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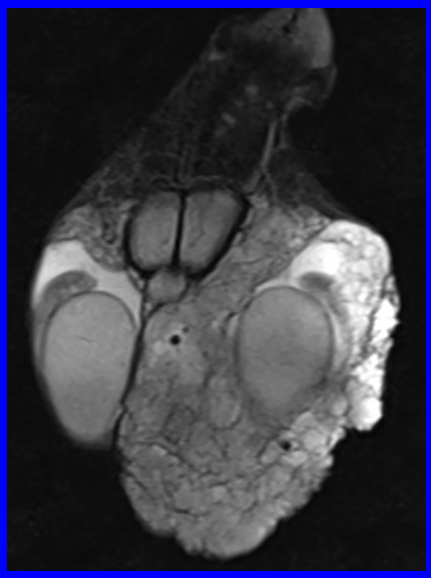

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Original Articles

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EDITORIAL

Dear Readers;

We are happy to be with you, our valued readers, in the last issue of 2024. We will have left behind a successful year and 4 issues with this issue. We have included publications from all fields of health. We will be with you again with intense effort and cooperation in the upcoming period, and we plan to get one step closer to our goals in the new year with the devoted work of our editorial board and referees.

We chose our cover image for this issue from the article titled "A Rare Case: Scrotal Hemangioma" by Coşkun et al.'s. In this article, which included MRI images of a 30-year-old male patient, it was stated that there was a testicular mass that had been present since birth, that it filled more than half of the left hemiscrotum on physical examination, that it was a painless, externally ecchymotic, approximately 10 cm vascular structure with palpation.

Other articles selected for the cover are as follows:

"Identification of CircRNA Complexes Acting on miRNA Sponges within the Exosome in Patients with Alzheimer's Disease: An In Silico Approach" by Kalkan Cakmak et al.,

"Ambulances Under Fire: A Cross-sectional Analysis of Terrorist Attacks on Ambulances and Their Medical Implications" by Kınık et al.,

"Efficacy Comparison of Ibuprofen 400 mg and 800 Mg in the Treatment of Renal Colic: Prospective Randomized Double-blind Clinical Study" by Dönmez et al.,

"Evaluation of Toxicity Associated with CAR-T Cell Therapy and Nursing Interventions" by Erdal et al.

We will be happy to be with you in our new issues in 2025. Writing your articles in good English next year will increase the likelihood of acceptance. We will continue our English editing service next year. I would like to thank our new assistant editors and section editors who joined us this year, and the new referees who were added to our staff. I would like to thank each one of them individually, stating that each of their contributions is very important to us.

Dear scientists and valuable readers, I would like to announce here that we will include one thesis study and/or student study in each issue in the new year.

I wish you all the best, until we meet in many new issues…

Kind regards,

Prof. Dr. Adem AKÇAKAYA
Editor in Chief

Original Article

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Investigation of the Chemical Composition and Antimicrobial Activity of Endemic *Seseli salsugineum* A. Duran and Lyskov Essential Oil

Endemik *Seseli salsugineum* A. Duran ve Lyskov Uçucu Yağının Kimyasal Bileşimi ve Antimikrobiyal Aktivitesinin Araştırılması

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ABSTRACT

Objective: The main purpose of this study was to determine the chemical compositions and antimicrobial activity of the essential oil from the aerial parts of the recently discovered endemic species *Seseli salsugineum (S. salsugineum)* A. Duran and Lyskov for the first time.

Methods: Essential oil from the aerial parts of *S. salsugineum* was isolated by using a Clevenger-type apparatus and the oil was analyzed by gas chromatography (GC) and GC-mass spectrometry (GC-MS), simultaneously. Furthermore, antimicrobial activity of the essential oil was tested against Gram-negative (*Pseudomonas aeruginosa* ATCC B888), Gram-positive (*Staphylococcus aureus* ATCC 6538, *Bacillus cereus* NRRL B-3711), and three fungal strains: *Candida albicans* ATCC 24433, *Candida parapsilosis* ATCC 22019 and *Candida krusei* ATCC 6258 by broth microdilution method. Chloramphenicol and amphotericin B were used as positive controls. Minimum inhibitory concentrations were determined.

Results: Dried aerial parts of *S. salsugineum* yielded 0.28% (v/w) essential oil. GC and GC-MS analyses resulted in the

ÖZ

Amaç: Yeni keşfedilmiş endemik bir tür olan *Seseli salsugineum (S. salsugineum)* A. Duran and Lyskov'un toprak üstü kısımlarından elde edilen uçucu yağın kimyasal bileşimi ve antimikrobiyal aktivitesinin ilk kez incelenmesi amaçlanmıştır.

Yöntemler: *S. salsugineum*'un toprak üstü kısımlarının Clevenger apareyi ile uçucu yağı elde edilmiş ve uçucu yağın bileşimi gaz kromatografisi (GK) ve GK-kütle spektrometresi (GK-KS) ile belirlenmiştir. Uçucu yağın antimikrobiyal aktivitesi mikrodilüsyon yöntemi ile Gram-negatif (*Pseudomonas aeruginosa* ATCC B888), Gram-pozitif (*Staphylococcus aureus* ATCC 6538, *Bacillus cereus* NRRL B-3711), ve üç fungus suşu: *Candida albicans* ATCC 24433, *Candida parapsilosis* ATCC 22019 ve *Candida krusei* ATCC 6258 suşlarına karşı çalışılmıştır. Pozitif kontrol olarak kloramfenikol ve amfoterisin B kullanılmış ve minimum inhibisyon konsantrasyonları belirlenmiştir.

Bulgular: *S. salsugineum* uçucu yağı verimi %0,28'dir (h/a). GK ve GK-KS analizinde uçucu yağın ana bileşenler olarak sabinen (%35,5), kessan (%10,5), α-pinen (%6,4) ve terpinen-4-ol (%5,0)

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ABSTRACT

characterization of sabinene (35.5%), kessane (10.5%), α-pinene (6.4%), terpinen-4-ol (5.0%) as main constituents. The essential oil was found to be effective against all tested strains (320-1280 µg/mL).

Conclusion: To the best of our knowledge, this study is the first report on the chemistry and biological activity of *S. salsugineum* essential oil.

Keywords: *Seseli salsugineum*, Apiaceae, essential oil, antibacterial activity, antifungal activity

ÖZ

bulunmuştur. Uçucu yağ test edilen tüm suşlara karşı (320-1280 µg/mL) antimikrobiyal aktivite göstermiştir.

Sonuç: Bildiğimiz kadarıyla, bu çalışma ile *S. salsugineum* uçucu yağının kimyası ve biyolojik aktivitesi ilk kez çalışılmıştır.

Anahtar Sözcükler: *Seseli salsugineum*, Apiaceae, uçucu yağ, antibakteriyel aktivite, antifungal aktivite

Introduction

The genus *Seseli* (Apiaceae) is represented by about 125-140 taxa in the world. It is widespread in Euro-Siberian and Eastern Mediterranean regions including Türkiye (1). The Flora of Türkiye comprises 13 taxa in Türkiye, including 8 endemic species (1-3). Recently a new *Seseli* species is identified. The newly described species, *Seseli salsugineum (S. salsugineum)* A. Duran and Lyskov is a narrow endemic species, confined to Lake Tuz area (Konya province) in Central Anatolia, Türkiye. It grows in salt marshes and salt steppes. It is an element of the Irano-Turanian phytogeographic region (4). Morphological features of *S. salsugineum* is described by some researchers (4-6).

This genus contains essential oil, coumarines, terpenoids and polyacetylenes (7). Essential oils and coumraines are major secondary metabolites of this genus and these may be responsible for their bioactivities (3,7). Seselinal, sesibiricol, sibirinol, sesibiricin, isosibiricin, osthol, coumurrayin, sesebrin, sesebrinol, sibiricin, imperatorin, bergapten, xanthotoxin, isopimpinellin and mexoticin were isolated from *Seseli* species (8). Sabinene, α-pinene, β-phellandrene, germacrene D and limonene were identified as major components in essential oils of *Seseli* sp. (9-11). It is widely used in traditional medicine due to its antibacterial, antifungal (3), insect repellent (3,12), emmenagogue, anti-bloating (12), anti-inflammatory (3,13), antinociceptive (13) anti-tumor (3,13-18), anti-rheumatic (19), and antioxidant (20) activities. It has importance due to the essential oil of the genus *Seseli* which is used in traditional medicine and has therapeutic properties especially antimicrobial activity (5).

The present study was designed to elucidate the chemical composition and antimicrobial activity of *S. salsugineum* essential oil.

Methods

Plant Material

Aerial parts of the recently discovered narrow endemic species *S. salsugineum* A. Duran and Lyskov was collected from near Lake Tuz (Türkiye. C4 Konya: Cihanbeyli, between Gölyazı-Lake Tuz, 9th kilometer, 923 m, salty marshes, 25.09.2011, Duran et al. (4) 9855 (Herbarium: HUB). The essential oil was obtained by water distillation for 3 h from 150 g air-dried material, using a Clevenger-type apparatus.

Gas Chromatography and Gas Chromatography Mass Spectrometry Analyses

Gas chromatography (GC) and GC-mass spectrometry (GC-MS) conditions were described previously (21). Identification of the essential oil components were carried out by comparison of their relative retention times with those of authentic samples or by comparison of their relative retention index to series of *n*-alkanes. Computer matching against commercial (Wiley GC/MS Library, MassFinder 4.0 Library), and in-house "Başer Library of Essential Oil Constituents" was built up by genuine compounds and components of known oils (22,23).

Antimicrobial Activity

Bacillus cereus NRRL B-3711 (NRRL-Agricultural Research Service Culture Collection), *Staphylococcus aureus* ATCC BAA 1026 (ATCC-American Type Culture Collection), *Pseudomonas aeruginosa* ATCC B888, *Candida albicans* ATCC 24433, *Candida parapsilosis* ATCC 22019, *Candida krusei* ATCC 6258 were used as test microorganisms.

The antimicrobial activity of the essential oil was evaluated by broth microdilution assay according to a modified Clinical and Laboratory Standards Institute method (25). Since this study was an *in vitro* study, ethics committee approval was not required. Mueller Hinton Agar (MHA), Mueller Hinton Broth (MHB) for bacteria, Potato Dextrose Agar (PDA) and Roswell Park Memorial Institute (RPMI) 1640 for fungi were provided and prepared by diluting with distilled water appropriately. Laboratory materials, mediums and contaminated materials used in antimicrobial activity experiments were sterilized in an autoclave at 121 °C under 1.5 atm pressure for 20 minutes. Bacteria were used as MHA medium and PDA medium for *Candida* strains. The prepared media were stored at +4 °C for a maximum of 2 weeks. Their purity was checked, and the microorganisms stored in 15% glycerol solution at -85 °C were inoculated into the prepared media and allowed to multiply by incubating in a bacteriological oven at 37 °C for 24 hours. Turbidity adjustment was made using a turbidimeter according to the developing cultures McFarland No: 0.5 (approximately 10^8 CFU/mL for bacteria) tube (24).

The essential oil (20-0.019 mg/mL) was dissolved in sterile dimethyl sulfoxide for the initial stock solution. One hundred µL of essential oil was applied to -96well microplates and 2 fold serial dilutions were performed. After the dilutions, 50 µL aliquots of turbidimetrically adjusted microorganisms were inoculated on to the plates. After incubation in MHB and RPMI mediums at 37 °C for 24 h, the first well was treated with 20 µL of resazurin, which insured on all microplates the minimum inhibitory concentrations (MIC) where the lowest concentration of the samples prevented visible growth. Solvent and microbial controls were also added to the assay plate. Antimicrobial assays were repeated at least three times for all the test samples. MIC of the samples were determined and compared with both positive controls. The reference drugs, chloramphenicol (for bacteria) and amphotericin B (for fungus) (Sigma-Aldrich) were used as positive controls (24).

Results

The yield of essential oil was obtained by hydrodistillation from the aerial parts of *S. salsugineum* was 0.28% (v/w). It was analysed by GC and GC-MS, simultaneously. A total of 42 compounds were identified in the essential oil of *S. salsugineum*, which represented 93.7% of the oil. Sabinene (35.5%), kessane (10.5%), α-pinene (6.4%) and terpinen-4-ol (5.0%) were characterized as main constituents. Other components are given in Table 1.

Antimicrobial activity of the essential oil (40-2560 µg/mL) compared to reference antibiotics (2-64 µg/mL), was tested against the following bacterial strains: Gram-negative (*Pseudomonas aeruginosa* ATCC B888), Gram-positive (*Staphylococcus aureus* ATCC 6538, *Bacillus cereus* NRRL B-3711), and three fungal strains: *Candida albicans* ATCC 24433, *Candida parapsilosis* ATCC 22019 and *Candida krusei* ATCC 6258; results are presented in Table 2.

The essential oil generally showed the best action (320 µg/mL) against the tested microorganisms except for *P. aeruginosa* strain (1280 µg/mL).

Discussion

In previous works on the other species of *Seseli*, the most abundant compound in the essential oil *S. rigidum* fruit oil was found as monoterpenes α-pinene (37.8%) and sabinene (13.5%) (25). Goncalves et al. (26) reported that α-pinene (24.8-24.9%), β-pinene (23.5-23.9%) and (*Z*)-β-ocimene (13.3-16.0%) were major constituents in the essential oils of *S. tortuosum*. The main constituents of the oils of *S. montanum* subsp. *peixotoanum* were α-pinene (36.0-37.1%), β-pinene (22.5-23.6%) and limonene (7.7-8.8%). (*Z*)-β-Ocimene was a minor component in *S. montanum* subsp. *peixotoanum* oils. Among the sesquiterpenes, β-elemene was the major one (5.2-5.8%) (26). In another study, the essential oils obtained from the different parts of *S. rigidum* (flower, leaf and fruit) were analysed and α-pinene (33.0, 26.3, 33.2%), sabinene (7.9, 7.8, 18.5%) and limonene (7.1, 5.4, 8.7%) were found as major components (27). According to

Table 1. Volatile constituents of essential oil of *Seseli salsugineum*

RRI	Compound	%
1032	α-Pinene	6.4
1035	α-Thujene	0.6
1076	Camphene	0.8
1118	β-Pinene	2.3
1132	Sabinene	35.5
1159	δ-3-Carene	0.3
1174	Myrcene	2.0
1188	α-Terpinene	0.4
1203	Limonene	2.0
1210	2-Methyl-2-butenal	0.1
1218	β-Phellandrene	0.8
1246	(Z)-β-Ocimene	0.4
1255	γ-Terpinene	1.3
1266	(*E*)-β-Ocimene	0.5
1280	*p*-Cymene	3.0
1290	Terpinolene	0.3
1553	Linalool	0.7
1556	*cis*-Sabinene hydrate	0.3
1571	*trans-p*-Menth-2-en-1-ol	0.2
1611	Terpinen-4-ol	5.0
1617	Lavandulyl acetate	0.7
1638	*cis-p*-Menth-2-en-1-ol	0.2
1668	(*Z*)-β-Farnesene	1.0
1704	γ-Muurolene	0.3
1709	α-Terpinyl acetate	0.6
1744	δ-Selinene	1.0
1726	Germacrene D	2.3
1755	Bicyclogermacrene	0.3
1772	Neryl isobutyrate	0.5
1773	δ-Cadinene	1.3
1786	Neryl propionate	0.6
1786	Kessane	10.5
2069	Germacren-D-4-ol	1.5
2144	Spathulenol	1.4
2187	T-Cadinol	0.5
2198	Thymol	0.3
2209	T-Muurolol	1.0
2228	Acorenone B	2.5
2232	α-Bisabolol	0.6
2255	α-Cadinol	2.7
2278	*cis*-Guaie-9-en-11-ol	0.5
2369	Eudesma-4(15),7-dien-1-β-ol	0.5
	Total	93.7

RRI: Relative retention indices calculated against *n*-alkanes, %: calculated from FID data

Table 2. Minimum inhibitory concentrations values (µg/mL)

	S. salsugineum	Chloramphenicol	Amphotericin B
B. cereus	320	4	-
S. aureus	320	8	-
P. aeruginosa	1280	16	-
C. albicans	320	-	8
C. parapsilosis	320	-	64>
C. krusei	320	-	64>

the Tosun et al. (28) the main constituents were determined as germacrene D (54.1%) and sabinene (22.4%) in *S. gummiferum* and *S. corymbosum* subsp. *Corymbosum,* and *β*-phellandrene (29.2%), *α*-phellandrene (8.2%) and germacrene D (2.5%) in *S. corymbosum* subsp. *corymbosum* essential oil.

According to a previous study, evaluation of MIC of the oils showed variability of inhibition among all the fungi tested, *Candida albicans* ATCC 10231, *C. tropicalis* ATCC 13803, and *C. parapsilosis* ATCC 90018. *S. rigidum* oil proved to be more active with MIC and MLC values ranging from 0.64 to 1.25 µL/mL (27). In another work, the antimicrobial activity of essential oils from *S. rigidum* (root, leaf, flower and fruit) were tested against *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 6538, *Bacillus cereus* ATCC 10876, *Candida albicans* ATCC 16404 and *Aspergillus niger* ATCC 10231. MIC values were found ranging from 0.02 to 3.24 mg/mL (26).

Conclusion

The *Seseli salsugineum* sp. was discovered and published by Duran et al.'s study in 2021. The essential oil was obtained and its antimicrobial activity was demonstrated. Compared with the literature, to the best of our knowledge, this is the first report on the chemical constituents and antimicrobial activities of the *S. salsugineum* essential oil.

Ethics

Ethics Committee Approval: Ethics committee approval is not required.

Informed Consent: Informed consent is not required.

Footnotes

Authorship Contributions

Concept: G.Ö., B.D., A.D., K.H.C.B., Design: G.Ö., B.D., A.D., K.H.C.B., Data Collection or Processing: G.Ö., G.G., Analysis or Interpretation: G.Ö., B.D., G.G., Literature Search: G.Ö., G.G., Writing: G.Ö., B.D., G.G., A.D., K.H.C.B.

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 Article

DOI: 10.14235/bas.galenos.2024.48108
Bezmialem Science 2024;12(4):406-12

Comparison of Microhardness and Surface Roughness of New Nanofiber Filled Flowable Composite

Yeni Nanofiber Dolduruculu Akışkan Kompozitin Mikrosertlik ve Yüzey Pürüzlülüğünün Karşılaştırılması

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ABSTRACT

Objective: This study aimed to compare the microhardness and surface roughness (Ra) of a resin composite, which was recently introduced to be the first flowable composite with the nano-sized fiber filler, with the particle filled composite resins with different properties, used in Class V cavities.

Methods: Totally 100 disc-shaped samples (diameter: 4 mm, height: 2 mm) were prepared and divided into five groups in accordance to the different types of composites (n=20): 1) Flowable composite with nano-fiber filler (Group N: NovaPro Flow, Nanova, USA); 2) Flowable bulk-fill composite [Group Estelite Bulk Fill Flow (EBF): Tokuyama, Japan]; 3) Flowable composite [Group G-aenial Universal Flo (GUF): GC Corp, Japan]; 4) Highly-filled flowable composite [Group G-aenial Universal Injectable (GUI): GC Corp, Japan]; 5) Micro-hybrid composite (Group Z250: Filtek Z250, 3M ESPE, USA). They were polished with aluminum oxide polishing discs. Ra measurements (μm) were made using contact profilometer (MarSurf M 300 C; Mahr GmbH, Germany) (n=10). Vickers microhardness evaluations were made using HMV microhardness tester (Shimadzu, Japan) (n=10). Three dimensional (3D) optic profilometer was used to evaluate the surface topography. One-way ANOVA, Shapiro-Wilk and Tukey tests were used for statistical analysis ($p<0.05$).

Results: At top and bottom surfaces, Group N showed significantly lowest microhardness values while Group Z250 showed significantly highest microhardness values than other groups ($p<0.05$). Group GUI showed significantly higher microhardness values than group

ÖZ

Amaç: Bu çalışmada, nano boyutlu fiber dolduruculu ilk akışkan kompozit olarak yakın zamanda piyasaya sürülen bir rezin kompozitinin mikrosertlik ve yüzey pürüzlülüğünün (Ra), Sınıf V kavitelerde kullanılan farklı özelliklere sahip partikül dolduruculu kompozit rezinlerle karşılaştırılması amaçlandı.

Yöntemler: Yüz adet disk şeklinde numune (çap: 4 mm, yükseklik: 2 mm) hazırlanarak farklı kompozit türlerine göre beş gruba ayrıldı (n=20): 1) Nano fiber dolduruculu akışkan kompozit (Grup N: NovaPro Flow, Nanova, ABD); 2) Akışkan bulk fill kompozit [Grup Estelite Bulk Fill Flow (EBF): Tokuyama, Japonya]; 3) Akışkan kompozit [Grup G-aenial Universal Flo (GUF): GC Corp, Japonya]; 4) Yüksek oranda doldurucu içeren akışkan kompozit [Grup G-aenial Universal Injectable (GUI): GC Corp, Japonya]; 5) Mikro hibrit kompozit (Grup Z250: Filtek Z250, 3M ESPE, ABD). Alüminyum oksit polisaj diskleri ile parlatıldı. Ra ölçümleri (μm) kontak profilometre (MarSurf M300C; Mahr GmbH, Almanya) (n=10) kullanılarak yapıldı. Vickers mikrosertlik değerlendirmeleri ise HMV microhardness tester (Shimadzu, Japonya) (n=10) kullanılarak yapıldı. Yüzey topografyasını değerlendirmek için üç boyutlu (3D) optik profilometre kullanıldı. İstatistiksel analiz için one-way ANOVA, Shapiro-Wilk ve Tukey testleri kullanıldı ($p<0,05$).

Bulgular: Üst ve alt yüzeylerde Grup N anlamlı olarak en düşük mikrosertlik değerlerini gösterirken, Grup Z250 diğer gruplara göre anlamlı olarak en yüksek mikrosertlik değerlerini gösterdi ($p<0,05$). Grup GUI, grup EBF ve GUF'ye göre anlamlı derecede

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ABSTRACT

EBF and GUF ($p<0.05$). Hardness ratio was found lower than 80% in Group N. No significant differences in Ra were found between the groups. 3D optic profilometer revealed that similar scratch appearances were detected in all groups.

