same) (9).	**Anal intercourse:** It is the penetration of the erectile penis into the anus (New) (19).
	Other anal sexual practices with body parts: They are the stimulation of the anus and/or rectum by the individual herself or her partner in order to obtain sexual pleasure with other body parts (e.g. finger, fist) other than the penis (Same) (19).
	Other anal sexual practices with inanimate objects: They are the stimulation of the anus and/or rectum by the individual herself or her partner to obtain sexual pleasure with inanimate objects (e.g. dildo) (Same) (19).
	Anodyspareunia: It is a complaint of pain or discomfort occurring during or during anal penetration attempt (New) (19).
	Anal laxity: It is a feeling of laxity in the anal tone (New) (19).
5.12. Other anorectal symptoms	**Rectal bleeding/mucus:** It is blood or mucus coming from the rectum (Same) (19).
	Perianal itching/pruritus: It is the sudden presence of itching in the perianal area (New) (19).
	Flaturia: It is gas leakage from the urethra (New) (19).
	Fecaluria: It is fecal incontinence from the urethra (New) (19).
	Vaginal flatus/feces: It is leakage of gas or stool from the vagina (New) (5).

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Review

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Psychological First Aid and Practice Principles in the Coronavirus (COVID-19) Outbreak Process

Koronavirüs (COVID-19) Salgın Sürecinde Psikolojik İlk Yardım ve Uygulama İlkeleri

Nurhayat KILIÇ BAYAGELDİ

Artvin Çoruh University Faculty of Health Sciences, Department of Psychiatric Nursing, Artvin, Turkey

ABSTRACT

The coronavirus (COVID-19) epidemic, which has recently become common in the world, caused many people to die. The World Health Organization has declared COVID-19 as a pandemic. Pandemic situations disrupt community life by creating collective stress in society. It is known that after the pandemics, there is an increase in the symptoms of anxiety, depression, stress and post-traumatic stress disorder, and the symptoms gain resistance and turn into permanent mental disorders. Psychosocial interventions are recommended using internet technology to prevent the negative consequences of pandemics from turning into permanent mental disorders. At this point, psychological first aid (PFA) is the first and emergency psychosocial support intervention. PFA helps individuals to recover by activating their own coping skills with empathic and pragmatic approaches. PFA can be given using internet technology as physical distance is mandatory during virus outbreaks. In this review study, the principles and practices of PFA that can be applied in the case of COVID-19 epidemic, which has been seen all over the world recently and caused the death of many people. This review article is thought to guide aid providers in epidemic situations and contribute to the development of aid skills.

Keywords: Coronavirus, psychological first aid, psychosocial interventions

ÖZ

Son zamanlarda dünyada yaygın görülmeye başlayan koronavirüs (COVID-19) salgını çok sayıda insanın yaşamını yitirmesine neden olmuştur. Dünya Sağlık Örgütü COVID-19 enfeksiyonunu pandemi olarak ilan etmiştir. Pandemiler, toplumda kolektif stres yaratarak toplum yaşamını sekteye uğratabilmektedir. Yaşanan pandemiler sonrasında toplumda anksiyete, depresyon ve travma sonrası stres bozukluğu belirtilerinde artışlar olduğu ve belirtilerin direnç kazanarak kalıcı ruhsal bozukluklara dönüştüğü bilinmektedir. Pandemilerin olumsuz sonuçlarının kalıcı ruhsal bozukluklara dönüşmesinin engellenmesi için internet teknolojisini kullanarak psikososyal müdahalelerin yapılması önerilmektedir. Bu noktada ilk ve acil psikososyal destek müdahalesi psikolojik ilk yardımdır (PİY). Psikolojik ilk yardım empatik ve pragmatik yaklaşımlar ile bireylerin kendi baş etme yeteneklerini harekete geçirerek iyileşmelerine yardımcı olur. Psikolojik ilk yardım virüs salgınları sırasında fiziksel mesafe zorunlu olduğu için internet teknolojisi kullanılarak verilebilmektedir. Bu derleme çalışmasında son günlerde tüm dünyada görülen ve çok sayıda insanın ölümüne neden olan COVID-19 salgını durumunda uygulanabilecek psikolojik ilk yardım ilke ve uygulamaları açıklanmaktadır. Bu derleme yazısının, salgın durumlarında yardım sağlayıcılarına rehberlik edeceği ve yardım becerilerinin gelişimine katkı sağlayabileceği düşünülmektedir.