Conclusion: Incorporation of flowable composite with nanofiber filler may not be advantageous for micro-hardness, hardness ratio and Ra properties.

Keywords: Class V restoration, flowable composite, nanofiber, microhardness, surface roughness

ÖZ

yüksek mikrosertlik değerleri gösterdi ($p<0,05$). Grup N'de sertlik oranı %80'in altında bulundu. Tüm gruplarda Ra anlamlı bir farklılık bulunmadı. 3D optik profilometrede tüm gruplarda benzer pürüzlü görünümler tespit edildi.

Sonuç: Akışkan kompozitin nanofiber doldurucu maddesi ile birleştirilmesi mikro sertlik, sertlik oranı ve Ra özellikleri açısından avantajlı olmayabilir.

Anahtar Sözcükler: Sınıf 5 restorasyon, akışkan kompozit, nanofiber, mikrosertlik, yüzey sertliği

Introduction

Direct resin composites have gained popularity as the preferred material due to their ability to restore tooth structure, while preserving the natural look of teeth with shape, color, and texture (1). Also, these restorative materials are frequently used for Class V cavities which may be caused by caries, erosion, abfraction and abrasion. However, enhancing restorative materials for Class V cavities presents challenges as they have distinct biomechanical demands compared to occlusal cavities. The stress caused by occlusal forces in Class V lesions can lead to restorative material fractures and debonding (2). To withstand the forces exerted during chewing, enhancing the mechanical properties of restorative materials has been achieved through modifications in filler particle size and morphology. These modifications include incorporating ceramic particles with random orientation, whiskers in single or multi-layer form, or fibers in continuous or discontinuous arrangements in various orientations. These adjustments have led to improved mechanical properties of the materials (3-5). For reinforcement, the fibers can enhance mechanical properties by acting mainly as crack stoppers and mimic the tooth structures (3,6). Microstructural parameters - such as fiber diameter, fiber length, fiber orientation, fiber loading, and the adhesion between fibers and the polymer matrix - play a crucial role in determining the characteristics of fiber-reinforced composite resins. These factors greatly influence the performance and properties of the composite material (7). Inorganic hydroxyapatite (HAp) nanofibers are used as one of the methods to reinforce dental resin composites (8). HAp nanofibers are calcium phosphate fillers that have been shown to offer more stress transfer by interaction between nanofibers and matrix and reduced polymerization shrinkage so improving the marginal integrity. These aesthetic materials that utilize glass fibers are used in various dental clinical procedures with a primary focus on restorative dentistry (9). Although novel restorative materials are launched in the markets, there is no gold standard procedure on the choice of best materials for Class V restorations.

Attaining a smooth and durable surface is of great clinical significance for composite resin restorations as it directly impacts the long-term survival (10). The filler particles of composite resin materials can influence the material's ability to be polished and its hardness, consequently affecting the surface roughness (Ra) and hardness of the restoration. To our knowledge, limited data are present for evalauting the microhardness and Ra of a resin composite, which was recently introduced to be the first flowable composite with the nano-sized fiber filler. Thus, the aim of this study was to compare the microhardness and Ra of new nanofiber reinforced flowable composite with flowable bulk-fill, highly-filled flowable composite, flowable composite and microhybrid composite resins used in Class V cavities.

The null hypothesis of this *in vitro* study was:

There would be no differences in microhardness and Ra of new nano-fiber reinforced flowable with other composite materials used in Class V cavities.

Methods

Sample Size Calculation

A power analysis was performed to establish the specimen size according to the literature (11). In this study, for each group, minimum 10 specimens were required to gain a medium effect size ($d=0.50$), with 95% power and a 5% type 1 error rate.

Sample Preparation

A list of the composite resins used in this study is given in Table 1. The colors of composites were selected as A2. Five different composite resins were used: Group N: NovaPro Flow (Nanova, USA); Group Estelite Bulk Fill (EBF): (Tokuyama, Japan); Group G-aenial Universal Flo (GUF): (GC Corp., Japan); Group G-aenial Universal Injectable (GUI): (GC Corp., Japan) and Group Z250: Filtek Z250 (3M ESPE, USA). Hundred disc-shaped samples, for each tested materials (n=20) (height: 2 mm and diameter: 4 mm) were prepared using teflon molds. To achieve a smooth, polymerized surface, the samples were sandwiched between two transparent polyester matrix strips (Mylar Strip, SS White Co., Philadelphia, PA, USA) and glass slides. The excess material was then removed by applying pressure using the glass slides. Then, the samples were polymerized with a light-emitting diode light-curing unit for 20 s according to the manufacturer's guidelines (light emitting diode, light curing unit) (Valo, Ultradent, South Jordan, UT, USA) (irradiance of 1000 mW/cm^2). The top surfaces of the samples underwent polishing using a sequence of aluminum oxide polishing discs. (Sof-Lex XT, Pop-On, 3M ESPE, USA) with a slow hand-piece.

They were stored in distilled water at 37 °C for 24 h in a dark vial.

All restorative procedures were done by a single operator (Z.C.O.) in accordance to the manufacturers' instructions.

Microhardness Measurement and Calculating Bottom/Top Hardness Ratio

Ten disc-shaped samples of each composite resin were used (n=10) and Vickers microhardness test was peformed with HMV microhardness tester (HMV-G, Shimadzu Corp., Japan) according to the ASTM E384-17 standard (12). Three measurements were obtained on the top (upper) and bottom (lower) surfaces of each specimen (200 g load and 10 s dwell time). Vickers Hardness values of each surface was recorded as the average of these measurements. The hardness number of the bottom surface was divided by the hardness number of the top surface to establish the hardness ratios (%), which were subsequently converted to a percentage. A second operator, who was unaware of the type of composite resin, performed all of the microhardness measurements (R.H.E.O.).

Surface Roughness Measurement

Ten disc-shaped samples of each composite resin were used (n=10) and Ra test was perfomed with a contact profilometer (MarSurf M 300 C; Mahr GmbH, Göttingen, Germany) in accordance with EN ISO 4288 (stylus tip Radius: 5 µm, a stylus driving speed: 0.5 mm/s, traversing length (Lt): 1.75 mm and five cut-off lengths: 0.250 mm) (13). Three measurements were performed in 4 different locations (in each quadrant in a clockwise direction) of the polished surface and arithmetic mean of the measurements (µm, Ra) were recorded. A second operator, who was unaware of the type of composite resin, performed all of the Ra measurements (B.O.).

Statistical Analysis

A software program (SPSS 22.0 Windows, SPSS Inc., IL, USA) was used for statistical analysis. Shapiro-Wilk test, and Levene's test were to determine the normality of variables and homogeneity of variances for microhardness and Ra data. Since the data were normally distributed, one-way analyses of variance (one-way ANOVA) were used to compare the the materials. All pairwise comparisons were performed with the Tukey HSD test at a significant level of 0.05.

Surface Topography Analysis

One specimen from each group was subjected to surface pretreatment to evaluate the three dimensional (3D) surface topography with an optic profilometer (Nanomap 1000WLI,

Table 1. The composite resins used in this study

Material	Filler type	Organic marix
Filtek Z250 (Z250) 3M ESPE, St Paul, MN, USA	Zirconia, silica 78 wt %, 60 vol % 0.01 µm to 3.5 µm with an average particle size of 0.6 µm	Bis-GMA, Bis-EMA, UDMA, TEGDMA
NovaPro Flow Nanova Inc, Missouri, USA	Barium borosilicate glass (0.7 µm), hydrophobic amorphous silica (40 nm), hydroxyapatite fibers. 60% wt	Bis-EMA, TEGDMA, UDMA
Estelite Bulk Fill Flow Tokuyama Dental Corp, Ibaraki, Japan	Spherical Silica-zirconia (200 nm) 70 wt %, 56 vol %	Bis-MPEPP, TEGDMA, Bis-GMA
G-aenial Universal Flo GC Corp., Tokyo, Japan	SO_2 (16 nm), Strontium glass (200 nm) 69 wt %, 50% vol	UDMA (15-20 wt %), TEGDMA (5-10 wt %), Bis-MEPP (5-10 wt %)
G-aenial Universal Injectable GC Corp, Tokyo, Japan	150 nm Barium glass, silica 69 wt %	Dimethacrylate monomers

BIS-GMA: Bisphenol A glycidyl methacrylate, UDMA: Urethane dimethacrylate, TEGDMA: Triethylene glycol dimethacrylate, Bis-MPEPP: Bis-methacryloxyethoxy phenyl propane, Bis-EMA: Bisphenol A diglycidyl methacrylate ethoxylated, µm: Micrometer, wt %: Weight percentage, vol %: Volume percentage, nm: Nanometer

Table 2. Mean microhardness values, hardness ratio and standard deviations (± SD) for all groups

Groups	Top	Bottom	Hardness ratio (%)
Group N	40.603±3.378^{A}	20.633±3.183^{A}	50.9±7.4
Group EBF	54.454±1.748^{B}	50.917±2.442^{B}	93.6±4.6
Group GUF	51.389±3.19^{B}	40.946±5.55^{B}	79.6±9.4
Group GUI	65.168±4.222^{C}	57.671±5.662^{C}	88.5±6.2
Group Z250	113.023±8.416^{D}	95.834±5.873^{D}	85.0±5.4
p-value	<0.001	<0.001	

*Different capital letters show the significant difference between the groups (p<0.05). N: NovaPro Flow, EBF: Estelite Bulk Fill Flow, GUF: G-aenial Universal Flo, GUI: G-aenial Universal Injectable, Z250: Filtek Z250, SD: Standard deviation

AEP Technology, Saratoga, CA, USA). The scan range was adjusted to 232 mm, the vertical dynamic range was adjusted to 500 mm and the stylus loading force was set to 12 mg. A color scale and graphics were used for interpretation of the images. Different values are represented with different colors. The negative values indicate the pits while the positive values resemble the peaks.

Results

Microhardness Measurement and Calculating Bottom/Top Hardness Ratio

Mean microhardness values, hardness ratio and standard deviations of all groups are presented in Table 2. At top and bottom surfaces, Group N showed significantly lowest microhardness values while Group Z250 showed significantly highest microhardness values than other groups ($p<0.05$). Group GUI showed significantly higher microhardness values than group EBF and GUF ($p<0.05$). However, no significant differences were determined in microhardness values between Group EBF and Group GUF ($p>0.05$).

Group Z250, Group GUI, Group GUF and Group EBF showed hardness ratio equal or exceeding the 80% threshold values although Group N exhibited lower hardness ratio (50.9%) than theshold values (80%).

Surface Roughness Measurement

Mean Ra values and standard deviations of all groups are shown in Table 3. There were no signicant differences in Ra between the groups ($p>0.05$).

Surface Topography Analysis

In all groups, similar micro-scratches and irregularities were detected (Figure 1).

Discussion

In this study, the microhardness and Ra of new nanofiber reinforced flowable composite with flowable bulk-fill, highly-filled flowable composite, flowable composite and microhybrid composite used in Class V cavities, were compared. Based on the results, the null hypothesis that there would be no differences in microhardness and Ra of new nanofiber reinforced flowable with other composite materials used in Class V cavities, was partially rejected since the differences in microhardness values of the composite resins used were significant while no significant differences in Ra were found among all composite resins.

Vickers hardness is a method used to determine the hardness value by measuring the depth or area of an indentation left by an indenter with a specific shape, force, and time of application. The hardness value is indicative of a material's ability to withstand applied loads (14). It represents a combination of deformation and elastic behavior. Several factors related to resin composites can influence hardness, including the size, shape, and fraction of fillers in the inorganic phase. Generally, hardness increases with a higher amount of fillers (15). This can be explained by the fact that as the volume fraction of fillers increases, a point is reached where particles come into contact with each other within the matrix. At this point, stress is transferred primarily through the interactions between hard particles (16,17). In this study, NovaPro Flow exhibited significantly the lowest microhardness values while Filtek Z250 showed significantly the highest microhardness values at the top and bottom surfaces. This finding could be attributed

Table 3. Mean surface roughness values and standard deviations (± SD) of all groups (µm, Ra)

Groups	Surface roughness (± SD)
Group N	0.198±0.06
Group EBF	0.161±0.053
Group GUF	0.126±0.044
Group GUI	0.145±0.053
Group Z250	0.166±0.055
p-value	0.051

N: NovaPro Flow, EBF: Estelite Bulk Fill Flow, GUF: G-aenial Universal Flo, GUI: G-aenial Universal Injectable, Z250: Filtek Z250, SD: Standard deviation

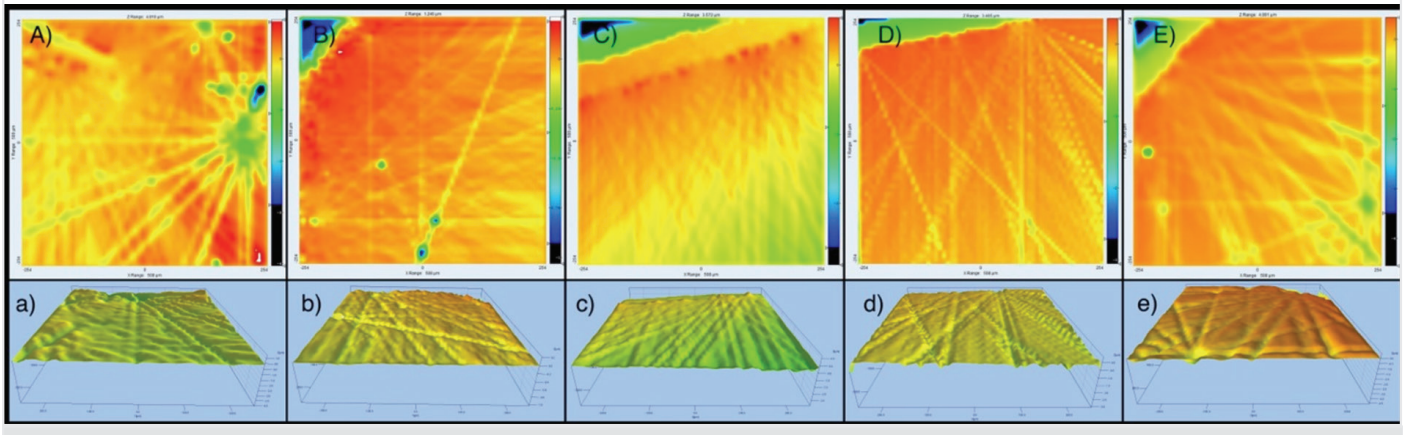

Figure 1. Optic profilometer images showing the 2D (A-E) and 3D (a-e) surface topography of all composite resins. A, a: NovaPro Flow, B, b: Estelite Bulk Fill Flow, C, c: G-aenial Universal Flo, D, d: G-aenial Universal Injectable, E, e: Filtek Z250, 2D:Two dimensional, 3D: Three dimensional

to the differences in inorganic filler amount and filler types of the composite resins used in this study. NovaPro Flow is a low-viscosity, visible-light cured, radiopaque, nanohybrid composite that contains 60% by weight HAp nanofiber filler (lowest filler load) while Filtek Z250 is a high-viscosity, microhybrid composite that contains 78% by weight silica-zirconia fillers (highest filler load). Besides, this is in line with McCabe and Wassell (18), who reported that microhardness of composite materials enhanced with increasing filler content. However, in this study, when G-aenial Universal Injectable, G-aenial Universal Flow and EBF, which have similar filler amount, were compared, it was determined that G-aenial Universal Injectible showed higher microhardness values than the other two composites at the top and bottom surfaces. It was indicated that composite resins with small filler particles increased surface microhardness (19). So, this finding could be explained by the fact that G-aenial Universal Injectable (150 nm) had smaller filler particles than the G-aenial Universal Flow (200 nm) and EBF (200 nm).

As light passes through the composite resins, light intensity is clearly reduced due to light absorbtion and attenuation (20). Hardness values of bottom/top surfaces generally can be used to measure the degree of polymerization (21). Direct methods, such as infrared and Raman spectroscopy, are not commonly employed in routine procedures due to their complex, expensive, and time-consuming nature (22). Thus, in this study, the Vickers microhardness measurement was preferred to determine the restorative material's degree of polymerization, in view of its ease of use, popularity and relative efficiency (23). Degree of polymerization is influenced by many factors, such as the chemical structure of the monomers, filler composition, curing time and light intensity (21). In the literature, it is indicated that an acceptable degree of polymerization is achieved if the bottom hardness corresponds to at least 80% of the top surface hardness (24). However, in this study, hardness ratio lower than 80% was found in only NovaPro Flow. This finding could be attributed to its lower translucency.

The esthetics of a restorative material may be compromised due to its Ra, leading to negative impacts on abrasion and wear resistance, plaque buildup, and the potential development of secondary caries (25). Especially in Class V restorations, plaque accumulation is very important in terms of gingival health in this region (26).

Various polishing systems are such as polishing discs, rubber wheels, cups, discs, and pastes, that can be used to finish and polish composite resin restorations (27). To ensure consistency and eliminate any potential variations caused by different polishing systems, the same polishing procedure was employed for all materials in the current study, despite individual manufacturers typically recommending specific polishing systems for each material evaluated. Previous studies indicated that smoothest surfaces were obtained with multistep aluminum oxide polishers (27,28). Thus, in this study, the multistep polishers with higher flexibility (Sof Lex) were preferred. In the literature, it was reported that 2D Ra above 0.2 μm resulted in an increase of plaque accumulation and higher risk for caries and periodontal inflammation (29). It was found that the majority of patients were capable of discerning differences of approximately 0.3 μm in terms of mean roughness (30). In this study, all composite resins exhibited lower Ra values than 0.2 μm.

Profilometers are commonly utilized to obtain roughness values, providing a quantitative assessment of surface irregularities. Mechanical profilometry, a widely used method for evaluating surface properties, offers a 2D representation of the surface, yielding limited information (31). On the other hand, optical profilometry, which is also employed to measure Ra after polishing composites (32), captures the 3D surface topography, thereby reflecting the natural characteristics of the surface (33). By utilizing 3D measurements, optical profilometry offers a more comprehensive and detailed description of surface topography compared to 2D measurements, providing a more complete understanding of the surface (31). In this study, the Ra of the composite resins was measured with a mechanical profilometer, then their surface topograpy was evalauted with an optical profilometer. NovaPro Flow offers optimal handling and finishing properties that does not require any special polishing tools that enable the dentist to achieve the desired finish and esthetics expected from a flowable composite. In this study, NovaPro Flow showed similar Ra values when compared to the other composite resins. Besides, no significant differences in Ra values were observed for other tested composites. The results of 3D optic profilometer images revealed that similar scratch appearances were detected in all groups, were consistent of the Ra values.

Study Limitations

In this study, multistep polishers were used and oral conditions such as bacteria, saliva or pH and temparatures changes were not considered. Another limitation of this study was that it was not accomplished using spectral analysis. Thus, further studies should focus on the effect of different polishing systems and clinical conditions on the surface properties of this new restorative material, additionally using different test techniques such as spectral analysis.

Conclusion

Despite the limitations of this study, it can be concluded that: At the top and bottom surfaces, new nanofiber reinforced flowable composite showed significantly lowest microhardness while microhybrid composite showed highest microhardness than other composites. At the top and bottom surfaces, highly-filled flowable composite showed significantly higher microhardness than flowable bulk-fill and flowable composite resin. Hardness ratio of a new nanofiber reinforced flowable composite was found lower than 80% threshold value that was the acceptable degree of polymerization. Similar Ra and surface topography were obtained for all composite resins.

Ethics

Ethics Committee Approval: Ethics committee approval is not required.

Informed Consent: Informed consent is not required.

Footnotes

Authorship Contributions

Concept: Z.C.O., Design: R.H.E.O., Z.C.O., Data Collection or Processing: R.H.E.O., Z.C.O., B.O.O., Analysis or Interpretation: B.O.O., E.E.D., Literature Search: R.H.E.O., B.O.O., Writing: R.H.E.O., B.O.O.

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Original Article

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Identification of CircRNA Complexes Acting on miRNA Sponges within the Exosome in Patients with Alzheimer's Disease: An *In Silico* Approach

Alzheimer Hastalarında Eksozom İçinde Süngerimsi miRNA'ya Etki Eden CircRNA Komplekslerinin Tanımlanması: Bir *In Silico* Yaklaşımı

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ABSTRACT

Objective: Alzheimer's disease (AD) is a neurodegenerative ailment that launches insidiously and progresses slowly, and its prevalence gradually increases with age. In a former study, we explored the plasma exosomal circular RNA (circRNA)-micro RNAs (miRNA) expression profiles in AD patients derived from *next-generation sequencing* data by scanning experimentally validated miRNA-target interaction databases. The current paper focused on computing the integration of circRNA-miRNA and RNA binding proteins (RBP) into the plasma exosomes using the output of our earlier study.

Methods: We identified 24 upregulated circRNAs and 12 downregulated miRNAs from our previous paper. These were then subjected to prediction of circRNA-miRNA via the circMine web server and MiRNet. Subsequently, the circRNA Interactome web server was used to integrate the 24 circRNAs with RBPs. Finally, the obtained numeric scale results were visualized using R studio, ensuring the validity and reliability of our findings.

Results: As an overall outcome of calculations, we found CNTN4, SH3BGRL, *THOC2*, CGGBP1, FLNA, ATP6V1A, and UBN1 from queried 24 circRNAs linked to AD pathology, proposing that circRNAs complexes composed of RBPs and miRNAs might be considered empirically under oncoming studies.

ÖZ

Amaç: Alzheimer hastalığı (AH), sinsi başlayan ve yavaş ilerleyen, prevalansı yaşla birlikte giderek artan nörodejeneratif bir hastalıktır. Önceki bir çalışmada, deneysel olarak doğrulanmış miRNA-hedef etkileşim veritabanlarını tarayarak *next-generation sequencing* verilerinden elde edilen AH'li hastalarda plazma eksozomal dairesel RNA (circRNA)-mikro RNA'lar (miRNA) ekspresyon profillerini araştırdık. Mevcut makale, önceki çalışmamızın çıktısını kullanarak circRNA-miRNA ve RNA bağlayıcı proteinin (RBP) plazma eksozomlarına entegrasyonunu hesaplamaya odaklandı.

Yöntemler: Önceki makalemizden 24 yukarı regüle edilmiş circRNA ve 12 aşağı regüle edilmiş miRNA tanımladık. Bunlar daha sonra circMine web sunucusu ve MiRNet aracılığıyla circRNA-miRNA'nın tahminine tabi tutuldu. Daha sonra, 24 circRNA'yı RBP'lerle entegre etmek için circRNA Interactome web sunucusu kullanıldı. Son olarak elde edilen sayısal ölçek sonuçları R studio kullanılarak görselleştirilerek bulgularımızın geçerliliği ve güvenirliği sağlanmıştır.

Bulgular: Hesaplamaların genel bir sonucu olarak, AH patolojisine bağlı sorgulanmış 24 circRNA'dan CNTN4, SH3BGRL, *THOC2*, CGGBP1, FLNA, ATP6V1A ve UBN1 bulundu.

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ABSTRACT

Conclusion: Basically, our calculations claim that it may be examined whether these complexes behave as sponge miRNA in AD.

Keywords: Alzheimer's disease, exosomes, miRNA sponges, circRNA

ÖZ

Sonuç: Temel olarak hesaplamalarımız, bu komplekslerin AH'de sünger miRNA gibi davranıp davranmadığının incelenebileceğini iddia etmektedir.

Anahtar Sözcükler: Alzheimer hastalığı, eksozomlar, miRNA süngerleri, sirkülerRNA

Introduction

Alzheimer's disease (AD) is an onward neurological illness, which nowadays influences more than 5.5 million people (1,2). Progressively recognized as a serious, global public health concern, the widespread consensus in the pathological diagnosis of AD is the accumulation of extracellular amyloid beta plaques and neurofibrillary tangles (3). As AD commenced insidiously and progressed unsteadily rise over the years, investigations in the discovery of candidate biomarkers for the early diagnosis of AD urgently need to be exerted seeing that lack of novel biomarkers has existed for many years.

Exosomes are tiny vesicles and different membrane structures of endosomal origin, based on biomarker scanning, are a trend and rapidly developing area in the diagnoses of neurodegenerative disease since they generally cross the blood-brain barrier and include many types of non-coding RNA (ncRNA)s (4).

Circular RNAs (circRNAs) that belong to the class of ncRNA molecules are covalently closed and endogenous biomolecules with neither 3' poly(A) tails nor 5' end caps (5). Given the advances in the next-generation sequencing (NGS) and bioinformatics techniques, researchers have identified a great number of circRNAs and found these RNAs in tissue and cell-specific expression patterns in eukaryotes (6). Since the milestone finding of ciRS-7/CDR1as (circRNA sponge for miR-7) in 2013, circRNAs have turned out a valuable issue in RNA research (7,8). CircRNAs are believed to be structurally more stable than other RNAs, owing to being covalently closed circular biomolecules. This stability makes them have longer half-life than linear RNAs and will probably display a model feature of circRNAs during their forthcoming evolution as biomarkers (9,10). This suggests that circRNAs may be considered as biomarkers in the early diagnosis of diseases. Additionally, circRNAs are established to be proper biomolecules as therapeutic targets for several disorders such as cancer, cardiovascular diseases, chronic inflammatory diseases, and neurological disorders (11-13).

The presence of circRNA accumulation in various species during aging suggests that circRNA may be a factor associated with aging and the pathogenicity of age-related disease such as AD (14). Moreover, a great number of differentially expressed circRNAs in the brains of AD patients have been reported (9). Some differentially expressed circRNAs have been monitored to ease AD-like pathological conditions in cellular and animal models of AD, suggesting that circRNAs are presumably related to regulating the neuropathophysiology of AD (15).

Even though little has been recognized about the functions of circRNAs as yet, two main functions have been identified as playing a role including as miRNA sponges and circRNA-protein interactions. The most notable proteins interacting with RNA molecules are the RBPs. RBPs are a category involved in the metabolic functioning of RNAs by mediating their translation, transport, localization, and maturation; in fact, these proteins take part in the shape of ribonucleoprotein complexes (10).

What we know about sponge miRNAs and RBPs is primarily based on empirical studies that investigate how they are. Besides, bioinformatics methods can predict the binding sites of RBPs and miRNAs on circRNAs, so providing the opportunity to perform preliminary analysis prior to proceeding to experimental studies. Thus, the main purpose of this paper is to compute the interaction with RBP proteins and miRNAs in the exosomal circRNA complex, and to describe with bioinformatics approaches that possible circRNAs may be the first target molecules for further experimental biomarker research.

Methods

Data Collection

As a work-schema of the present study is summarized in Figure 1, we extended our previous data to demonstrate the possible interaction of miRNA and RBP with circRNA. The list of differential expression of miRNAs and circRNAs was obtained from analysis of dataset (ID: MTAB-11222) in the repository of ArrayExpress (16) (https://www.ebi.ac.uk/arrayexpress/experiments/E-MTAB-11222).

Prediction of Interaction of miRNA and RBPs with circRNAs

The list of differential expression of miRNAs and circRNAs was submitted to MiRNet 2.0 (17) as multiple query types to observe possible integrations in the network with selecting no specific tissue. To identify the potential interactions between miRNA and circRNA based on their sequence matching feature, the circRNA-miRNA prediction tool was employed by utilizing the miRanda, miRBase, and circBase databases through circMine web server (18). The parameters of score cutoff and energy cutoff were set at 140 and -7 to assign the significant interacting miRNA. To determine the possible binding sites of RBPs to the circRNAs, circRNA Interactome (19) that serves for RBPs binding to the circRNA junctions using Targetscan prediction tool was utilized. Furthermore, the flanking regions of circRNAs were evaluated and considered the highest number of binding sites for each RBP.

Data Visualization

The interaction network of the miRNAs and circRNAs was constructed using the Cytoscape 3.9.1 (20), which is one of the most well-known network visualizations. Heatmap plots representing the numerical scale of the interaction of circRNAs with both miRNAs and RBPs were conducted with R studio software (version 4.1.2, https://rstudio.com/).

Results

In the present computational study, 24 upregulated circRNAs and 12 downregulated miRNAs derived from our previous paper (21) were used for the possible integration of circRNA and miRNAs, and calculating the potential miRNA sponges through circMine with binding energy scores. As shown in Figure 2, 14 miRNAs and 18 circRNAs were integrated via miRnet web server, whilst the heatmap in Figure 3 that a total of 23 micro RNAs and 24 circRNAs were computed according to their energy score. However, there is no integration of HDAC1 from circRNAs and 9 miRNAs in the network in Figure 2. There is also a main network indicating potential circRNA to miRNAs and miRNAs to their target genes in a Supplementary Figure 1.

It is apparent from Figure 4 that Heatmap provides an account of potential 23 RBP binding proteins with interacting 129 circRNAs with distinct IDs. In the supplementary file, overall, 22 circRNAs are indicated by 129 circBase IDs.

Discussion

The circRNAs have been reported to incline to augment in the aging-brain and to be abundant in mammalian brain, however their function and expression in the nervous system are poorly understood (22). To conduce to the content of further studies related to AD pathology, as regarding two main tasks of circRNAs, the present study explores the plasma exosomal upregulated circRNAs and downregulated miRNAs in AD patients from our former study by calculating potential sponge miRNAs and RNA Binding proteins (RBPs) through computational tools. The main reason for analyzing the upregulated circRNAs is that miRNAs may be downregulated when circRNAs act as sponges to miRNA.

In the present study, in Figure 3, there is a hierarchically top binding energy score in some circRNAs including CNTN4, SH3BGRL, *THOC2*, CGGBP1, FLNA, ATP6V1A and UBN1 in the heatmap. In CNTN4 and CGGBP1 from these circRNAs, there is no any integration with miRNAs in Figure 2. Despite no correlation with miRNAs in the network analysis in Figure 2, interestingly, CNTN4 is predominantly expressed in brain, particularly in cerebellum (23). Also, CGGBP1 is extently expressed in cerebellum and cerebral cortex (24). In accordance with the calculation in Figure 3, CNTN4 interacts with hsa-miR-485-5p and hsa-miR-185-5p at peak value, as well as it is interacting with all miRNAs significantly, but CGGBP1 is interacting with hsa-miR-374a-3p at the highest matching score. In Figure 4, fused in sarcoma (FUS), Methyltransferase like-3 (METTL3) and TAR DNA *binding* protein 43 (TDP43) have much more binding sites on CNTN4 but not CGGBP1. CNTN4 is a member of the Contactin family, and functionally serves as one of the axon guidance molecules critical for the accurate construction of the optic system. Despite there are presently very limited studies that display a correlation between CNTN4 and AD pathology, a correlation has been reported in a few papers between CNTN4 and APP by dysregulation of expression of Contactin protein in the autopsy brain tissue of AD patients (25,26).

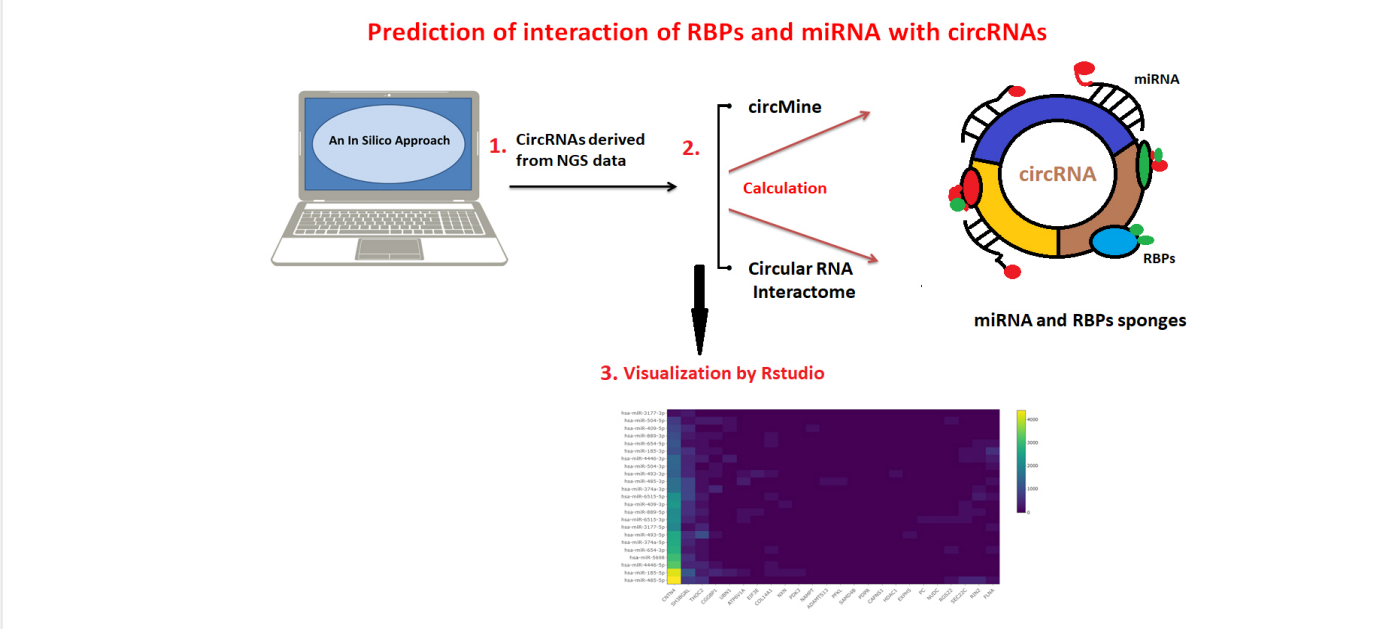

Figure 1. The work flow of the current study is composed of three main steps

NGS: Next-generation sequencing, RBPs: RNA binding proteins

THOC2 is also a highly expressed gene in the brain, and calculations have shown that it potentially interacts with a dozen of miRNAs as circRNAs. Importantly, *THOC2* is a functionally mRNA transport protein and has been uncovered to be associated with the pathogenesis of ALS (27). *THOC2* is integrating with both has-mir-374a-3p and hsa-miR-485-3p in Figure 3, but it is potentially interacting with hsa-miR-493-5p and hsa-miR-485-5p as the highest matching score by far than other miRNAs in Figure 3. In Figure 4, it can be clearly observed that the number of binding sites for Argonaute 2 (AGO2) as well as Eukaryotic initiation factor 4A-3 (EIF4A3) is by far greater for *THOC2*.

ATP6V1A, which is in many sites in the subcellular location as well as mostly located in secretory vehicles, has been reported to be down-regulated in AD and related to Synaptic Vesicle Cycle, Phagosome, and Oxidative Phosphorylation (28). Surprisingly, Wang et al. (29) have also claimed that the deficiency of ATP6V1A might be associated with neuronal disorder and neurodegeneration. In Figure 2, ATP6V1A is interacting with

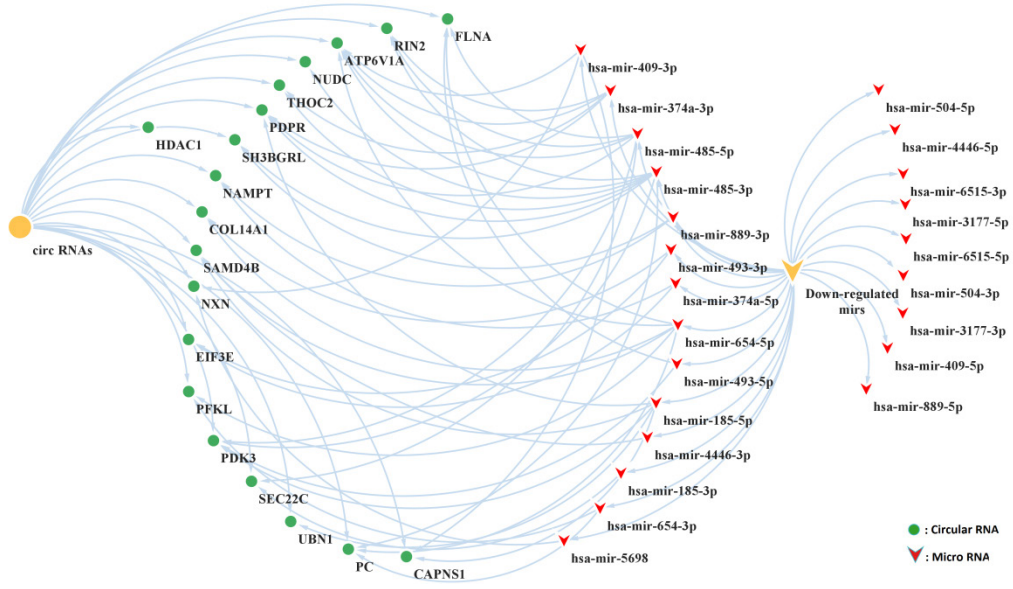

Figure 2. It represents the results obtained from multiple queries of exosomal circRNAs and miRNAs, the prediction of interaction network in the MiRNet 2.0 web server. Upregulated circRNAs are colored as circular nodes in green whereas downregulated miRNAs are colored by red nodes. The type of network is a source >> target that is indicated by the blue arrow edges. The network was designed as two subgroups circRNAs and Down-regulated miRs

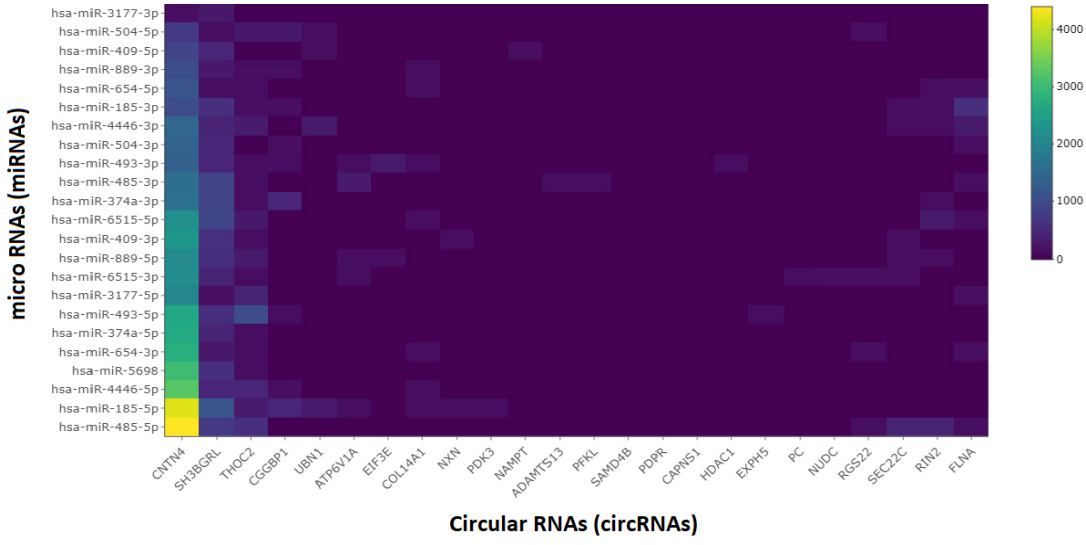

Figure 3. It gives an account of that Heatmap analysis of exosomal circRNAs and miRNAs that outcomes acquired from circMine server with respect to total energy score scale indicating in the range of 0 to 4407. The coloring varies from navy blue to yellow regarding the total score energy

has-mir-409-3p, has-mir-374a-3p, has-mir-485-5p, and has-mir-654-5p, whereas it is possibly interacting with hsa-miR-185-5p, hsa-miR-889-5p in Figure 3. However, the results of the correlational analysis for ATP6V1A set out a commonly interaction with hsa-miR-485-3p in Figures 2, 3. Taken together, just as AGO2 and EIF4A3 are commonly having highest binding sites on ATP6V1A, so DiGeorge syndrome critical region 8 gene, Fragile X mental retardation protein (FMRP), FUS and Hu

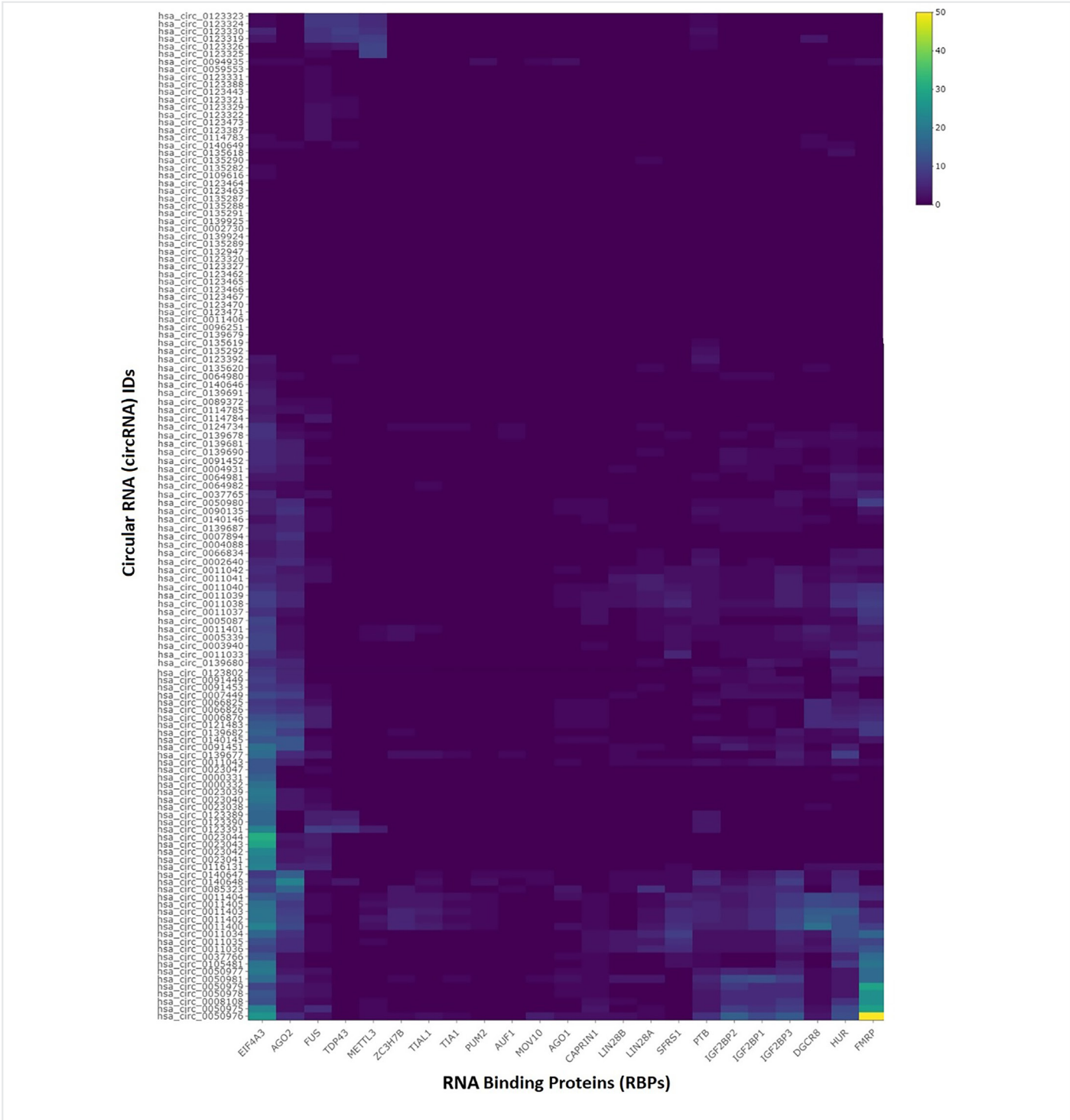

Figure 4. It presents that the possible correlations in the Heatmap analysis among the circRNAs and miRNAs that outcomes retrieved from Circular RNA Interactome server concerning the count of RBPs to the circRNA on a numeric scale indicating in the range of 0 to 50. The coloring varies from navy blue to yellow between the numerical scale of circRNAs and RBPs

RBP: RNA binding proteins

antigen R (HUR) in RBPs in Figure 4 have significant binding sites as well.