Anahtar Sözcükler: Koronavirüs, psikolojik ilk yardım, psikososyal müdahaleler

Address for Correspondence: Nurhayat KILIÇ BAYAGELDİ, Artvin Çoruh University Faculty of Health Sciences, Department of Psychiatric Nursing, Artvin, Turkey
E-mail: nurhayatkilic@yahoo.com **ORCID ID:** orcid.org/0000-0002-2790-5227

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Introduction

In every period of history, there were many epidemic diseases that caused societies to be at risk of mass death. The coronavirus pandemic, which has recently started to be seen in Wuhan, China and spread rapidly to many countries, has caused a large number of people to die. At this point, World Health organization (WHO) has declared coronavirus disease-19 (COVID-19) as a pandemic (1-3). It is known that pandemics have psychosocial effects on the individual, society and at the international level (4,5). Studies have reported that after the restriction measures in the pandemic and pandemic process, there are increases in the symptoms of anxiety, depression, anger bursts and post-traumatic stress disorder in the society, and even after the pandemic is over, the symptoms gain resistance and turn into permanent mental disorders (4-12). It is known that psychological interventions are recommended and performed using telephone and web-based online applications and internet technology in order to cope with the psychological problems experienced by individuals during the pandemic process (4,7). Psychological support interventions can help reduce anxiety in the early stages of the pandemic and can also be useful in dealing with the negative effects of situations such as witnessing death and loss of a loved one in the later stages (13). At this point, experts have stated that the first and immediate psychosocial support intervention is psychological first aid (PFA) after disasters, including major pandemics (14-22). PFA interventions are humanitarian and supportive interventions offered to individuals who are in pain and need support and assistance (20,22). PFA is a type of support that can be provided by individuals who will not harm the affected individuals in safe places where respect and privacy are provided (20-23). PFA can be done by phone or online to reduce the spread of viruses during the COVID-19 pandemic. When it is done face to face by healthcare professionals or other individuals in exceptional conditions, physical distance should be maintained and security should be provided (24,25). The purpose of PFA interventions is to help individuals feel calmer, better cope with their difficulties, improve their ability to manage the situation and make informed decisions by reducing the initial distress experienced in disaster situations. PFA can help normalize anxiety and other feelings that individuals experience. It can also contribute to the development of feelings of security, calmness and hope (20-26).

PFA is not a diagnosis, an integrated mental health treatment, or any of the therapies and psychological interpretations performed by professionals. PFA is an early psychosocial intervention approach applied after disasters. PFA is a supportive and practical aid intervention that helps reduce the distress experienced by individuals after events causing serious crisis. PFA does not evaluate serious mental health problems and long-term recovery difficulties of all individuals affected by the disaster and does not include any treatment element. It focuses on understanding the reactions of individuals who have experienced the disaster and have been affected by it. Therefore, it stimulates the individual's ability to cope with empathic and pragmatic approaches and enables individuals to recover (22,25,27-30).

In recent years, various PFA guidelines and models have been published in many disasters, including the COVID-19 pandemic (20,22,24,25,31-36). At the same time, studies have also been conducted to prove that PFA interventions are beneficial and effective psychosocial support interventions for both affected individuals and PFA providers in many catastrophic events in the COVID-19 pandemic (28-30,35-41). Cheng et al. (35) stated that online psychological support interventions were effective for inpatients in their study which they performed to provide PFA to hospitalized patients during the COVID-19 pandemic. Francis et al. (36) stated that both PFA providers and PFA takers were satisfied with the online service as a result of the PFA support given by phone for 4 weeks during the COVID-19 pandemic process in Malaysia, in line with the principles set by the International Federation of Red Cross and Red Crescent Societies (look, listen, link). They stated that PFA interventions were important during the pandemic period because of their wide applicability with short training (36).

In this review, the PFA implementation principles that could be applied in the case of the COVID-19 pandemic, which has recently spread all over the world and caused the death of many people, were explained in line with the principles set out by WHO and the International Federation of Red Cross and Red Crescent Societies. In this review, online PFA interventions for adults were discussed. It is thought that this review can guide PFA providers in pandemic situations and contribute to the development of assistance skills.