More importantly, FLNA, the most interacting one with miRNAs in circRNAS in Figure 3, has been recently elicited by Tsujikawa et al. (30) enriching tau pathological lesions in post-mortem *Progressive supranuclear palsy*-PSP brains as well as tau aggregation through their interactions with F-actin. Given that obtained data from Figure 2 and 3, hsa-miR-654-5p, hsa-miR-485-5p are commonly interacting with FLNA, however, the calculation output of FLNA with hsa-miR-185-3p is higher than the other miRNAs (Supplementary Figure 1). In spite of flanking region on FLNA, FMRP has 35 binding sites on FLNA by far compared to other RBPs in Figure 4.

As far as concern the computational Heatmap in Figure 4, there is an unambiguous relationship between CNTN4 and EIF4A3, FUS, METTL3, TDP43 with a higher number of binding sites than other RBPs. Considerably, reliable evidence display that TDP43 and FUS proteins related to the AD pathology, emphasizing the function of RBPs in neurodegeneration (31,32). Besides, TDP43 protein acts as a neuropathological marker in Alzheimer's disease (33). On the other hand, EIF4A3 possesses the highest number of binding sites in RBPs. Particularly, HDAC1, PC, *THOC2*, and PFKL from circRNAs have far more binding sites of EIF4A3 than other circRNAs. This output points out a need to understand the various perceptions of EIF4A3 that exist among the first genes to be evaluated in experimental analysis steps such as *in vitro* or *in vivo*.

SH3BGRL is a protein that is regularly localized in vesicles and has been reported to be highly expressed in Parkinson's disease with the method of MALDI-ToF-MS (34). Correspondingly, we also found the SH3BGRL in the exosome by the differential gene expression analysis via NGS in AD patients. From both data in Figures 2 and 3, potential interactions between hsa-miR-485-5p, hsa-miR-889-3p and SH3BGRL are obvious. In Figure 2, however, hsa-miR-185-5p is the best matching score with SH3BGRL, suggesting as acting sponge by its much more value. Considering potential circRNA complexes, as shown in Figure 4, AGO2, EIF4A3, HUR, and Insulin-like growth factor 2 mRNA-binding protein 1-2-3 (IGF2BP1-2-3) have much more binding sites on SH3BGRL (Supplementary Figure 1).

In Figure 4, FMRP with the largest number of binding sites has 50 binding sites, specifically on SAMD4B. It is an mRNA binding protein associated with translational inhibition of target transcripts of the amyloid precursor protein mRNA. Regarding the data, it has been suggested by Renoux et al. (35) that FMRP expression is not directly to be a primary contributor AD pathogenesis, despite decreased expression of FMRP in the brain resulting in Fragile X Syndrome. In Figure 2, SAMD4B has interactions with hsa-miR-185-5p and hsa-miR-4446-3p, but no matching score related to SAMD4B in Figure 3, proposing that variable outputs might be depended on different algorithms in web servers. Additionally, FUS, HUR, PTP and IGF2BP1-2-3 from RBPs have more binding sites on SAMD4B by far than other RBPs in Figure 4.

On the other hand, UBN1 is the most statistically significant gene expression in our former study (21) and has the potential to interact alternatively with hsa-miR-185-5p, hsa-miR-409-5p, hsa-miR-504-5p and hsa-miR-4446-3 in Figure 3 (Supplementary Figure 1), whereas it solely interacts with hsa-miR-185-5p in the network of Figure 2 that is also the highest matching score with the other miRNAs in Figure 3. Hence, UBN1 might be strongly described as a correlation with hsa-mir-185-5p concerning both calculation outcomes in Figure 2 and 3. Moreover, AGO2, EIF4A3, FMRP, FUS, HUR and IGF2BP3 are RPBs that have much more binding sites on UBN1 than other RBPs, suggesting that these RPBs should be given primarily consideration in further experimental analysis.

In this study, we attempt to explore the probable complexes that exosomal circRNAs related to AD pathology can form with RBPs and miRNAs by the computational biology approach of preliminary analysis, which will assist in the detailed analysis in oncoming experimental studies. However, there has been limited research on RBPs and miRNAs composed of circRNAs complexes, and more documents are needed. To raise the accuracy and reliability of our preliminary analysis, we were based on the inspiration of the findings in our earlier study (21) and employed more than one bioinformatics tool including circMine (18), circRNA Interactome (19) and MiRNet (17) to integrate all related data, therefore our calculations resulted meaningfully.

Study Limitations

The main limitations of the study need to be validated by further experimental investigations, due to the lack of adequate data in the databases, all miRNAs and circRNAs could not be integrated into those associated with AD, and the sample size of the NGS utilized here was not big sufficiently, solely comprising 9 tissue samples.

Conclusion

Given the functions and stability of circRNAs, they are promised as target molecules to be explored in diseases such as AD, where early diagnosis is crucial. Hence, circRNAs are paving the way for further investigation of new biomarkers in AD. To this end, prior to undertaking further experimental investigations, we sought to predict the miRNAs and RBPs involved in the structure of circRNA complexes in AD patients by sequence-based matching calculation. Our present report points out several potential integrations of circRNA-miRNAs and circRNA-RBPs that contribute to the pathology of AD.

Ethics

Ethics Committee Approval: Ethics committee approval is not required.

Informed Consent: Informed consent is not required.

Footnotes

Authorship Contributions

Concept: R.K.C., N.B., U.K., Design: R.K.C., N.B., Data Collection or Processing: R.K.C., N.B., Analysis or Interpretation: B.S., N.E., H.S.P., U.K., Literature Search: B.S., N.E., H.S.P., Writing: R.K.C., N.B.

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

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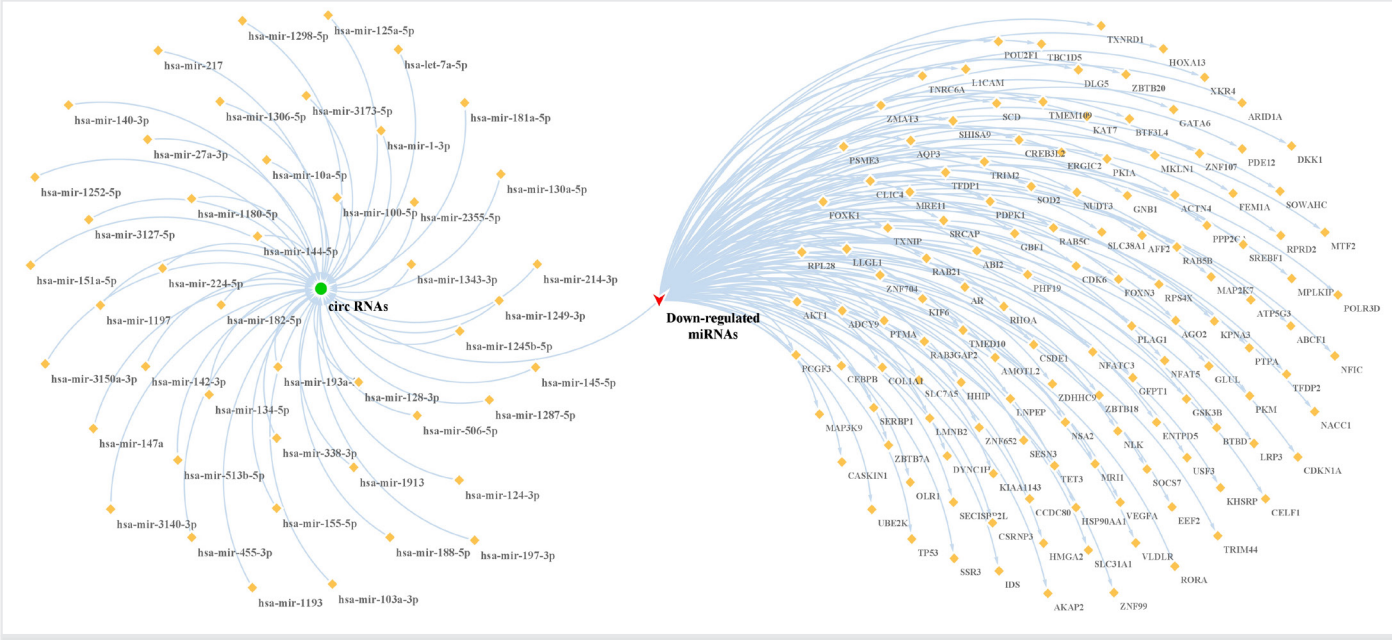

Supplementary Figure 1. The network reveals potential circRNA-miRNA interactions and miRNA-target gene interactions

Original Article

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Clinical Utility of Molecular Diagnostics in Children with Respiratory Infections

Çocuklarda Solunum Yolu Enfeksiyonlarında Moleküler Tanı Yöntemlerinin Klinikte Kullanımı

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ABSTRACT

Objective: Lower respiratory tract infections (LRTI) are among the major causes of mortality in children worldwide. Our study aimed to determine viral agents in children under five years of age who were followed up with acute LRTI.

Methods: Nasopharyngeal swab samples were taken from children aged 1 month to 5 years who were diagnosed with LRTI at the Bezmialem Vakıf University pediatric emergency department between March 1, 2015 and January 31, 2016. Cases with underlying chronic diseases were excluded from the study. The presence of the agent was investigated in the samples taken using the real-time polymerase chain reaction method. The distribution of cases in which the agent was detected according to age groups and seasons was examined. The relationship between the agent and the clinical and laboratory findings was investigated.

Results: Of the 95 patients included in the study, 51 (53.7%) were male, 44 (46.3%) were female, and the mean age was 26.2 months. The presence of viruses was shown in 50 cases (52.6%). The most frequently detected viruses were rhinovirus (28%), human bocavirus (HBoV) (26%) and respiratory syncytial virus (RSV) (24%). While RSV infections were more common in winter months, HBoV was observed to persist throughout the year. No significant relationship was found between the clinical and laboratory findings of the patients and the agents.

Conclusion: Viral etiology was detected in 52.6% of the cases. Molecular tests are valuable in detecting more than one virus in the distribution of viruses among different age groups.

Keywords: Infections, respiratory tract, multiplex polymerase chain reaction

ÖZ

Amaç: Alt solunum yolu enfeksiyonları (ASYE) tüm dünyada çocuklarda mortalite nedenleri arasında önemli yer tutmaktadır. Çalışmamızda akut ASYE tanısıyla izlenen beş yaş altındaki çocuklarda viral etkenlerin belirlenmesi amaçlanmıştır.

Yöntemler: Bezmialem Vakıf Üniversitesi çocuk acil servisinde 1 Mart 2015-31 Ocak 2016 tarihleri arasında ASYE tanısı alan 1 ay-5 yaş arası çocuklardan nazofarengeal sürüntü örnekleri alınmıştır. Altta yatan kronik hastalığı olan olgular çalışma dışı bırakılmıştır. Alınan örneklerde gerçek zamanlı polimeraz zincir reaksiyonu yöntemiyle etken varlığı araştırılmıştır. Etken tespit edilen olguların yaş gruplarına ve mevsimlere göre dağılımı incelenmiştir. Klinik ve laboratuvar bulguları ile etken ilişkisi araştırılmıştır.

Bulgular: Çalışmaya dahil edilen 95 hastanın 51'i (%53,7) erkek, 44'ü (%46,3) kız olup yaş ortalaması 26,2 aydır. Virüs varlığı 50 olguda (%52,6) gösterilmiştir. En sık saptanan virüsler, rinovirüs (%28), insan bocavirüs (HBoV) (%26) ve respiratuvar sinsiyal virüstür (RSV) (%24). RSV enfeksiyonları daha çok kış aylarında görülürken HBoV'nin yıl boyu sürdüğü gözlenmiştir. Hastaların klinik ve laboratuvar bulguları ile ajanlar arasında anlamlı ilişki saptanamamıştır.

Sonuç: Olguların %52,6'sında viral etiyoloji saptanmıştır. Moleküler testler virüslerin farklı yaş grupları arasındaki dağılımında birden fazla virüsün tespitinde değerlidir.

Anahtar Sözcükler: Enfeksiyonlar, solunum yolu, multipleks polimeraz zincir reaksiyonu

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Introduction

Lower respiratory tract infection (LRTI) is one of the most common conditions encountered in pediatric emergency departments during the winter months. Viruses are the most common etiological agents of LRTIs in infants and children under 5 years of age (1,2). Respiratory syncytial virus (RSV) is responsible for more than 50% of bronchiolitis cases (3). RSV and influenza viruses have been reported to cause 66,000-111,000 deaths annually in developing countries (3,4). In Türkiye, LRTI is the 6th most common disease exposed by children 0-6 years age (5).

Knowledge of the viral etiology may help cohorting in hospitalized patients or deciding on antiviral therapy. Although routine use of rapid diagnostic tests during emergency department is not recommended, it may decrease antibiotic use (1). In a retrospective cohort study among hospitalized children, multiplex polymerase chain reaction (PCR) tests for identification of respiratory viruses led to a decrease in utilization of healthcare resource (6).

Studies investigating viral pathogens in children with LRTI are limited in Türkiye (7-15). In this study, we sought to determine the predominant viral etiologies of LRTI in children at a tertiary care center in İstanbul. We also searched viral-viral detections and associations with the clinical findings.

Methods

Study Setting

Bezmialem Vakıf University Hospital is one of the sentinel hospitals in İstanbul. During the study period of March 2015 and January 2016, 271,625 children were treated at the emergency department.

Sample Detection

One hundred children attending to emergency department between March 2015 and January 2016 were tested with PCR methods for respiratory pathogens. Informed consent was requested to enter the study.

A nasopharyngeal sample was obtained from patients aged between 1 month and 5 years who had diagnosis of bronchitis, bronchiolitis, and pneumonia characterized by acute-onset cough or difficulty in breathing with fast breathing for age. Consolidation, infiltrate or effusion on chest X-ray (CXR) were considered as radiographic evidences of pneumonia (1). Patients with underlying diseases including cystic fibrosis, congenital heart disease, recurrent wheezing, neuromuscular diseases, and immunodeficiency; and those who had been hospitalized during the last 14 days were excluded. Data including age, sex, symptoms and signs at presentation, blood leukocyte count, C-reactive protein (CRP), CXR findings were recorded. Seventeen viruses: RSV; influenza A and B viruses; human parainfluenza virus (HPIV) types 1-4; human adenovirus (HAdV); human rhinovirus (HRV); human metapneumovirus (HMPV); human coronavirus (HCoV) (NL63, 229E, OC43, HKU1), human bocavirus (HBoV), human enterovirus (EV), and parechovirus (PeV); and five bacteria: *Chlamydia pneumoniae, Streptococcus pneumoniae, Haemophilus influenzae* type B, *Staphylococcus aureus,* and *Mycoplasma pneumonia*) were investigated with real-time PCR (RT-PCR). Among bacteria, only *C. pneumoniae* and *M. pneumoniae* were accepted as pathogens. The samples which were taken from the patient's nose by nasopharyngeal swab with 2-3 rotations were sent to the microbiology lab within maximum 30 minutes. After extraction of clinical samples, a commercial RT-PCR kit (FTD respiratory 21 (Fast Track) was used for identification of DNA and RNA of the respiratory pathogens. This kit used a set including primers and TaqMan probes which can detect one or more of these viruses. When the viral nucleic acid was of an RNA virus, a reverse transcription process occurred for production of cDNA. Then cDNA was duplicated by PCR using a primary probe specific to that virus. During PCR, amplicons were detected by measuring fluorescent radiation. None of the patients needed hospital treatment.

This study was approved by Bezmialem Vakıf University Clinical Research Ethics Committee with decision no 610/21 (date: 04.06.2014). The study was funded by Bezmialem Vakıf University (project number: 18245212-108.99).

Statistical Analysis

The SPSS 15.0 statistical program was used for analysis of the data. Demographic and clinical characteristics of patients with no virus detection, virus positive patients, and patients with viral-viral co-detection were compared using the Pearson chi-square and Kruskal-Wallis tests according to variable type.

Results

Patient Characteristics

Among 100 patients evaluated five were excluded because of underlying comorbidities. Among 95 children, mean age was 26.2 months and 53.7% were male (Table 1). Cough, wheezing, and fever were the most common clinical findings (100%, 70%, and 60% respectively). Mean leukocyte count was 11,600/mm^3 and CRP level was 2.84 mg/dL. Radiographic findings were hyperinflation, interstitial infiltrations, lineary atelectasis, lobar consolidation, peribronchial thickening, and lobar atelectasis. None were hospitalized or needed intensive care management.

Respiratory Viruses

Sixty two respiratory viruses from 50 patients were detected. The most commonly detected viruses were human rhinovirus (28%) followed by hBoV (26%), and RSV (24%) (Table 2). The mean age of children with human rhinovirus, HBoV and RSV infections were 25.7 months, 26.7 months, and 24.7 months, respectively. Co-detection of ≥2 viral pathogens was found in 11 patients (Table 3). The most common viral co-detection was HBoV and HMPV (3 patients). While the majority (72.7%) of HBoV infections presented as viral co-infections, all samples with HPIV positivity were single infections. RSV infections were mostly seen in the winter. However, HBoV was found throughout the year (Figure 1).

Table 1. Demographic and clinical characteristics of pediatric patients with LRTI

Variable (%)	No virus detected (n=45)	Single virus positive (n=39)	Viral-viral coinfection (n=11)	p-value
Age (months)[a]	28.06	21.79	35.09	0.107
Symptoms (%)				
Wheezing	73.3	64.1	81.8	0.445
Fever	51.1	64.1	81.8	0.140
Nasal discharge	44.4	38.5	45.5	0.834
Difficulty in breathing	20	17.9	27.3	0.792
Nasal obstruction	6.7	7.7	0	0.645
Cyanosis	0	2.6	9.1	0.164
Signs (%)				
Rales	60	71.8	72.7	0.465
Prolonged expirium	48.9	69.2	54.5	0.165
Rhonchi	42.2	25.6	63.6	0.051
Retractions	28.9	30.8	18.2	0.713
Lab findings				
WBC	11.545	12.422	12.100	0.577
PNL (%)	50.52	50.05	57.90	0.540
CRP	1.71	2.30	1.56	0.581
CXR ordered (%)	80	89.7	100	0.161
Use of antibiotics (%)	36.4	53.8	50	0.265

[a]Mean, WBC: White blood cells, PNL: Polymorphonuclear leukocytes, CRP: C-reactive protein, CXR: Chest X-ray, LRTI: Lower respiratory tract infection

Table 2. Distribution of viruses according to age groups

	Total n (%)	Single infection n (%)	Viral-viral co-infection n (%)	Mean age, months (SD)	Age (months)			
					1-6	7-12	13-24	25-60
HRV	14 (28)	13 (92.8)	1 (7.1)	25.7 (17.1)	1 (7.1)	4 (28.5)	2 (14.2)	7 (50)
HBoV	13 (26)	5 (38.4)	8 (61.5)	26.7 (20.1)	2 (15.3)	4 (30.7)	1 (7.6)	6 (46.1)
RSV	12 (24)	7 (58.3)	5 (41.6)	24.7 (18.2)	1 (12.5)	4 (23.5)	2 (28.5)	5 (16.6)
HPIV	8 (16)	8 (100)	0	26.8 (22.7)	3 (37.5)	1 (5.8)	0	4 (13.3)
HMPV	4 (8)	1 (25)	3 (75)	33 (12.3)	0	2 (11.7)	0	2 (6.6)
HAdV	3 (6)	1 (33.3)	2 (66.6)	48 (12)	0	0	0	3 (10)
PeV	3 (6)	2 (66.6)	1 (33.3)	10.8 (6.3)	1 (12.5)	0	2 (28.5)	0
HCoV	3 (6)	2 (66.6)	1 (33.3)	27 (15.5)	0	1 (5.8)	0	2 (6.6)
Human EV	2 (4)	0	2 (100)	24 (16.9)	0	1 (5.8)	0	1 (3.3)
Total	50	39 (78)	11 (22)	26.3 (20.7)	8 (16)	15 (30)	5 (10)	22 (44)

SD: Standard deviation, HRV: Human rhinovirus, HBoV: Human bocavirus, RSV: Respiratory syncytial virus, HPIV: Human parainfluenza virus, HMPV: Human metapneumovirus, HAdV: Human adenovirus, PeV: Parechovirus, HCoV: Human coronavirus, EV: Enterovirus

Comparison of Patients with No Virus Detection, Single Virus Positive Patients, and Patients with Viral-viral Co-detection

Mean age of subjects with viral-viral co detection was higher than those with single infections (35 months vs 21.7 months) but without reaching statistical significance (Table 1). Clinical findings, order of chest radiographs, and use of antibiotics were not significantly different between the three groups.

Viral-bacterial Co-detection

Twenty one patients had simultaneous detection of bacteria with viruses. *M. pneumoniae* (1 of 5), *H. influenzae* type b (5 of 7), *S. aureus* (4 of 8), and *S. pneumoniae* (19/28) were detected as co-detections with viruses.

Table 3. Distribution of viruses in patients with viral-viral co-infection

Pathogens detected	Number (%)	Mean age, months
Co-infection, two viruses	10	
HBoV+HMPV	3 (27.2)	40
RSV+HboV	2 (18.1)	36
HBoV+HAdV	1 (9)	36
HBoV+human EV	1 (9)	12
RSV+PeV	1 (9)	14
RSV+HAdV	1 (9)	60
HRV+human EV	1 (9)	36
Co-infection, three viruses	1	
RSV+HBoV+HCoV-NL63	1 (9)	36
Total	11 (100)	35

HBoV: Human bocavirus, HMPV: Human metapneumovirus, RSV: Respiratory syncytial virus, HAdV: Human adenovirus, EV: Enterovirus, PeV: Parechovirus, HRV: Human rhinovirus, HCoV: Human coronavirus

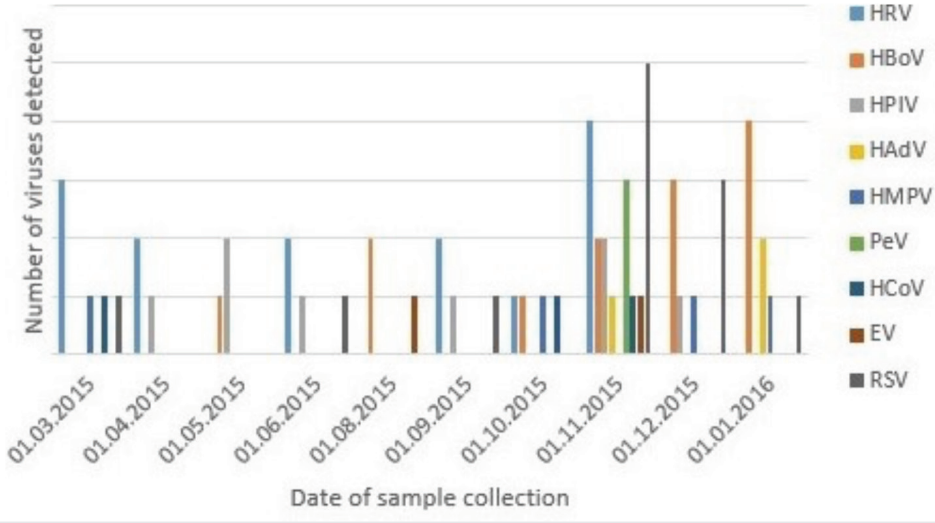

Figure 1. Comparison of viral seasonality

HBoV: Human bocavirus, HMPV: Human metapneumovirus, RSV: Respiratory syncytial virus, HAdV: Human adenovirus, EV: Enterovirus, PeV: Parechovirus, HRV: Human rhinovirus, HCoV: Human coronavirus

Discussion

Here we investigated a plenty of respiratory pathogens (17 viruses) among children with LRTI. Viruses were detected in 52.6% of patients. HRV (28%), HBoV (26%), and RSV (24%) were the most commonly identified pathogens. Other detected viruses were HPIV (16%), HMPV (8%), HAdV (6%), PeV (6%), HCoV (6%), and human EV (4%). RSV infections were mostly seen in the winter. However, HBoV infections were found throughout the year. Adenoviral infections occurred in older children, and PeVs were detected in younger children (mean age 48 months vs 10.8 months, respectively. Among infants and young children with bronchiolitis and pneumonia, RSV is the most common etiological agent which can lead to epidemics in winter and early spring. An observational and prospective study in Spain identified RSV in 52.9% of children with LRTI (16). A previous study from Türkiye identified RSV as the leading pathogen (55.6%) followed by HPIV (27.8%), HMPV (13%), influenza A virus (9.3%), and HAdV (5.6%) (7). Gökçe et al. (8) examined 316 children younger than 24 months who were hospitalized for acute viral bronchiolitis. Of the 316 children, 237 showed at least one respiratory tract pathogen (75% of the participants). RSV was the most common virus (40.1%) followed by HRV (n=78, 24.6%). HRV, the etiologic agent of common cold, may contribute to community acquired pneumonia. It is one of the most commonly detected respiratory viruses among childhood respiratory diseases (12-79%) (17). Since it is frequently identified as a co-infecting pathogen and among asymptomatic children, the role of HRV in severe LRTI is unclear (18). HBoV infection prevelance was reported as

5-19% in children with acute LRTI (19). Midilli et al. (14) reported that HBoV was identified in 6.5% of children with LRTI. Uyar et al. (13) evaluated 62 children with bronchiolitis in comparison to 33 healthy children for the presence of respiratory viruses. HBoV was detected in 4.8% of patients. HRV was the most commonly detected virus in the control group while none of the samples was positive for HBoV (13). Demirci et al. (15) showed HBoV positivity rate as 6.7% for children 1 month to 56 months. Having of a sick sibling and number of children at home increased the risk (15).

We identified that 10.5% patients had two or more respiratory viruses. Among co-detected viruses HBoV was the leading virus. HBoV is reported as an infrequent sole cause of acute bronchiolitis (20). Children with viral co-detections tended to be older than those with single infections. Co-infection with multiple viruses has been reported in 8.4-36.1% of children with LRTI (21). Cebey-López et al. (16) conducted an observational, prospective study in Spain among children with LRTI and showed that 45.1% of children had viral co-infections (11). The most frequent co-infection patterns were RSV-HRV. Among a comparison cohort of 97 children from United Kingdom, 5 patients (5.2%) had viral co-infections (RSV-HBoV/HBoV-influenza). Gökçe et al. (8) performed a cross-sectional, descriptive study in İzmir, with 316 children younger than 24 months who were hospitalized for acute viral bronchiolitis. RSV and HRV had the highest co-infection rate (22.3%).

There are conflicting data on whether viral co-infection is associated with increased severity in children with respiratory infections (21-23). We did not find any significant differences in clinical or laboratory findings, order of chest radiographs, and use of antibiotics among patients with no virus detection, virus positive patients, and patients with viral co-detection. There is substantial diversity in the management of LRTI reflecting the absence of a consensual treatment. Although combinations of clinical features have been proposed to distinguish viral from bacterial diseases, none are sufficiently sensitive to be reliable or widely used. Subramony et al. (6) reported that identification of a viral etiological agent decreased use of antibiotics and chest radiographs among children hospitalized.

Viruses and bacteria residing in the upper respiratory tract have complex interactions. In this study *S. pneumoniae* and *H. influenzae* were more likely to be present in the nasopharynx in combination with respiratory viruses.

Study Limitations

A limitation of our study was that there was no control group. Many types of bacteria and viruses can be present as part of the natural flora without causing an infection. A systematic literature review of 23 studies showed that RSV, influenza viruses, parainfluenza viruses, and HMPV had strong evidence for LRTI (24). HRV was only weakly associated with LRTI symptoms.

The second limitation was that patients were enrolled during only 11 months. The cost of broadly multiplexed molecular test is high and only 100 patients could be selected for sampling. Surprisingly, there were no influenza positive cases. According to Turkish Ministry of Health General Directorate of Public Health, influenza activity in Türkiye started at October, reached peak at December, and finished at the end of February in 2015. In 2016, influenza cases cumulated between February and March. Since the study was completed at January we could have missed influenza cases. Long term follow up is needed to obtain more important information.

Conclusion

We detected viruses in more than half of children with LRTI. Although some of these viruses might represent carriage we thought that at least one third were true pathogens. There was no significant difference in clinical findings or clinical course between patients with no virus detection, with single virus detection, and with double/multiple detection. Interestingly, respiratory symptoms such as wheezing and fever were slightly higher in the "coinfection" group and there was a higher rate of antimicrobial prescriptions (36.4-53.8%) in patients with identified viruses. Molecular tests are helpful to identify the etiology of LRTI. However the efficacy was limited in the emergency department setting unless results were obtained quickly and clinicians were notified.

Ethics

Ethics Committee Approval: This study was approved by Bezmialem Vakıf University Clinical Research Ethics Committee with decision no 610/21 (date: 04.06.2014).

Informed Consent: Informed consent was requested to enter the study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: İ.T., B.S., B.B.D., U.E., F.U.K., Ö.T., Concept: İ.T., B.S., B.B.D., U.E., F.U.K., Ö.T., Design: İ.T., B.S., B.B.D., U.E., F.U.K., Ö.T., Data Collection or Processing: İ.T., B.S., B.B.D., U.E., F.U.K., Ö.T., Analysis or Interpretation: İ.T., B.S., B.B.D., U.E., F.U.K., Ö.T., Literature Search: İ.T., B.S., B.B.D., U.E., F.U.K., Ö.T., Writing: İ.T., B.S., B.B.D., U.E., F.U.K., Ö.T.

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

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Original Article

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Ambulances Under Fire: A Cross-sectional Analysis of Terrorist Attacks on Ambulances and Their Medical Implications

Ateş Altındaki Ambulanslar: Ambulanslara Yönelik Terörist Saldırılarının ve Tıbbi Etkilerinin Kesitsel Bir Analizi

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ABSTRACT

Objective: This study aims to conduct an epidemiological analysis of the country, race, weapon type, and type of attack, along with the medical consequences of terrorist attacks on ambulances.

Methods: The population of this retrospective cross-sectional epidemiological study consisted of terrorist attacks on ambulances, as documented in the Global Terrorism Database provided by The National Consortium for the Study of Terrorism and Responses to Terrorism, covering the period from 1970 to July 2021. Incidents, deaths, and injuries from these terrorist attacks were analyzed according to country, attack type, and weapon type. Additionally, incidents, deaths, injuries, and material losses from attacks against ambulances were analyzed by year.

Results: In the last 52 years, 80 terrorist incidents occurred, and 444 people were adversely affected medically in 75 of these incidents. In 96.3% of these cases, the terrorist organization achieved its desired goal, and 71.3% resulted in material loss. During this period, terrorist attacks on ambulances were reported in 27 countries. In 17 of these incidents, the nationality of the attacker was different from that of the target person, and 70 people were adversely affected medically. These incidents featured seven different types of attacks and five different types of weapons. The most preferred type of attack was bombing (n=38), while the most commonly used weapon type was explosives (n=39).

ÖZ

Amaç: Bu çalışma, ambulanslara yapılan terörist saldırıların tıbbi sonuçları ile birlikte ülke, ırk, silah türü ve saldırı türünün epidemiyolojik bir analizini yapmayı amaçlamaktadır.

Yöntemler: Bu retrospektif kesitsel epidemiyolojik çalışmanın evreni, 1970'ten Temmuz 2021'e kadar olan dönemi kapsayan, Ulusal Terörizm ve Terörizme Tepki Araştırmaları Konsorsiyumu tarafından sağlanan Küresel Terörizm Veri Tabanı'nda belgelendiği gibi, ambulanslara yönelik terör saldırılarından oluşuyordu. Bu terör saldırılarından kaynaklanan olaylar, ölümler ve yaralanmalar ülkeye, saldırı türüne ve silah türüne göre analiz edildi. Ayrıca, ambulanslara yönelik saldırılardan kaynaklanan olaylar, ölümler, yaralanmalar ve maddi kayıplar yıllara göre analiz edildi.

Bulgular: Son 52 yılda 80 terör olayı meydana geldi ve bu olayların 75'inde 444 kişi tıbbi olarak olumsuz etkilendi. Bu olguların %96,3'ünde terör örgütü amacına ulaşmış, %71,3'ü maddi kayıpla sonuçlanmıştır. Bu dönemde 27 ülkede ambulanslara yönelik terör saldırıları bildirildi. Bu olayların 17'sinde saldırganın uyruğu hedef kişininkinden farklıydı ve 70 kişi tıbbi olarak olumsuz etkilendi. Bu olaylarda yedi farklı saldırı türü ve beş farklı silah türü yer aldı. En çok tercih edilen saldırı türü bombalama (n=38), en sık kullanılan silah türü ise patlayıcılar (n=39) oldu.

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ABSTRACT

Conclusion: In the last six years, there has been an increase in terrorist attacks, adversely affecting many people medically. The main reasons for this increase include growing civil unrest in countries, easy access to ambulances, perceived lack of threat, and a rise in individual armament. Due to these issues, it is crucial to train health workers, take precautions, make preparations, and create emergency action plans to mitigate terrorist attacks.

Keywords: Prehospital care, healthcare security, ambulance services, terrorist attacks, public health safety

ÖZ

Sonuç: Son altı yılda, birçok insanı tıbbi olarak olumsuz etkileyen terör saldırılarında bir artış oldu. Bu artışın başlıca nedenleri arasında ülkelerde artan sivil huzursuzluk, ambulanslara kolay erişim, algılanan tehdidin eksikliği ve bireysel silahlanmadaki artış yer alıyor. Bu sorunlar nedeniyle terör saldırılarını azaltmak için sağlık çalışanlarının eğitilmesi, önlem alınması, hazırlıkların yapılması ve acil eylem planlarının oluşturulması büyük önem taşımaktadır.

Anahtar Sözcükler: Hastane öncesi bakım, sağlık güvenliği, ambulans hizmetleri, terör saldırıları, halk sağlığı güvenliği

Introduction

Terrorist attacks involve acts of threat, fear and violence, both nationally and internationally. This attack is made up of a pre-designed structure with the aim of causing mass casualties (1), injuries, destruction, and excessive fear within society (2). This structure can target the health system both directly and indirectly. The reason for this is that health services are accessible 24/7, are a public sphere and contain a large number of people, materials and information. In other words, healthcare is considered as a soft target for terrorist (3).

Attackers carry out terrorist acts against health services in various ways. These acts have a negative impact on the health system. For example, as a result of terrorist acts directly or indirectly targeting the health system, a large number of people may be in urgent need of medical care and endanger health personnel (4). In addition, attacks on health services cause physical and psychological injuries, destruction of infrastructure, disruptions in maternal and child health care, restricted access and fear of going to health services (5). Attacks targeting healthcare workers occur both intentionally and unintentionally. These attacks include delaying, obstructing, damaging ambulances, stealing medical devices such as ambulances, or directly attacking or kidnapping personnel (6). In addition, after a terrorist attack, ambulance crews arriving at the scene are at risk of becoming the target of a secondary attack.

In the literature, it is noted that terrorists sometimes target or use ambulances because these vehicles can increase the destructive power of an attack, do not arouse suspicion, provide access to secure areas, and can carry a large number of bombs (1,7). Therefore, it is extremely important to detect, prevent and manage terrorist acts against ambulances and to develop data collection and reporting mechanisms. In the literature, there are studies that retrospectively analyse the impact of terrorist attacks on the health system (1,5,8-12). However, there are no studies that medically analyse the terrorist attacks against ambulances. In this study, it is aimed to analyse the medical dimension of terrorist attacks against ambulances in the world with various variables.

Methods

This study is a cross-sectional epidemiological study. The population of the study consists of terrorist attacks against ambulances between 1970 and 2021 taken from the Global Terrorism Database (GTD) data file provided by The National Consortium for the Study of Terrorism and Responses to Terrorism (START). An epidemiological analysis of the scene (country), year distribution, weapon, type of attack, attacker and material damage and medical consequences of terrorist attacks on ambulances was aimed.

Data Source

The data source of the study was the GTD data file available on the START website, which is a database on terrorism. GTD is a free access database containing information on terrorist incidents that occurred in the world between 1970 and July 2021 (except 1993). The database systematically records information on terrorist incidents, both national and international, and currently contains more than 200,000 incidents. For each incident, information is available on a number of variables such as the year of the incident, the location, the number of injured and killed, the target and the group responsible. Publications produced from GTD data are regularly available on the START website (13). This study constitutes a secondary survey of GTD data.

Definitions

START, also known as START, is a research and education centre that conducts scientific research on the causes and medical consequences of national and international terrorism. START website has many databases such as START datasets, IVEO Knowlege Matrix, GTD, Big Allied and Dangerous, TEVUS Portal, PIRUS dataset, Nuclear Facilities Attack Database (14).

The GTD, available on the START website, is a database containing systematic data on national and international terrorist incidents. According to this database, a terrorist attack is defined as the unlawful threat or use of force and violence by a non-state person or group (actor) to achieve a political, economic, religious or social objective through fear, coercion or intimidation (15). In order for an incident to be included in the GTD, it must be intentional and the incident must constitute a certain level of violence or an immediate threat of violence.

Inclusion Criteria

In order for an event to be considered as a terrorist attack, it is included in GTD's raw data file if it meets the three inclusion criteria predetermined by GTD:

- Criterion 1: The act must be aimed at attaining a political, economic, religious, or social goal.

- Criterion 2: There must be evidence of an intention to coerce, intimidate, or convey some other message to a larger audience (or audiences) than the immediate victims.

- Criterion 3: The action must be outside the context of legitimate warfare activities.

Events are excluded when an event does not fulfil any of GTD's three inclusion criteria or when there is uncertainty. These criteria of GTD are determined within the database, not by the authors.

In order to achieve the aim of the study, the researchers developed inclusion criteria among 135 variables in the GTD (Table 1). Events including these criteria were included in the study.

Data Preparation

The GTD metadata file was downloaded from the START website in November 2023. The downloaded GTD metadata file was uploaded to IBM SPSS Statistic Version 19 and a search was performed for terrorist attacks targeting ambulances. As a result of the search, the data suitable for the inclusion criteria of the study were saved in the Microsoft Excel file. At the stage of downloading the GTD metadata file, data for the period between August 2021 and 2023 were not yet available, so data for the relevant years were not recorded.

Statistical Analysis

Frequency analysis was performed to determine the number of incidents, deaths and injuries of terrorist attacks against ambulances by year, country, weapon and type of attack. The data of the study was designed to retrospectively analyse the scene, weapon, attack, perpetrator information, material damage and medical outcomes of terrorist attacks on ambulances based on an open access data set. Therefore, ethics committee review was not sought for the data of the study. The identities of the attackers in the GTD metadata file were removed from the study data file. The accuracy of GTD data was not evaluated.

Results

General Results

There were 80 terrorist incidents against ambulances in the world between 1970 and July 2021, which meet the inclusion criteria of the study. In 96.3% of these terrorist attacks, the terrorist organisation achieved the desired target and 71.3% of these attacks resulted in material loss. However, since 16% of the terrorist incidents against ambulances were lost due to various reasons in GTD data, 75 terrorist attacks were analysed. Four hundred forty four people were adversely affected medically by these terrorist attacks.

Table 2 analyses the number of incidents, injured and dead according to logistical, ideological and miscellaneous variables of terrorist attacks against ambulances. The logistical variable made a comparison between the nationality of the attacker and the country where the attack took place. This table revealed that among 7 terrorist incidents, the attackers' nationalities diverged from the regions where the attacks occurred, resulting in adverse

Table 1. GTD variables and subcategories in the study

Variables	Subcategories
Date	Year (1970- July 2021)
Inclusion criteria	Criterion 1, criterion 2, criterion 3
Country	All
Attack type	Assassination, hijacking, kidnapping, barricade incident, bombing/explosion, armed assault, unarmed assault, facility/infrastructure attack, unknown
Succes of attack	Yes/no
Suicide attack	Yes/no
Weapon type	Biological, chemical, radiological, nuclear, firearms, explosives, fake weapons, incendiary, melee, vehicle, sabotage equipment, other, unknown
Casualties and consequences	Total number of fatalities Total number of injured Property damage (yes/no/unknown) Extent of property damage
International - logistical	Yes/no/unknown
International - ideological	Yes/no/unknown
International - miscellaneous	Yes/no/unknown

GTD: Global Terrorism Database

medical consequences for 14 individuals. Ideological variable enabled a comparison between the nationality of the attacker and the nationality of the target. As per the data presented in this table, it was evident that among the 17 terrorist incidents examined, the nationality of the perpetrator diverged from that of the target individual, resulting in adverse medical repercussions for 70 individuals. Miscellaneous variable showed whether the attacker targeted a person of a different nationality. According to this table, it was concluded that there was no incident in which the attacker targeted a person of a different nationality.

Medical Outcomes and Property Damage by Year and Country

Table 3 shows the distribution of incidents, injuries, deaths and material losses according to the years of terrorist attacks against ambulances. According to this table, the first terrorist attack against ambulances occurred in 1985. With the increase in terrorist attacks between 2000 and 2014, 219 people were adversely affected medically during these years. In the last six years, it was seen that the number of terrorist attacks and material loss was the highest compared to other years. While the highest number of attacks on ambulances occurred in 2013 and 2019, the highest number of deaths (n=26) and injuries (n=53) occurred in 2018 compared to other years.

Table 4 shows the medical analysis of the distribution of terrorist attacks against ambulances according to the countries. According to this table, terrorist attacks on ambulances occurred in 27 countries. Iraq was the country where the highest number of terrorist incidents occurred among 27 countries and the most medically affected country. Libya was the most medically affected country after Iraq. The most medically affected countries after Libya were Thailand, Yemen and Syria respectively. It was concluded that Türkiye, Israel, India, Cameroon and Central Africa Republic countries were not medically affected by terrorist attacks on ambulances.

Analysis of Medical Outcomes by Type of Attack and Weapon

Table 5 shows the medical analysis of terrorist incidents against ambulances in the world according to attack and weapon types.

Table 2. Analysis of medical results based on logistical, ideological, and miscellaneous categories

Logistical	Event	Wound	Kill
International	7	9	5
Domestic	38	121	82
Unknown	30	153	74
Ideological	**Event**	**Wound**	**Kill**
International	17	43	27
Domestic	28	87	60
Unknown	30	153	74
Miscellaneous	**Event**	**Wound**	**Kill**
International	-	-	-
Domestic	72	274	161
Unknown	3	9	-

According to this table, it was seen that 7 different attack types and 5 different weapon types were preferred in terrorist incidents against ambulances. While the most preferred type of attack in the incidents against ambulances was bombing, the most preferred weapon type was explosive weapons. Among the attack types, bombing and armed assault caused more deaths and injuries than other attack types. Among the weapon types, explosive and firearms types had the highest number of deaths and injuries.

Discussion

This study holds significance as the inaugural attempt to epidemiologically delineate the weaponry, attack modality, and medical ramifications across 75 terrorist incidents targeting ambulances worldwide from 1970 to July 2021. Moreover, the study encapsulates three distinct subcategories: the overarching findings of the 75 terrorist assaults; medical outcomes and material damages categorized by annual distribution and impacted nations; and a nuanced analysis of medical consequences contingent upon attack and weapon typology.