Psychological First Aid in the Coronavirus Pandemic

PFA is evidence-based interventions that focus on the mental health of individuals and address the psychosocial needs of individuals during the COVID-19 pandemic (24,26,42). PFA interventions can improve individuals' ability to cope with an event by increasing individual resilience after trauma (24,35). For this reason, it is important to apply PFA during the pandemic and to teach individuals methods of coping with stress (13,43).

During the pandemic; quarantined individuals, individuals seeking information and support, healthcare professionals, individuals recovering from COVID-19 or losing their relatives, risky groups who may need special assistance, individuals with mental health or substance abuse problems may wish to receive an online PFA (25). It is important to determine the appropriate communication tools, hours and assistance services for effective online assistance. Tools to be used for communication in PFA interventions should be safe, easy to use and have features that can be repaired in a short time in case of technical problems (24,25).

In online PFA interventions, there are individuals who need support, and PFA providers. For PFA interventions, PFA providers must first be identified and trained before starting assistance service. Working files should be prepared for PFA providers and their work should be followed. PFA providers should be able to evaluate their readiness to provide PFA support during the pandemic (25). In order to be able to help the individuals in need in the best way, the PFA provider must be physically and emotionally well-equipped (20,22,24).

During the COVID-19 pandemic, interviews within the scope of PFA can be conducted by phone or online in order to prevent the spread of the virus (24,25). During the COVID-19 pandemic, PFA providers may need to work from home due to curfew or restrictions (25). In this case, it is important to choose a quiet room before answering a call in order to harmonize the physical environment and not to be disturbed (24,25). The PFA provider should also ensure that confidentiality and privacy are protected during the call. Since it is known that individuals with mental and physical illnesses can call in 24/7 call centers, especially at night, PFA providers who provide night assistance services may need more training and inspection (24,25). Guidelines should be set for answering calls during the COVID-19 pandemic. If it is necessary to record any statistical information such as age or gender, verbal consent is required from the caller. Contact information about health services and health officials should be determined in detail (25). Official sites where up-to-date and accurate information about the COVID-19 pandemic can be accessed, should be known (24). The procedures regarding when and in which situations individuals with severe mental problems should be referred during the pandemic should be clear (25). Since psychoeducational materials can be sent to the callers when necessary, documents containing up-to-date and accurate information should be prepared. Practices should be prepared for PFA providers to protect their health in the face of difficult calls (24-25). The PFA provider should know the implementing regulations and their roles. It is also important to evaluate the aid service process by holding meetings by team members (20,22,25).

The International Red Cross and Red Crescent Societies (2020) published a guide that could be useful in the COVID-19 pandemic, and the basic principles of PFA were explained in the guide as: look, listen and link (25). In the principle of looking; current situation, individuals seeking support, risks, needs of affected individuals and reactions of individuals are addressed (20,25). In the principle of listening; the principles of starting a conversation, listening to the individual effectively, accepting the feelings experienced by the individual, calming the individual experiencing intense stress, asking questions about the individual‘s needs and concerns, and determining the appropriate solutions for the individual’s problems and needs are discussed (24,25). In the principle of linking; information is given about the principles of accessing the right information, encouraging people to connect with their loved ones and social supports, coping skills, and connecting with the special assistance services needed (24,25). All these principles of action provide guidance to the PFO provider in assessing a disaster situation and providing effective assistance to individuals (20,23). WHO stated that PFA providers should know all the stages while fulfilling 3 basic principles and they should help with a sense of responsibility (20).

The Principle of Looking for Assessment of Needs

In the COVID-19 pandemic period, the needs of the callers, the situation they are in and the real or perceived risks they experience are evaluated. It is important to plan remote support in this evaluation process. At this point, the PFA provider should know how to ask appropriate questions about the individual‘s needs and concerns, to listen actively, and to respond appropriately without judging the affected individual and creating a new trauma in the individual (20,22-25). They should have sufficient knowledge about effectively managing difficult calls when necessary. (25).