In the literature on health services, it is emphasised that there are many terrorist attacks targeting facilities rather than personnel (16). Within the array of attacks directed at facilities, ambulance services, which administer initial care to patients and facilitate their transfer to appropriate hospitals, are included. Attacks on ambulances result in the potential deprivation of urgent care

Table 3. Incident, death, and injury counts, and material damage by year distribution

Year	Event	Wound	Kill	Property
1985	1	6	4	1
1987	2	1	2	2
1989	1	-	-	1
1990	1	-	1	-
1994	1	-	2	-
1997	1	-	2	1
1998	1	2	-	1
2007	2	1	2	2
2008	1	5	-	-
2009	4	27	13	2
2011	2	45	18	2
2012	3	37	9	3
2013	9	42	20	5
2014	6	5	5	5
2015	7	3	10	6
2016	6	11	7	4
2017	4	6	3	3
2018	8	53	26	5
2019	9	19	9	5
2020	6	7	12	5
2021	5	13	16	4
Total	**80**	283	161	57

for individuals or the community, pose threats to healthcare professionals, and contribute to the system's weakening (4). In the analysis of this study, it was observed that 444 individuals were adversely affected medically due to attacks on ambulances. Moreover, it was concluded that these terrorist attacks occurred frequently in the last six years (Table 3). This is believed to stem from assailants having easy access to ambulances, minimal threat perception, and possessing significant capacity for carrying weapons (17). However, the reason for the increase in attacks on ambulances in recent years may be due to the under-reporting of terrorist incidents in previous years (3).

Attacks on healthcare services tend to be prevalent in countries experiencing internal conflicts and wars (3). For example, it is emphasised that terrorist attacks on health care have increased, as reported in the Russian-Ukranian war, the attack on the occupied Palestinian territories and the wars in Syria (5). In the findings of the study, it was seen that terrorist attacks on ambulances occurred in 27 countries and the most affected countries are the regions where there was war such as Iraq, Yemen, Libya and Syria. Another factor that causes ambulances to be targeted in regions where there is civil unrest or war is that healthcare workers come from a different country or ethnic group (18). In this study, analysis revealed that in 17 instances of terrorist attacks, the nationality of the assailant differed from that of the target, resulting in adverse medical effects on 70 individuals. Additionally, findings indicated attacks on ambulances in countries without ongoing warfare or internal strife. This occurrence was attributed to disruptions during various protests, impeding the ambulances' operations at the scene. For instance, a report detailing an attack on an ambulance highlighted the assailants' intent to hinder the transport of an injured police officer to the hospital by assaulting the ambulance (19).

Table 4. Incident counts and medical analysis by country

Country	Incident	Wound	Kill
Afghanistan	3	3	5
Algeria	2	-	9
Burkina Faso	3	-	6
Cameroon	1	-	-
Central African Republic	1	-	-
Colombia	2	1	-
Egypt	8	9	2
El Salvador	1	-	2
India	1	-	-
Iraq	16	129	58
Israel	1	-	-
Kenya	3	7	5
Libya	2	40	21
Mali	2	-	7
Myanmar	1	-	1
Nicaragua	2	1	-
Pakistan	5	4	5
Philippines	4	6	3
Somalia	3	5	1
Sri Lanka	1	6	4
Sudan	1	2	0
Syria	2	17	11
Thailand	1	35	4
Türkiye	1	-	-
Ukraine	2	2	1
Yemen	5	13	16
Yugoslavia	1	2	-

Various types of attacks and weapon usage are employed against the healthcare system. In incidents targeting ambulances within the study, the most commonly preferred attack type was bombing, while the predominant weapon type was explosive devices. In an analysis of attacks on primary healthcare services, it was emphasized that 55% of the assaults involved bombings, while among weapon types, 55% comprised the use of explosive devices (12). The reason for the widespread use of this type of weapon is that it is relatively easy and inexpensive to manufacture, simple to use and easy to execute, requiring a small number of attackers (9). However, it has been observed that the use of these types of weapons has increased in recent years due to their easy production, easy crossing of international borders and easy availability (20). This increase leads to a greater medical impact on those at the scene and more people dying.

Reducing easy access and developing incident preparedness/response plans are important to reduce the frequency and medical consequences of terrorist attacks on ambulances (21). A study on health care emphasises that ambulances are often left unguarded and unlocked (7). Therefore, in the event of a terrorist attack, medical personnel should leave the ambulance in a safe place, minimise unattended time, choose safe routes, lock the vehicle and know what to do in the event of an attack. The International Committee of the Red Cross, in its report Ambulance and Prehospital Services in Risky Situations, emphasises that to prevent

Table 5. Incident, wound, and kill counts by type of attack and weapon

Attack type	Event	Wound	Kill
Assassination	2	-	3
Armed assault	25	21	18
Bombing	38	256	137
Hijacking	3	1	2
Kidnapping	1	-	-
Facility/Infrastructure attack	4	-	-
Unknown	2	5	1
Weapten type	**Event**	**Wound**	**Kill**
Firearms	30	21	20
Explosives	39	138	257
Incendiary	3	-	-
Melee	1	-	2
Unknown	2	5	1

and mitigate terrorist attacks on ambulances, local legislation for ambulances should be strengthened, the roles and responsibilities of stakeholders should be clearly defined and coordination with all stakeholders, including the armed forces, should be ensured (18). These actions are considered to contribute to the reduction of terrorist attacks against ambulances.

Study Limitations

There are several limitations in this study. First, as mentioned in the methodology, the data of the study were obtained from the GTD database. GTD comprehensively records global terrorist incidents in its database. This database uses media reports to obtain data on terrorist incidents. Therefore, there is a possibility of bias and data gaps in the available data and the study constitutes a secondary analysis of GTD data. However, as the database records terrorist attacks perpetrated by non-state actors, not all terrorist attacks on ambulances may be included. The GTD database does not include terrorist attacks that occurred after July 2021. Therefore, terrorist incidents against ambulances in the last two years were not included in the study. Finally, there were 80 terrorist attacks against ambulances in the GTD database. However, since the data on 5 terrorist attacks were missing, the medical outcomes of 75 terrorist attacks were analysed.

Conclusion

Terrorism threatens health care both nationally and internationally. This threat to health care causes many people to die, disrupting the system and restricting access. Ambulances are the first team to arrive at the scene after an incident. Therefore, they are at risk of being exposed to terrorist attacks both directly and indirectly. In the last fifty-two years, there were 80 terrorist attacks on ambulances and 444 people were adversely affected medically in 75 incidents. These terrorist incidents occurred mostly in countries where there was civil unrest or war and were the most medically affected countries. The most common type of explosive weapon was used in terrorist incidents and this type of weapon caused more people to be adversely affected medically than other types of weapons. When compared by years, there has been an increase in terrorist incidents in the last six years. The main reasons for this increase are the increase in civil unrest in the countries, easy access to ambulances, lack of threat and increase in individual armament. Due to these problems, it is necessary to train healthcare workers, take precautions, make preparations and create emergency action plans against terrorist attacks against ambulances. In addition, strategies for healthcare services against terrorist attacks should be developed.

Ethics

Ethics Committee Approval: Ethics committee approval is not required.

Informed Consent: Informed consent is not required.

Footnotes

Authorship Contributions

Concept: K.K., C.Ç., Design: K.K., C.Ç., Data Collection or Processing: N.D., C.Ç., H.K., Analysis or Interpretation: K.K., N.D., C.Ç., H.K., Literature Search: N.D., C.Ç., H.K., Writing: K.K., N.D.

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Original Article

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A Mobile Application Designed for Adults at Risk of Developing Diabetes: A Study Protocol for a Randomized Controlled Trial

Diyabet Riski Olan Erişkinlere Yönelik Olarak Mobil Uygulama Geliştirilmesi: Randomize Kontrollü Çalışma Protokolü

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ABSTRACT

Objective: The primary aim of this study is to develop a prediabetes mobile application (PREDIABE-T^{R}) designed in Turkish containing information and advice for individuals at risk of developing diabetes; the secondary aim is to determine whether the use of this application can make a difference in the participants' eating according to the Mediterranean diet plan, or in their physical activity and other diabetes-related metabolic parameters.

Methods: The adults in the experimental group will be using the PREDIABE-T^{R} mobile application for a period of 6 months. The application consists of a pedometer, a diet diary, and sections on diabetes risk, an instructor and a body mass index calculator. Individuals can use the mobile app to contact a public health nurse or academic on a 24/7 basis. Public health nursing can thus perform a consulting role within this framework. Over the same period, the control group will use the Turkish Nutrition Guide and the Diabetes Checklists mobile application distributed by the Turkish Ministry of Health. At the end of the six-month period, a review will be made of the diabetes metabolic data, physical activity levels and the Mediterranean diet eating behaviors.

Results: The benefits of interventions to promote a healthy lifestyle are evident in terms of preventing a transition from prediabetes to diabetes and maintaining present status.

ÖZ

Amaç: Bu çalışmanın birincil amacı diyabet riski olan bireylerde sağlıklı beslenme, fiziksel aktivite yapılması gibi bilgilendirme ve öğütler içeren Türkçe geliştirilmiş bir prediyabet mobil uygulama (PREDIABE-T^{R}) geliştirilmesi, ikincil amacı ise kullanımının katılımcıların Akdeniz tipi beslenme, fiziksel aktivite ve diyabete ilişkin metabolik parametrelerinde değişim yaratıp yaratmayacağını belirlemektir.

Yöntemler: Deney grubunda yer alan erişkinlere 6 ay boyunca PREDIABE-T^{R} mobil uygulaması kullandırılacaktır. Mobil uygulama adımsayar, beslenme takibi, diyabet riski, bilgilendirme ve beden kitle indeksi hesaplama bölümlerinden oluşmaktadır. Bireyler mobil uygulama aracılığıyla 7/24 araştırmacı olan halk sağlığı hemşiresi ve akademisyen ile iletişim kurabilmektedir. Bu çerçevede halk sağlığı hemşireliği danışmanlık rolü yerine getirilebilmektedir. Kontrol grubu ise aynı süreçte Sağlık Bakanlığı'nın Türkiye Beslenme Rehberi ve Diyabet Kontrol Listeleri mobil uygulamasını kullanacaktır. Altı aylık süreç sonunda her iki grubun da diyabet metabolik değerleri, fiziksel aktivite düzeyleri, Akdeniz tipi beslenme davranışları incelenecektir.

Bulgular: Prediyabetik dönemden diyabete geçiş sürecinin engellenmesinde ve mevcut durumun korunmasında sağlıklı yaşam tarzı müdahalelerinin yararlı rolü ortadadır.

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ABSTRACT

Conclusion: This study describes the effect of the use of a mobile application by individuals with prediabetes on metabolic parameters. If reductions can be achieved in metabolic parameters (such as HbA1c), it can be concluded that the mobile app is effective.

Keywords: Mediterranean diet, metabolic variables, mobile application, physical activity, prediabetes

ÖZ

Sonuç: Bu çalışma prediyabetli bireylerde mobil uygulama kullanımının metabolik parametreler üzerine etkisini yansıtmaktadır. Eğer metabolik parametrelerde (A1C gibi) düşme sağlanırsa mobil uygulama etkindir sonucu ortaya çıkacaktır.

Anahtar Sözcükler: Akdeniz tipi beslenme, metabolik değişkenler, mobil uygulama, fiziksel aktivite, prediyabet

Introduction

The "prediabetic" stage that is the interval leading to manifest diabetes from normal glucose metabolism is observed to switch into Type 2 diabetes in 5-10% of individuals each year (1). According to the data of the 10th Edition of the International Diabetes Federation (IDF) Diabetes Atlas, the prevalence of Impaired Glucose Tolerance (IGT) is 541 million (10.6%) while this rate corresponds to 319 million (6.2%) for Impaired Fasting Glucose (IFG). It is expected that these rates will reach 730 million (11.4%) for IGT and 441 million (6.9%) for IFG in 2045. While the incidence of prediabetes increases with age, it is estimated that there will be a pronounced rise in this rate in the next two decades. Projections for 2045 foresee that the prevalence of IFG will increase in all age groups and that in particular, IGT will display a rise in the young adult (<45 years) and older adult (70 years) populations (2). The data of Türkiye's Nutrition and Health Survey for 2017 reveal that the percentage of IFG, defined as fasting serum glucose level of 100-125 mg/dL, is 16.3% among individuals aged 15 and above, and 27.3% among those aged 19 years and above in Türkiye. The prevalence of IFG in individuals aged 15 and above is 15.9% among women and 16.8% among men (3).

There are a number of studies that have been conducted in Türkiye assessing various complications and variables related to prediabetes (4). We found in the accessible resources in the Turkish literature that there are a few studies on mobile applications designed to monitor individuals with diabetes (5), and others that evaluate the effect of such applications (5), but we have not found any research published on a Turkish mobile app for individuals with prediabetes or about the effect of such an app on metabolic variables. The aim of this study is to determine whether participants at risk of diabetes making use of a prediabetes mobile application (PREDIABE-T^{R}) designed in Turkish to inform and advise about healthy eating and physical exercise can record a difference in their implementation of the Mediterranean diet, their engaging in physical activity and in other diabetes-related metabolic parameters.

Hypotheses

Our hypotheses were formulated in line with population – intervention – comparison – outcome - study; significance was set at 0.05 (6). In addition to standard applications, the intervention group will be using the PREDIABE-T^{R} mobile app. The control group will only use standard applications. In this context, our research hypotheses are the following:

H1a: When compared with the control group, the eating behaviors with regard to adopting the Mediterranean diet of prediabetic adults using the PREDIABE-T^{R} app will be at a higher level.

H1b: When compared with the control group, the physical activity (MET, number of steps) of prediabetic adults using the PREDIABE-T^{R} app will be at a higher level.

H1c: When compared with the control group, the metabolic parameters (HbA1c, IFG, IGT) of prediabetic adults using the PREDIABE-T^{R} app will be at lower levels.

H1d: When compared with the control group, prediabetic adults using the PREDIABE-T^{R} app will lose more weight.

Methods

Study Design

This study protocol was drawn up for a single center, single-blind (participant), pretest-posttest, follow-up, parallel group (1:1 ratio) randomized controlled trial (Figure 1). The study protocol was prepared on the basis of the following standard protocol items: Recommendations for Interventional Trials (SPIRIT) guidelines (7) and the Consolidated Standards of Reporting Trials, Non-pharmacological Treatment Interventions Checklist (CONSORT-NPT) (8). The study was registered on ClinicalTrials.gov on NCT05592288.

Study Setting and Population

This study will be carried out at the Yıldırım Beyazıt Family Health Center (FHC) no. 9, a facility in the city of Kütahya, in western Türkiye. No. 9 is an FHC that addresses a large city population and where 5 physicians and 5 midwives work. The target sample will consist of prediabetic adults. The study will be conducted in the FHC between March and June 2024.

Sample Size Determination

The study population will consist of adults of the ages 40-65 who have received a diagnosis of prediabetes and are registered at the Yıldırım Beyazıt FHC no. 9. The sample size determined by

power analysis was based on calculations made with the G*Power program that were used in a similar study. Effect size was 0.666 (9); level of significance, 0.05; confidence interval limit 95%; testing power, 90%. The optimal number of participants calculated in the two-way hypothesis review was found to be 49 for each group. In previous studies (9), the size was increased by 10% to allow for losses. Ultimately, the decision was to enroll 59 participants in each group.

Inclusion Criteria

- Individuals to be included will be those who: are prediabetic (IFG=100-125 mg/dL-mmol/L, HbA1c=5.7-6.4% or IGT=140-199 mg/dL-mmol/L),

- are active Android/IOS cell phone users,

- are not pregnant or have any malignancy,

- have no hearing or vision impairment,

- are at least primary school graduates and fluent in Turkish.

Exclusion Criteria

- Individuals who have a diagnosis of diabetes or are using an insulin pump or oral antidiabetic agents,

- have vision impairment,

- are pregnant,

- have any condition that precludes engaging in physical activity,

- have psychiatric issues or problems with communicating, will be excluded.

Randomization

The stratified block randomization (1:1) will be used to attain objectivity in assigning individuals to the experimental group that will be using the mobile app and to the controls who will be following routine practices (10). The variable "gender" will be used as the control variable in the stratified block randomization (Figure 1).

Blinding and Preventing Bias

The researchers cannot be blinded in this study since they were the parties to develop the application and will be involved in the training. On the other hand, the participants can be blinded because they are to be using either the PREDIABE-T^{R} app or the Türkiye Nutrition Guide. In order to prevent bias, the collection of the randomized assignment data will be handled by a researcher who is not actively involved in the study; this researcher will submit the data in opaque envelopes to the implementing researcher.

Intervention Group

PREDIABE-T^{R} Mobile App Group

Prediabetic individuals 40 years of age and over will be asked to use the PREDIABE-T^{R} mobile app. The researcher will demonstrate how the application can be downloaded to the participants' phones and how to use it. Before the PREDIABE-T^{R} app is sent out to the participants, its content will be reviewed for suitability and content validity by health professionals and experts in health communications, informatics, and social media. The mobile app will be used and the process monitored for a period of 6 months. Each week, healthy eating and physical activity messages will be sent over the mobile app and a daily step count will be announced. Education about prediabetes, the risk of diabetes, the calculation of the body mass index (BMI), as well as other motivating self-evaluation tools and notifications will be sent out via the PREDIAB-T^{R} mobile app. The adherence of the experimental group to the intervention will be assessed in the software and encouraging messages will be sent out to ensure continued use of the app. For example: Bracelets inscribed with "Congrats! You've reached your daily steps goal!" or "Keep Active, Keep Protected from Diabetes!" will be distributed as gifts. If requested, the researcher will come to the FHC one day every week to meet with the participants.

The application consists of a total of 3 parts and 4 modules (Figure 2).

- Module 1: Personal Data

Containing data on the participant's age, gender, telephone number, email and perception of his/her health (bad, so-so, good, very good).

- Module 2: Medical History of the Participant

In this module, the participants tick the items that apply to themselves or their first-degree relatives by marking the conditions in their medical history that may increase the risk of prediabetes. Additionally, in line with the recommendations of the Turkish Association of Endocrinology and Metabolism, this module contains the Finnish Diabetes Type-2 Risk Score (FINDRISK) which assesses an individual's risk of diabetes (11).

- Module 3: Healthy Lifestyle Behaviors

The sub-sections of the module are devoted to nutrition, height-weight-BMI and physical activity.

Nutrition

Users can enter the foods they eat into the application and are informed of the benefit/harm of these foods with a red/green light alert (12). The weight-to-height risk table of the Turkish Diabetes Foundation has been added to the BMI section, where additionally, individuals can automatically calculate their BMI according to the entered. Furthermore, this section will also provide users with 14 recommendations from the Mediterranean Diet Adherence Screener (MEDAS).

Physical Activity

Healthcare providers emphasize at every opportunity the importance of engaging in regular physical activity during the transition from prediabetes to diabetes (13). Physical activity will be followed up with a step-count and a scheduling of the recommended physical activity the participant should engage in during the week. A gold star icon will be sent to app users when they satisfy their physical activity requirement. At the end

of the study, those who have collected 18 or more gold stars will be gifted bracelets inscribed with "Keep Active, Keep Protected from Diabetes!"

- Module 4: Communications

This module will allow users to send direct messages to the researcher, 24/7 for any information they may need.

Notifications

This section contains information based on the guidelines of the IDF, the American Diabetes Association, the Turkish Ministry of Health, Association of Turkish Dieticians, the Turkish Diabetes Foundation, Turkish Diabetes Society, and the Turkish Diabetes Nursing Association on the signs and symptoms of diabetes and prediabetes, diagnosis, treatment, complications, nutrition, physical activity, BMI monitoring and the normal BMI range. The section is designed to inform users about the content of the guidelines and raise awareness and knowledge levels about diabetes.

Safety

This section contains the data and passwords users will use to access the system.

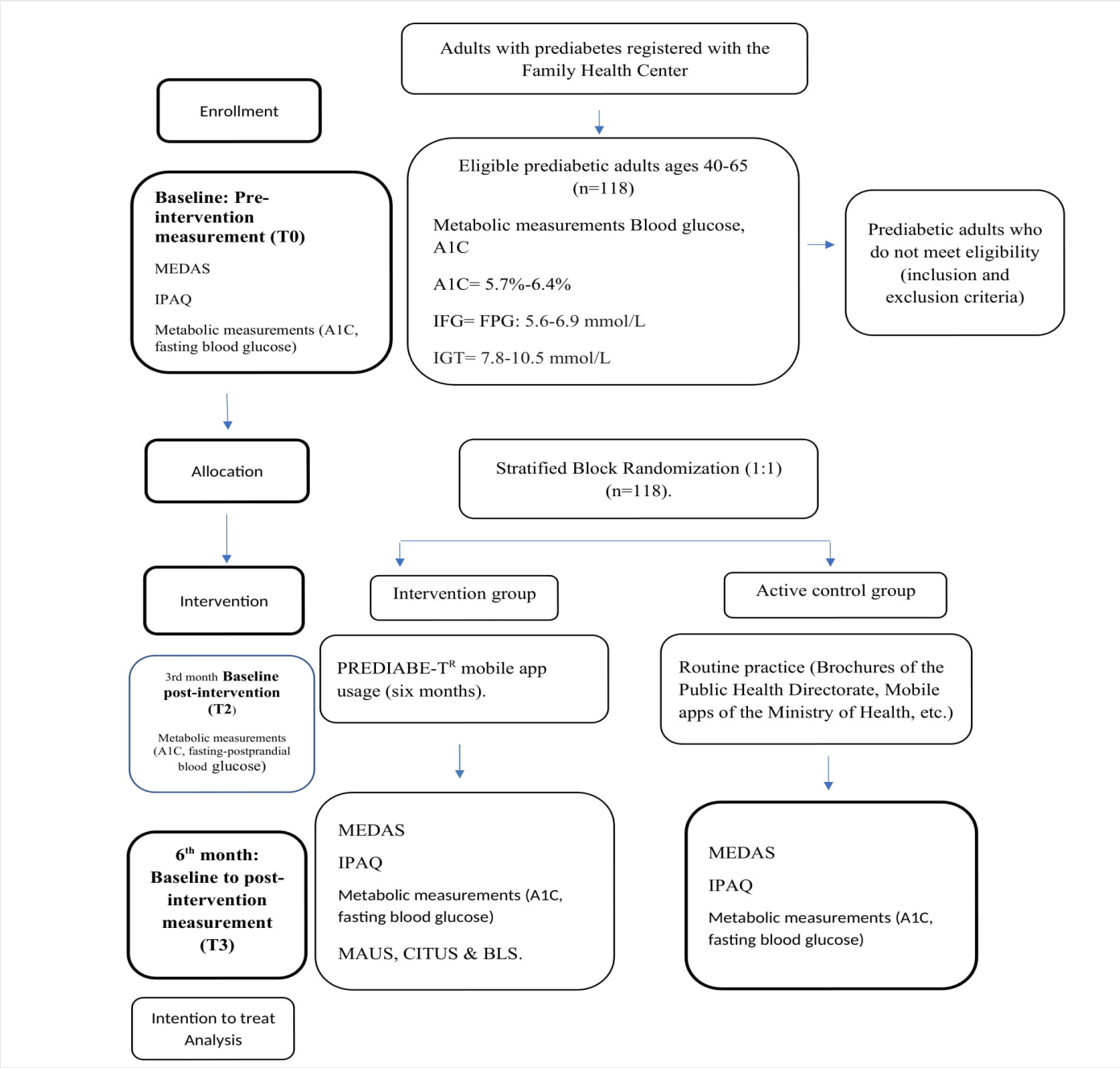

Figure 1. Research flow chart

Figure 2. Prediabetes mobile application draft

Active Control Group

Besides the routine health monitoring of the active controls, this group will be instructed in how to download and use the Ministry of Health's Türkiye Nutrition Guide Mobile Application.

Data Collection

Data will be collected on the individuals' sociodemographic features, gender, age, education, profession and medications they are taking. Their diabetes risk will be measured by means of the FINDRISK. The data collection form will be filled out on the basis of self-reporting. FINDRISK was developed in 1987 by Tuomilehto and Lindström to identify individuals at risk of Type-2 diabetes mellitus without laboratory testing (14). The Turkish validity and reliability study for FINDRISK was performed by Etbaş Demirağ (15) in 2016. The participants' metabolic measurements will be taken by a researcher using the Accu-Chek® Performa Nano device from a capillary blood sample; HbA1c values will be retrieved from FHC records.

Outcome Criteria

The expected primary outcome is a change in the adherence to the Mediterranean diet, physical activity levels and in the prediabetes metabolic values of the adults. The secondary outcome is a change in the adults' ability to achieve weight loss.

Primary Outcome Criterion

Mediterranean Diet Adherence Screener

The Turkish validity and reliability studies for MEDAS, which was originally developed in 2012 by Martínez-González et al. (16), were performed by Pehlivanoglu Ozkan et al. (17) in 2020. MEDAS consists of 14 questions. Each question is scored 1 or 0 points, depending on consumption. A total score of 7 or above indicates that the individual is adhering to the Mediterranean diet at an acceptable rate; a score of 9 or above indicates strict adherence. Cronbach's alpha coefficient for the scale is reported to be 0.829.

International Physical Activity Questionnaire

The International Physical Activity Questionnaire (IPAQ)-Short Form was developed in 1996 by Booth in order to identify the risk factors of inactivity and physical activity levels in prediabetic patients. The Turkish version of the IPAQ-Short Form consisting of 7 questions will be used. The Turkish validity and reliability studies for this questionnaire were conducted by Saglam et al. (18).

Metabolic Measurements

The participants' HbA1c, fasting blood glucose-postprandial blood glucose (whichever is appropriate for the individual) values will be measured.

The HbA1c measurement represents a 3-month mean value (19). The HbA1c testing does not require fasting conditions. The blood sample can be taken at any time of day (20). Over the period of the study, the HbA1c level the participant has obtained from being tested at any health facility will be taken from the personal health system records.

Blood Glucose Measurement: The researcher will measure the participants' blood glucose with a Roche Accu-Chek® Performa Nano device (21). A minimum eight-hour fasting period will be taken as a criterion for fasting blood glucose (22); a postprandial blood glucose test will be administered 2 hours after a meal.

Secondary Outcome Criterion

Height-weight Measurement and Body Mass Index

The researcher will measure the individuals' height and weight with calibrated devices. BMI will be calculated with the formula: Weight (kg)/height (m^2) (23).

Mobile App Usability and Usage Assessment Scale

Three measures developed by Hoehle et al. (24) to assess the usability and usage of a mobile application will be used. The validity and reliability study for the scales was performed by Güler (25).

Mobile Application Usability Scale

This is a measure used to assess and understand how the mobile app can be improved and how it may be made more user-friendly. The scale is a 7-point Likert-type and has a total of 40 items (1= definitely disagree, 7= completely agree). Cronbach's alpha coefficient for the scale is reported as 0.80-0.94 (25).

Continued Intention to Use Scale

This measure was developed to assess how eager individuals are to use the app. It comprises a total of 6 items and is a 7-point Likert-type (1= definitely disagree, 7= completely agree). There are no reversely scored items on the scale. Cronbach's alpha coefficient for the scale is reported as 0.90 (25).

Brand Loyalty Scale

This scale was developed to determine the extent of individuals' loyalty to the mobile app. It comprises a total of 5 items and is a 7-point Likert-type (1= definitely disagree, 7= completely agree). There are no reversely scored items on the scale. Cronbach's alpha coefficient for the scale is reported as 0.86 (25).

Validity-reliability

The study protocol was drawn up based on the SPIRIT (7) and CONSORT-NPT (8) guidelines. Randomization and blinding will reduce bias in the results. The measuring instruments used in this study are valid and reliable (15,17,18,25).

Ethics Statement

Ethical approval for the conduct of the study was obtained from Akdeniz University Faculty of Medicine Clinical Studies Ethics Committee (decision no.: KAEK-192, date: 16.03.2022) and institutional approval from the Kütahya Provincial Health Directorate (no.: 2022/47, date: 30.05.2022). Additionally, the written informed consent of the individuals to be included in the study will be collected. The study was conducted in compliance with the ethical principles of the Declaration of Helsinki.

Statistical Analysis

The data will be analyzed with the SPSS (Statistical Package for the Social Sciences) 23.0 package program licensed by Akdeniz University Faculty of Medicine Department of Biostatistics. The G*Power 3.1 program was used in determining sample size. Intention to Treat analysis will be employed for the analysis of lost data. Descriptive statistics will be defined by means and standard deviation. Numbers, percentage distribution, the chi-square and t-test will be used to identify homogeneous groups. To test normality, skewness and kurtosis values will be taken as a basis for the Shapiro-Wilk test. The one-way ANOVA, two-way ANOVA and ANCOVA tests will be used for dependent and independent groups. Non-parametric equivalents of correlation and regression analyses will be considered in the non-parametric analysis.

Discussion

This study will investigate the efficacy of a mobile application developed under the leadership of public health nursing staff on metabolic factors such as HbA1c and on physical activity and nutrition. Each message sent to the participants via the app will be evaluated on an individual basis. This strategy will have a potential key role in the development of healthy lifestyle applications in the long term. The control group will be asked to use the Diabetes Checklists and the Turkish Nutrition Guide of the Ministry of Health (26,27). The experimental group in the same study will use the PREDIABE-T^{R} mobile app and the metabolic factors, physical activity levels and Mediterranean diet nutritional behaviors of both groups will be compared. This course of action was planned in the light of current studies (28).

In the development stage of the mobile app, it was designed for use within the healthcare system of the Ministry of Health. Furthermore, it is important that the mobile app will be used for a period of six months, which will provide a time frame to determine the shortcomings of the app. Since the mobile app is free of charge, it can be used on a wide scale by nurses (28). The app can also be considered a facilitating alternative to face-to-face examinations, consultations, and follow-ups over the course of the current pandemic. It therefore opens the door to early diagnosis for individuals with prediabetes (29). The research is a randomized study of experimental design. The evidential level of the results to be obtained will be high. The developed mobile app will consequently offer individuals with prediabetes, socially disadvantaged groups, and health personnel an instrument that can be conveniently and safely employed. At the end of the study, this mobile application will be integrated into the Google Play Store database.

Study Limitations

The limitations of the study include the inclusion of individuals aged 40-65 years, prediabetic, using only Android mobile phones, without visual and hearing problems, with a minimum primary education level, who came to Kütahya City Center Family Health Center No. 9, the technical features of the mobile application, and the limitation of monitoring individuals for six months.

Conclusion

This study describes the effect of the use of a mobile application by individuals with prediabetes on metabolic parameters. If reductions can be achieved in metabolic parameters (such as HbA1c), it can be concluded that the mobile app is effective. In this context, information about this application will be sent out to administrators and policy-makers to ensure that more people make use of the app, thereby supporting communities in protecting and improving public health. This study is the first to elaborate on the role of public health nurses in prediabetes and preventive health.

Ethics

Ethics Committee Approval: Ethical approval for the conduct of the study was obtained from Akdeniz University Faculty of Medicine Clinical Studies Ethics Committee (decision no.: KAEK-192, date: 16.03.2022).

Informed Consent: Written informed consent was obtained from the individuals to be included in the study.

Footnotes

Authorship Contributions

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

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

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Original Article

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Effect of Body Mass Index on Balance, Trunk Muscle Endurance, Functional Mobility and, Physical Activity in College Students

Üniversite Öğrencilerinde Vücut Kitle İndeksinin Denge, Gövde Kas Enduransı, Fonksiyonel Mobilite ve Fiziksel Aktivite Düzeyine Etkisi

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ABSTRACT

Objective: The increasing prevalence of obesity at all ages in recent years also increases the need for physiotherapy and rehabilitation. Obesity can negatively affect physical fitness and college students are prone to obesity due to stress and anxiety they experience during their university years. This study aims to investigate the effect of body mass index (BMI) on balance, trunk muscle endurance, functional mobility, and physical activity in college students.

Methods: College students were divided into four groups according to BMI values: underweight, normal, overweight and obese. Balance was assessed using biodex balance system, trunk muscle endurance was measured through Biering-Sorensen and McGill trunk flexor endurance tests, functional mobility was evaluated with 30-second sit-to-stand test, and physical activity determined using International Physical Activity Questionnaire-Short Form.

Results: The study included 73 college students (mean= 20.76±1.11 years, 72.6% women). Normal weight students were significantly better than obese students regarding trunk muscle endurance scores ($p<0.01$). Obese students spent significantly more time sitting than underweight students ($p<0.05$). No significant correlation was found between BMI and balance and functional mobility ($p>0.05$).

Conclusion: Increased BMI can negatively affect trunk muscle endurance and sitting time. Exercises to improve trunk muscle

ÖZ

Amaç: Son yıllarda obezitenin her yaşta görülme sıklığının artması, fizyoterapi ve rehabilitasyona olan ihtiyacı artırmaktadır. Obezite fiziksel uygunluğu olumsuz yönde etkileyebilmektedir ve üniversite öğrencileri, üniversite yıllarında yaşadıkları stres ve kaygı nedeniyle obeziteye yatkın hale gelmektedir. Bu çalışma, üniversite öğrencilerinde vücut kitle indeksinin (VKİ) denge, gövde kas enduransı, fonksiyonel mobilite ve fiziksel aktivite üzerine etkisini araştırmayı amaçlamaktadır.

Yöntemler: Üniversite öğrencileri VKİ değerlerine göre zayıf, normal kilolu, hafif kilolu ve obez olmak üzere dört gruba ayrıldı. Denge, Biodex Denge Sistemi ile; gövde kas enduransı, Biering-Sorensen ve McGill gövde fleksör endurans testleri ile; fonksiyonel mobilite, 30 saniye otur-kalk testi ile; fiziksel aktivite, Uluslararası Fiziksel Aktivite Anketi-Kısa Form kullanılarak değerlendirildi.

Bulgular: Çalışmaya 73 üniversite öğrencisi dahil edildi (ortalama yaş= 20,76±1,11 yıl, %72,6 kadın). Normal kilolu öğrencilerin gövde kas endurans skorları obez öğrencilere göre anlamlı düzeyde daha iyiydi ($p<0,01$). Obez öğrenciler, zayıf öğrencilere göre oturarak anlamlı olarak daha fazla zaman harcıyorlardı ($p<0,05$). VKİ ile denge ve fonksiyonel mobilite arasında anlamlı bir ilişki bulunamadı ($p>0,05$).

Sonuç: Artan VKİ gövde kas enduransını ve oturma süresini olumsuz yönde etkileyebilir. Obez öğrencilerin fizyoterapi ve

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ABSTRACT

endurance should be added to the physiotherapy and rehabilitation programs of obese students. Therefore, it is important for universities to adopt an approach that supports exercise habits and physical activity to prevent obesity.

Keywords: Young adults, university students, obesity, body composition

ÖZ

rehabilitasyon programlarına gövde kas enduransını geliştirmeye yönelik egzersizler eklenmelidir. Bu nedenle üniversitelerin obeziteyi önlemek için egzersiz alışkanlığını ve fiziksel aktiviteyi destekleyen bir yaklaşım benimsemesi önemlidir.

Anahtar Kelimeler: Genç yetişkinler, üniversite öğrencileri, obezite, vücut kompozisyonu

Introduction

Obesity is defined as excessive accumulation of adipose tissue in the human body at a level that threatens health (1). As a result of imbalances in calorie exchange, genetic and environmental factors, calorie intake is greater than calorie burned, resulting in a tendency to weight gain and the development of obesity (2). With the effect of increasing technology use and sedentary lifestyle, there is an enhance in the prevalence of obesity and weight gain problems (3). As the prevalence of obesity raises, the frequency of obesity-related diseases also increases. Obesity is associated with diseases that concern a wide range of systems, such as metabolic-hormonal complications, cardiovascular system diseases, respiratory system diseases, digestive system diseases, and musculoskeletal system diseases (4).

Obese individuals may face challenges in maintaining balance. Obesity changes body structure, enhances the mass of distinct body segments, and can result in biomechanical complications. In obese individuals, the abnormal distribution of body fat changes the center of gravity, resulting in a decrease in control of balance (5). Especially anthropometric measurements such as height and weight significantly affect both postural sway and postural stability (6).

Obesity not only impacts metabolism but also profoundly affects musculoskeletal health. Excessive adiposity leads to compromised skeletal muscle function, diminishing strength and muscle endurance due to metabolic dysregulation and inflammation (7). Additionally, obesity alters gait kinetics and kinematics, resulting in deviations from normal walking patterns and increased risk of injury. The mechanical burden of excess weight can also lead to postural abnormalities and malalignments, exacerbating functional impairment. Overall, the multifaceted effects of obesity extend beyond metabolic dysfunction to encompass significant challenges to musculoskeletal integrity and functional mobility (8).

University years are especially important times in shaping physical activity, diet and lifestyle habits (9). Young adults are prone to gaining weight during college years due to increased stress and anxiety levels (10). It has been stated that college students gain weight, especially in the first year of university (11). College students may face depression and anxiety due to reasons such as academic performance, pressure to be succeed, and post-graduation plans (12). As life expectancy is expected to continue to increase in all regions of the world, healthy aging becomes more important day by day (13). Since exercise habits and healthy dietary can prevent obesity, it is important to acquire healthy nutrition and physical activity habits at an early age during the healthy aging process (14). Because obesity, which occurs in youth and adolescence, tends to progress in later ages (15). Therefore, obesity is an important public health problem that occurs worldwide as a disease of all ages (16).

Activities provided by universities and institutions can increase college students' participation in physical activity and help prevent weight gain. On the other hand, the fact that the majority of lessons at universities consist of classroom lectures and require sitting to study may lead students to a sedentary lifestyle (17). People who don't engage in regular physical activity are more prone to gaining weight and facing an increased risk of obesity compared to those who maintain an active lifestyle. Reduced physical activity contributes to obesity, and obesity, in turn, results in decreased physical activity (18).

The reasons explained above indicate that college students and young adults are at risk of overweight and obesity. Numerous studies in the literature investigate the impacts of obesity on a wide variety of systems, structures and functions, but most of the studies have been conducted in the geriatric individuals (19,20). There are a limited number of studies on the multidimensional effects of obesity on physical fitness and functions in healthy college students or young adults. We think that investigating the effects of body mass index (BMI) on college students is important in terms of increasing the approaches of universities toward gaining physical activity habits. We also predict that our study will shed light on the need for physiotherapy and rehabilitation for obese individuals. This study aims to investigate the effect of BMI on balance, trunk muscle endurance, functional mobility, and physical activity in college students.

Methods

Study Design

This prospective and cross-sectional study was approved by Bezmialem Vakıf University Non-interventional Research Ethics Committee (decision number: 23/307, date: 18.12.2018). The study was conducted in compliance with the Declaration of Helsinki. The study was carried out in Bezmialem Vakıf University and conducted with college students studying at

the Faculty of Health Sciences. College students who agreed to participate in the study were informed about the research and an informed consent was obtained from the students.

The inclusion criteria of the study were being a college student between the ages of 18-25, not having an orthopedic and/or neurological disease that could hinder the evaluation process, and agreeing to participate in the study. Exclusion criteria were being diagnosed with a musculoskeletal system disease, having recently undergone an orthopedic operation, using insoles, and playing a sport professionally as this could affect the results.

Outcomes

College students' age, gender, presence of comorbidities, orthopedic surgery history and insoles use were recorded via a demographic information form constituted by the researchers. Balance was assessed using the biodex balance system (BBS) with the results of postural stability test (PST), limits of stability test (LOST), and clinical test of sensory integration of balance (CTSIB). Trunk muscle endurance was measured through the Biering-Sorensen test and McGill trunk flexor endurance test, functional mobility was evaluated with the 30-second sit-to-stand test (30STS), and physical activity levels were determined using the International Physical Activity Questionnaire-Short Form (IPAQ-SF).

BMI

Weight and height were measured to compute the BMI. These measurements were conducted with students standing, barefoot, and dressed in light clothing after fasting for a minimum of two hours. Tanita CO Tokyo-Japan (TANITA MC 180 MA) model digital scale was used for weight measurement and the values were recorded in kilograms (kg). Height measurement was made with a tape measure and recorded in centimeters (cm). BMI was calculated with the kilogram/meter^2 (kg/m^2). According to the calculated BMI, the college students were divided into four groups: underweight (BMI <18.5 kg/m^2), normal (18.5≤ BMI ≤24.99 kg/m^2), overweight (25.0≤ BMI ≤29.99 kg/m^2), and obese (BMI ≥30 kg/m^2) (21).

Balance

Balance evaluation performed with PST, LOST and CTSIB with help of the BBS (Biodex, Inc., Shirley). Ability to maintain the center of gravity within the support surface was evaluated with PST. Overall stability index, anterior-posterior stability index, and medial-lateral stability index were recorded during PST. Greater scores indicate poorer postural stability. The assessment of the ability to shift the center of gravity in five directions was conducted using LOST. LOST was applied on a static platform and the directional control of the students was evaluated as overall, forward, backward, left, right, forward-right, forward-left, backward-right and backward-left, recorded as a percentage. Higher scores in LOST results indicate better performance and dynamic postural stability. With CTSIB, it was evaluated how different senses contribute to balance and how well balance can be compensated when one or more of these senses are eliminated. CTSIB was applied in four different positions with static platform setting: eyes open firm surface, eyes closed firm surface, eyes open foam surface, and eyes closed foam surface. Oscillations for each position were calculated as a sway index, and high sway index scores refer to the increase with balance deterioration (22).

Trunk Muscle Endurance

Trunk extensor muscle endurance evaluated with the Biering-Sorensen test. During the test, the pelvis and lower extremities were fixed to the bed by the researcher. Students were asked to cross their hands on opposite shoulders and keep their trunks outside the bed in a position parallel to the floor for the maximum amount of time. Trunk flexor muscle endurance evaluated with the McGill trunk flexor endurance test. During the test, the individuals' feet remained in contact with the bed surface, the hips and knees were positioned in 90° flexion, and the trunk was positioned in 60° flexion. Students were asked to maintain this position for the maximum amount of time they could. For both tests, when the students could not control their posture or reached the maximum time of 240 seconds, the test was finished and the test result was recorded in seconds (23,24).

Functional Mobility

Functional mobility assessed with 30STS. The assessment was conducted using a standard chair with a height of 43 cm. Students were asked to cross their hands on opposite shoulders and perform maximum sit-to-stand activity by maintaining this position throughout the test period. At the end of the test, the number of repetitions for 30 seconds was recorded. The 30STS is also applicable for assessing lower extremity muscle strength in young adults (25).

Physical Activity

IPAQ-SF was used to measure physical activity levels of college students. IPAQ-SF includes a total of 7 questions in 4 separate sections regarding activities done for at least 10 minutes in the last 7 days. Walking, moderate, vigorous physical activity and, sitting time scores were determined and the total physical activity score was obtained by summing the all values. The activities in the questionnaire are scored as "metabolic equivalent (MET)-minutes/week (MET-min/wk) unit" (Total physical activity min/wk: time spent on vigorous + moderate + walking; MET min/wk: 8 × vigorous + 4 × moderate + 3.3 × walking) (26).

Statistical Analysis

G-power v3.1 program (Universitat Kiel, Germany) was used to determine the sample size. Based on the results of a study in the literature (27), we estimated that a minimum of 52 participants in total should be included in the study for 4 groups, 13 participants for each group. Sample size calculation was made based on the mean and standard deviation values of the Biering-Sorensen test within 80% power and 95% confidence interval. Data analysis was conducted using Statistical Package for Social Sciences (SPSS) Statistics v.26 (SPSS Inc., USA). Normal distribution characteristics of all groups were examined with the Shapiro-Wilk test. One-way analysis of variance (ANOVA)

test was used to compare normally distributed data and for variables with significant differences, Tukey honestly significant difference test was used as a post hoc test to determine which groups the difference was between. Kruskal-Wallis test was used to compare data that did not show normal distribution. Since there is no post hoc test that can be used in nonparametric tests, Mann-Whitney U test was used as two comparisons for four groups to determine which groups the variables with significant differences were between. Relationships between normally distributed data were examined with the Pearson correlation coefficient, and relationships between non-normally distributed data were examined with the Spearman correlation coefficient. Correlation strength level was evaluated according to Cohen's Kappa coefficient. Correlation strength was categorized as "very weak" (0.00-0.19), "weak" (0.20-0.39), "moderate" (0.40-0.59), "strong" (0.60-0.79), and "very strong" (0.80-1.00). In all analyses, $p<0.05$ (two-sided) was considered statistically significant.