The PFA support can be provided by visual or audio communication tools. When using visual communication tools, it is necessary to communicate empathically, warmly and respectfully with a tone that makes individuals feel cared (25). During communication, it should be spoken clearly. Simple words should be used, jargon and technical terms should not be used. Be a good listener and make individuals feel listened to by giving feedback (20,22,24,31). Focus should be on what the callers want to tell and their needs should be determined. Individuals should be given the information they need in a realistic way. False promises and false assurances should not be given against unknowns. In the aid process; individuals should not be told about the stories or problems of others, and the personal problems and troubles of the assistant should not be stated (20,22,24,25,31).

Due to the pandemic, individuals may be intensely worried and can call PFA providers for support. In this case, the emotions experienced by the individuals should be accepted and should not be judged. Making individuals feel understood can reduce the negative emotion experienced. PFA providers can support the development of feelings of calmness and security in individuals by using a calm tone of voice. Being calm and showing understanding can help people under stress to feel understood, respected, and cared for (16,20,22,25,31). Questions should be asked openly during the PFA interview. Questions should not be asked consecutively and individuals' answers should not be interpreted. Non-judgmental language should be used during the PFA interview (24,25). If visual contact is used, simple visuals should be preferred for psychoeducational messages (24,25).

The Principles of Listening to be Used when Answering a Call

The PFA provider should be able to communicate compassionately with individuals and develop positive cooperation (22). The PFA provider should know well when and how to initiate contact with individuals, taking into account the readiness of the individuals. When starting the first contact, the PID provider should introduce herself/himself and indicate her/his position and institution (22,23,25,44). In order to address the caller at the first contact, his/her name may be asked. If the individual does not want to say his or her name, the PFA provider should not be insistent. The issues that the individuals are seeking for help should be determined. The PFA provider should be informed about the duration and subject of the call according to the needs of the individuals (25). During the calls, PFA provider helps individuals identify their problems, determines their urgent needs, determines urgent solutions to relieve the anxiety of individuals, and enables the individual to take action (22,23-25,31).

Since there are uncertainties about the virus, individuals who experience feelings of stress and fear should be explained that

their reactions are normal. It is known that correct and up-to-date information can calm people in case of disasters. At the same time, correct and complete information can improve the feelings of security and control in the individual (16,22,24,25). Therefore, the information they need should be conveyed to individuals in a realistic way and individuals should be directed to the right sources for information (24).

The PFA provider should talk to the individual about the way of management of emotions experienced during the pandemic. Usually, methods of coping with difficult situations can be learned (24,25). When necessary, information can be given about simple solutions about useful and harmful coping methods (16,22). Correct breathing and relaxation exercises may be recommended to reduce anxiety and calm down when callers need help with calming techniques (22,24,25,31). Giving information to the individual about stress reactions and teaching stress management can accelerate the return of the individual to a normal life (22,26,40). If the callers are quarantined or isolated, they can be asked what they are doing to protect their physical and mental health, and if necessary, the following recommendations can be given:

Being socially close while maintaining physical distance: In the process of physical distance and social isolation, it can be beneficial to be in contact with family members, business and social friends through e-mail, telephone and social media applications (24,25,45). Watching the same movies with loved ones, reading the same books, meeting in virtual meetings and having a virtual chat while drinking coffee or tea together can be beneficial (25).

Maintaining daily routines: It is useful to plan a daily routine, try to maintain it and make new planning in the coming days.

Setting goals and being active: Setting goals and achieving them increase the sense of control and competence. Goals should be in accordance with realistic conditions. Making a to-do list during the day and marking what has been done as the day progresses can increase the feeling of satisfaction. Things that the individual enjoys such as books to be read or written, music to listen to, coloring, knitting, learning a new language or skill, and relaxing are beneficial (25).

Scheduling time individually or together: It can be helpful to create a to-do list that can be performed solo and together. Activities such as reading aloud to each other, playing board games, watching TV, listening to radio, and taking care of children can be given as examples.

Adding humor to situations: Since smiling reduces anxiety and frustration, it can be helpful to use humor in situations.

Maintaining hope: Making the individual remember the values they believe in and helping them to own them can be helpful in preserving hope.

Using stress-coping techniques: Relaxation techniques can reduce stress levels and can also be useful for managing pain and emotional turmoil (22,25).

Accepting emotions: In stressful situations, many emotions such as anger, disappointment, anxiety, regret, and self-blame can be experienced. Acceptance of emotions may be beneficial, as experienced emotions are normal responses to an abnormal situation (24,25).

Principles of Linking, Referring and Ending a Call

In disaster situations, daily functionality of individuals may deteriorate in an advanced degree and individuals may need special assistance (16). If the sleep patterns of the callers are impaired, if their daily functionality is severely impaired due to the problems experienced, if they have lost control over their behavior, if they have thoughts of harming themselves and others, if they have intensive substance abuse, if they have existing chronic diseases, if they are in risky groups, if they are exposed to violence and sexual harassment, they may need special assistance. At this point, the PFA provider should direct individuals to the help services they need and to experienced employees who will provide effective service (20,22,25). While referring, the needs and problems of the individuals should be clearly summarized in line with the interviews made with the individuals (22,23,27). Directing individuals to appropriate help centers in line with their urgent needs can help develop a sense of hope in the individual (44).

Physical distance is important during a virus outbreak. However, social interaction is important in reducing the negative impact of trauma. For this reason, it may be asked how the callers communicate with their family, friends or other individuals in a virtual environment. At this point, individuals who are isolated, in quarantine or staying at home should be helped to contact individuals or groups that provide social support in the shortest way (24,25). Internet applications such as telephone, e-mail, skype and other social media tools can be important in providing communication (22,25,44).

Providing information to the individual about stress reactions and teaching stress-reducing methods accelerate the return of the individual to a normal life (22,44). Therefore, the PFA provider can provide information to the affected individual about stress reactions, post-traumatic psychological reactions, useful and harmful coping methods (22). The information given may be effective on individuals‘ stress and problem solving skills (44).

While continuing to talk using the connection action principle, individuals may be asked what they know about the virus and how they can find new information. In case of need, the individual can be directed to the right sources with up-to-date information. The caller can be asked about the problem he/she wants to state during the conversation process. If necessary, information can be given about current contact numbers, e-mail or web pages regarding health and social services to be admitted. During the termination phase, the interview can be summarized briefly and the interview is terminated by offering good wishes to the individual (24,25).

Looking Angry Callers, Listening to Them, and Linking with Them

In pandemic, individuals' inability to maintain their daily routine, decrease in social and physical contact with those around them, and a significant deterioration of their life routine may cause an uncomfortable feeling (45). As time passes and authorities impose severe restrictions, there may be an increase in calls from angry callers (20,45). In this case, PFA providers should be sufficient in proper communication with angry individuals (25). During the interview with the intensely angry individual, the PFA provider should not use sedative techniques at the beginning of the conversation. Active listening can be helpful in calls with angry callers. When the caller's speech is completed, it is important to give feedback that the PFA provider listens and understands. It is necessary to empathize and not judge (25). When expressing that what the caller has said is understood, a much softer tone of voice and words should be used, not with the tone and words used by the caller. At the same time, trying to express what the caller is saying in other words may help calm down (25). If the conversation progresses to the linking stage, it may be asked whether the caller has talked to other people who experience this situation, whether there is someone to help on social media, and whether the caller has ability to cope with difficult decisions (25). The topics discussed are briefly summarized in the termination phase and the conversation is concluded gently. In some cases, it may not be possible to end the conversation gracefully. In such a case, the PFA provider may terminate the interview by stating that he/she is sorry for not being able to benefit the individual, and by expressing that he/she will gladly help in future interviews and by thanking (25).

Conclusion

COVID-19 pandemic can cause traumatic effects in society due to many reasons such as widespread transmission potential, high mortality rate, restriction measures and unusual ways of protection (social distancing, isolation). The need for effective psychosocial interventions is increasing in disaster events that have the potential to cause traumatic effects in society, including the COVID-19 pandemic. At this point, PFA interventions are the first and important step of effective emergency psychosocial intervention. PFA intervention and knowledge are known to be beneficial for both PFA providers and affected individuals. At this point, it is recommended to conduct researches that prove the effectiveness of PFA intervention and knowledge during the COVID-19 pandemic. It is thought that the principles of assistance presented in this study will provide guidance to PFA practitioners during the pandemic.

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