Results

College students studying in the departments of the faculty of health sciences were invited to the study (n=110). According to the study's inclusion-exclusion criteria, 37 college students were excluded from the study (Figure 1). A total of 73 college students, 53 female (72.6%) and 20 male (27.4%), participated in the study. The college students participating in the study were studying in the departments of nutrition and dietetics (n=9), occupational therapy (n=12), physiotherapy and rehabilitation (n=26), nursing (n=11), audiology (n=7), and health management (n=8). Mean age of the participants was 20.76±1.11 years, and the mean BMI was 25.02±1.53 kg/m^2. The demographic characteristics of the participants in the study are shown in Table 1.

No statistically significant difference was found in the PST, LOST and CTSIB results of balance measurements between groups with different BMI ($p>0.05$). Comparison of the values in balance among groups is given in Table 2. Mean scores of Biering-Sorensen and McGill trunk flexor endurance tests were 110.05±42.35 and 122.07±70.52 seconds, respectively. In Biering-Sorensen and McGill trunk flexor endurance tests, a statistically significant difference was observed between the groups ($p<0.001$; $p=0.001$). According to the Biering-Sorensen test results, obese students had significantly lower scores than underweight and normal weight students ($p<0.001$; $p<0.001$). At the same time, obese students had significantly lower McGill trunk flexor endurance test results than underweight and normal weight students ($p=0.002$; $p=0.003$). Mean scores of 30STS, which used to evaluate functional mobility, was 27.17±3.68 repetitions. There was no statistically significant difference between the groups in 30STS scores ($p=0.243$). While there was no statistically significant difference between the groups in vigorous physical activity, moderate physical activity, walking and total score in IPAQ-SF ($p>0.05$), there was a statistically significant difference between the groups in sitting time ($p=0.005$). Underweight students had less sitting time compared to normal weight and obese students ($p=0.004$; $p=0.004$). Comparison of the values in trunk muscle endurance, functional mobility, and physical activity among groups are given in Table 3.

BMI and Biering-Sorensen test and McGill trunk flexor endurance test showed a significant negative correlation ($r=-0.477$, $p=0.000$; $r=-0.487$, $p=0.000$). At the same time, BMI showed a statistically significant relationship with vigorous physical activity and sitting time ($r=0.272$, $p=0.028$; $r=0.411$, $p=0.001$). However, no significant relationship was found between BMI and balance, functional mobility, and total physical activity scores ($p>0.05$). Correlation between BMI and balance, trunk muscle endurance, functional mobility, and physical activity is given in Table 4.

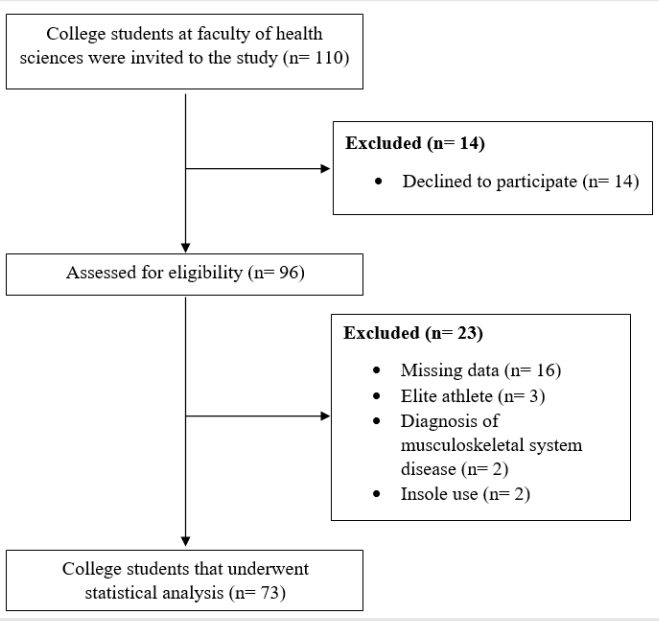

Figure 1. Flow chart of the study

Table 1. Demographic characteristics of groups

Characteristics	Total (n=73)	Underweight (<18.5 kg/m²) (n=18)	Normal (18.5-24.99 kg/m²) (n=22)	Overweight (25.0-29.99 kg/m²) (n=16)	Obese (≥30 kg/m²) (n=17)
Age (years)	20.76±1.11	20.61±1.14	20.50±1.05	21.18±1.60	20.77±0.66
Height (cm)	166.95±8.48	167.69±7.75	162.36±7.08	165.31±8.56	172.44±10.56
Weight (kg)	70.42±8.37	50.37±4.19	57.84±7.88	72.93±9.37	100.56±12.06
BMI (kg/m²)	25.02±1.53	17.88±0.49	21.85±1.90	26.56±1.20	33.81±2.55

Data are presented as mean ± standard deviation. BMI: Body mass index

Table 2. Comparison of the values in balance among groups

	Underweight (<18.5 kg/m²) (n=18)	Normal (18.5-24.99 kg/m²) (n=22)	Overweight (25.0-29.99 kg/m²) (n=16)	Obese (≥30 kg/m²) (n=17)	p-value
PST/overall	0.32±0.14	0.30±0.11	0.35±0.14	0.23±0.10	0.205
PST/anterior/posterior	0.22±0.12	0.21±0.10	0.27±0.12	0.16±0.07	0.113
PST/medial/lateral	0.12±0.05	0.14±0.07	0.16±0.09	0.08±0.06	0.101
LOST/overall	47.33±10.86	47.27±9.21	45.93±10.09	44.44±12.27	0.955
LOST/forward	64.05±10.72	59.68±15.28	64.34±15.86	59.44±13.80	0.634
LOST/backward	50.50±16.81	53.72±13.24	50.06±17.39	58.55±15.50	0.544
LOST/left	53.94±18.47	54.27±13.50	52.68±12.66	53.77±20.22	0.980
LOST/right	55.27±16.06	54.81±11.82	50.68±14.04	45.11±19.51	0.310
LOST/forward right	53.11±13.80	49.77±13.00	54.56±11.40	46,66±21,18	0.510
LOST/forward left	46.72±12.60	48.86±15.72	50.56±15.90	54.55±9.15	0.585
LOST/backward right	48.22±13.06	45.00±13.69	44.81±15.73	43.22±16.16	0.721
LOST/backward left	47.72±17.49	50.81±15.30	43.12±14.10	50.33±11.40	0.462
CTSIB/eyes open firm surface	0.47±0.23	0.55±0.23	0.54±0,21	0.49±0.16	0.475
CTSIB/eyes closed firm surface	0.72±0.20	0.93±0.46	0.87±0.27	0.91±0.20	0.178
CTSIB/eyes open foam surface	0.90±0.10	0.94±0.30	0.96±0.25	0.84±0.16	0.596
CTSIB/eyes closed foam surface	2.28±0.35	2.50±0.57	2.46±0.47	2.43±0.50	0.549
CTSIB/composite score	1.09±0.17	1.23±0.27	1.25±0.25	1.17±0.19	0.190

Higher scores on the PST, and CTSIB indicate greater balance impairment. Lower scores on the LOST indicates greater dynamic balance dysfunction. Data are presented as mean ± standard deviation.
PST: Postural stability test, LOST: Limits of stability test, CTSIB: Clinical test of sensory integration of balance

Table 3. Comparison of the values in trunk muscle endurance, functional mobility, and physical activity among groups

	Underweight (<18.5 kg/m²) (n=18)	Normal (18.5-24.99 kg/m²) (n=22)	Overweight (25.0-29.99 kg/m²) (n=16)	Obese (≥30 kg/m²) (n=17)	p-value
Biering-Sorensen test (s)	138.25±63.82	137.40±40.38	107.25±35.98	57.33±29.21	**0.000**
McGill trunk flexor endurance test (s)	181.72±82.87	148.55±77.85	99.88±70.80	58.11±50.58	**0.001**
30STS (reps)	26.22±4.25	26.95±3.10	28.75±3.37	26.77±4.02	0.243
Vigorous (MET-min/week)	102.22±265.76	450.90±1096.22	630.00±1058.45	343.66±642.66	0.306
Moderate (MET-min/week)	251.25±342.02	346.36±480.05	302.50±300.29	53.33±89.44	0.216
Walking (MET-min/week)	1639.32±1356.43	1735.10±990.14	1949.35±1632.41	2052.16±2182.71	0.834
Sitting (MET-min/week)	459.33±198.11	645.54±195.65	618.75±222.04	757.33±222.36	0.005
Total (MET-min/week)	1992.79±1427.43	2532.37±1431.66	2881.85±2028.67	2449.16±2708.90	0.384

Data are presented as mean ± standard deviation. Bold denotes p<0.05. 30STS: 30-second sit-to stand test, s: seconds, reps: Repetitions, MET: Metabolic equivalent, min: Minute. Higher scores on the Biering-Sorensen test, McGill trunk flexor endurance test and, 30STS indicate greater performance

Table 4. Correlation between BMI and balance, trunk muscle endurance, functional mobility, and physical activity

Outcomes	BMI	
	r-value	p-value
PST/overall	-0.012	0.926
LOST/overall	-0.014	0.909
CTSIB/composite score	0.147	0.243
Biering-Sorensen test	-0.477	**0.000**
McGill trunk flexor endurance test	-0.487	**0.000**
30STS	0.153	0.223
IPAQ-SF/total (MET-min/week)	0.137	0.278

P-values were calculated from pearson correlation test. Bold denotes p<0.05. BMI: Body mass index, PST: Postural stability test, LOST: Limits of stability test, CTSIB: Clinical test of sensory integration of balance, 30STS: 30-second sit-to stand test, IPAQ-SF: International Physical Activity Questionnaire-Short Form, MET: Metabolic equivalent, min: Minute

Discussion

In our study which conducted with college students, the effect of BMI on balance, trunk muscle endurance, functional mobility and physical activity was investigated. Our study showed that among college students with different BMIs, both trunk extensor and trunk flexor muscle endurance values of underweight and normal weight students were better than obese students. In terms of physical activity, normal weight and obese students spent more sedentary time compared to underweight students. At the same time, this study revealed that there were no differences in balance and functional mobility between students with different BMI.

There are studies investigating the effects of obesity on balance in healthy individuals, but most of the studies were conducted with children, adults and elderly groups. The number of studies investigating the effects of obesity on balance in healthy college students or young adults is limited in the literature. In a study conducted on sedentary female college students, it was found that anthropometric characteristics such as BMI and waist-hip ratio did not affect dynamic and static balance (28). Likewise, Suvarna et al. (29) showed in their study on college students that increasing BMI did not cause any impact on dynamic balance. In this respect, our study results are parallel to the literature. We think that the lack of relationship in our results may be due to the similarity of the lower extremity muscle strengths of the college students in our study, evaluated with 30STS. In their research, Hue et al. (30) identified a notable correlation between increased body weight and decreased postural stability among adult males. The relationship between BMI and balance in geriatric individuals was investigated and it was revealed that increasing body weight negatively affected balance and postural stability (31). Du Pasquier et al. (32) examined the correlation between age and postural stability and found that increasing age had negative effects on postural stability, regardless of height, weight and gender. Considering the results of the studies in the literature and our study, it is seen that increasing body weight in young adults does not significantly affect balance. However, when there are negative effects of aging in addition to increasing body weight, balance may be more affected in obese individuals.

In a study investigating the correlation between BMI and transversus abdominis (TrA) muscle endurance in college students, it was reported that students with higher BMI had lower TrA muscle endurance (33). In the study of Malayil et al. (34), a negative relationship was found between abdominal muscle endurance and BMI in sedentary college students. A study conducted on Korean college students revealed that obesity negatively affected both muscle endurance and cardiorespiratory endurance (35). In their study with healthy young adults, Pasupatham et al. (36) investigated the effect of BMI on the endurance of the lower back extensor muscles in underweight, normal weight and overweight individuals. The authors reported in their study that there was a negative correlation between BMI and lower back extensor muscle endurance. The data obtained from our study were compatible with the literature. We think that the positive, moderately significant correlation between the BMI and sedentary time of the college students in our study may affect the students' trunk muscle endurance, and in addition to the increase in BMI, spending more time sitting may reduce trunk muscle endurance. Mayer et al. (37) investigated the effects of obesity on back and core muscle endurance in adult firefighters using the modified Biering-Sorensen test and plank test. In that study, the authors found that non-obese adults had significantly better results on both trunk extensor and trunk flexor muscle endurance tests compared to the obese group. Pardeshi et al. (38) examined the relationship between BMI and core muscle endurance in adult sedentary women and reported that obese women had weaker core muscle endurance compared to normal weight women. In a study conducted on children aged 10-13, it was stated that BMI showed a negative correlation with abdominal muscle endurance (39). Studies in the literature show that increased BMI begins to negatively affect trunk muscle endurance starting from childhood. Studies conducted on adult and geriatric individuals reveal that increasing BMI continues to negatively affect trunk muscle endurance in later ages. Trunk muscle endurance has a critical role in maintaining balance and functional performance (40). Decrease in trunk muscle endurance can lead to falls, postural disorders and decreased functionality, especially in later ages (41). For this reason, we believe that preventing obesity at young ages is important for the preservation of trunk muscle endurance and public health.

To the best of our knowledge our study is the first to investigate the relationship between BMI and functional mobility in college students. A significant portion of the studies in the literature on this subject have been conducted with adult and geriatric individuals. Brady et al. (42) evaluated functional mobility in elderly women with 30STS and stated that the results were better in normal weight women than overweight women. Pataky et al. (43) investigated the effect of obesity on functional mobility in women and reported that obese women had significantly lower functional mobility levels compared to normal weight women. In a study among adult women by Hergenroeder et al. (44), being of normal weight was associated with better sit-to-stand test results. Ryder et al. (45) investigated the effect of bariatric surgery on functional mobility in obese young people. According to the results of that study, it was revealed that functional mobility results in young people after bariatric surgery were better at the sixth month and second year than before surgery. Contrary to the literature, in our study, there was no significant difference in functional mobility test scores between college students with different BMIs. We think that this may be due to the similar physical activity levels of the students participating in the study.

Contrary to our study, many studies that used IPAQ in the evaluation and investigated the relationship between BMI and physical activity have shown that increasing body weight negatively affects the level of physical activity. Cleland et al. (46) affirmed that prolonged sitting duration and insufficient physical activity levels were linked to obesity among young adults. It has also been observed that the possibility of obesity increases in men who have lower step counts. In a study which conducted with university students, a significant negative correlation was found between BMI and duration of vigorous physical activity that assessed by

IPAQ-SF (47). Sulemana et al. (48) investigated the relationship between physical activity levels and BMI values in their study on adolescent girls aged between 14-17. The authors reported that the overweight adolescents had lower physical activity levels compared to the normal weight adolescents. They also stated that there was a significant negative correlation between BMI values and total physical activity score of all participants in the study. However, there are also studies in the literature that support our results. Arslan et al. (49) reported in their study with university students that no significant relationship was found between BMI and physical activity level assessed by IPAQ-SF. Göger et al. (50) found that obesity in adult women significantly reduced healthy lifestyle behaviors but did not affect physical activity level. We think that since the college students participating in our study are educated in health sciences, their knowledge about the possible complications of obesity may motivate overweight and obese students to do physical activity and exercise. We also think that the fact that IPAQ-SF is a subjective physical activity assessment questionnaire based on self-report may also affect the results.

Study Limitation

In our study, the relationship between BMI and physical fitness of college students was evaluated multidimensionally. Balance was evaluated with an objective method, and the effects of BMI on many systems and structures were revealed. At the same time, our study achieved the aim of providing universities and institutes with a perspective on obesity prevention. These are among the strengths of our study. However, our study has some limitations. The study was single-centered, physical activity was not assessed with an objective measurement method, and the sample size was small. We think that there is a need for studies in the literature on BMI, where physical activity levels are evaluated with objective measurement methods, with a larger sample size, multi-centered and with different age groups.

Conclusion

According to the results of this study, although increasing BMI negatively affects trunk muscle endurance and increases sitting time in college students, there is no effect on balance, functional mobility and physical activity. For this reason, exercises to improve trunk muscle endurance should be added to the physiotherapy and rehabilitation programs of students with high BMI. However, studies conducted with geriatric individuals show that increasing body weight negatively affects balance, functional mobility and physical activity. Considering the changes that occur with aging in addition to increasing body weight, we can say that obese individuals will experience the negative effects of the parameters examined in the study more severely in later ages. We suggest that universities should enhance activities and events aimed at increasing college students' physical activity habits in order to prevent a sedentary lifestyle that may increase with age.

Acknowledgments

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Ethics

Ethics Committee Approval: This prospective and cross-sectional study was approved by Bezmialem Vakıf University Non-interventional Research Ethics Committee (decision number: 23/307, date: 18.12.2018).

Informed Consent: College students who agreed to participate in the study were informed about the research and an informed consent was obtained from the students.

Footnotes

Authorship Contributions

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

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Original Article

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Inhibitory Efficacy of *Thymus vulgaris* and *Origanum onites* Essential Oils on *Enterococcus faecalis* Biofilm

Thymus vulgaris ve *Origanum onites* Esansiyel Yağlarının *Enterococcus faecalis* Biyofilmi Üzerindeki İnhibitör Etkinliği

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ABSTRACT

Objective: The crucial aim of irrigation solutions used in root canal treatments is the elimination of biofilm which is considered an important virulence factor of *Enterococcus faecalis*. Essential oils have been investigated to understand their efficacy for biofilm elimination in recent years. This study aimed to evaluate the antimicrobial and antibiofilm effects of *Thymus vulgaris* essential oils (TEO) and *Origanum onites* essential oils (OEO) on *E. faecalis*.

Methods: The antimicrobial effectiveness of TEO and OEO against *E. faecalis* (ATCC 29212) was investigated by broth microdilution method, and minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined. The effect of TEO and OEO on preventing biofilm formation was evaluated by measuring biofilm biomass using crystal-violet method, and its effect on biofilm viability was evaluated by determining the number of living cells in the biofilm as colony-forming units. Biofilm viability was analyzed with one-sample t-test with statistical significance accepted as $p<0.05$.

Results: The MIC values of TEO and OEO were determined as 0.078 µL/mL, and MBC values were determined as 0.156 µL/mL for OEO and 0.078 µL/mL for TEO. The percentage inhibition of biofilm formation at MIC value for OEO and TEO was determined as 53.9% and 55.6%, respectively. Both essential oils caused a significant reduction in the number of viable cells within the biofilm.

Conclusion: It is concluded that TEO and OEO show high antimicrobial and antibiofilm activity against *E. faecalis* biofilm.

ÖZ

Amaç: Kök kanal tedavilerinde kullanılan irrigasyon solüsyonlarının en önemli amacı *Enterococcus faecalis'*in önemli bir virülans faktörü olan biyofilmi ortadan kaldırmaktır. Sahip oldukları antimikrobiyal aktiviteyle esansiyel yağların çoğu patojenik mikroorganizmaya karşı alternatif olarak kullanılabilecekleri düşünülmektedir. Bu çalışmanın amacı da *Thymus vulgaris* (kekik) esansiyel yağı (TEO) ve *Origanum onites* (sivri kekik) esansiyel yağlarının (OEO) *E. faecalis* üzerindeki *in vitro* antimikrobiyal ve antibiyofilm etkilerini değerlendirmektir.

Yöntemler: TEO ve OEO'nun *E. faecalis*'e antimikrobiyal etkinliği sıvı mikrodilüsyon yöntemiyle araştırılmış, minimum inhibitör konsantrasyonu (MİK) ve minimum bakterisidal konsantrasyonu (MBK) belirlenmiştir. Çalışmalarda *E. faecalis* ATCC 29212 suşu kullanılmıştır. TEO ve OEO'nun biyofilm oluşumunu engelleme etkisi kristal-viyole yöntemiyle biyofilm biyokütlenin ölçülmesi ve biyofilm canlılığına etkisi ise biyofilm içerisindeki canlı hücrelerin sayısının koloni oluşturan birim olarak belirlenmesiyle değerlendirilmiştir. Biyofilm canlılığı one-sample t-testi ile analiz edilmiş ve istatistiksel anlamlılık $p<0,05$ olarak kabul edilmiştir.

Bulgular: TEO ve OEO'nun MİK değerleri 0,078 µL/mL, MBK değerleri ise OEO için 0,156 µL/mL ve TEO için 0,078 µL/mL olarak belirlenmiştir. OEO ve TEO için MİK değerinde biyofilm oluşumu inhibisyonunun yüzdesi sırasıyla %53,9 ve %55,6 olarak belirlenmiştir. Her iki esansiyel yağ da biyofilm içindeki canlı hücre sayısında önemli bir azalmaya neden olmuştur.

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ABSTRACT

Therefore, these essential oils can be considered an alternative irrigation solution for eliminating resistant root canal infections.

Keywords: Antibiofilm activity, biofilm, *Enterococcus faecalis*, minimum inhibitory concentration, minimum bactericidal concentration

ÖZ

Sonuç: TEO ve OEO'nun *E. faecalis* biyofilmine karşı yüksek antimikrobiyal ve antibiyofilm aktivite gösterdiği ve bu esansiyel yağların *E. faecalis* biyofilminin ortadan kaldırılmasını sağlayabileceği ve dirençli kök kanal enfeksiyonlarının eliminasyonunda alternatif irrigasyon solüsyonu olarak değerlendirilebileceği düşünülmektedir.

Anahtar Sözcükler: Antibiyofilm aktivitesi, biyofilm, *Enterococcus faecalis*, minimum inhibitör konsantrasyon, minimum bakterisidal konsantrasyon

Introduction

Microorganisms, found in the oral flora, can adhere better to hard and soft tissue surfaces due to their biofilm formation ability. *Enterococcus faecalis* is one of the important biofilm-forming bacteria, which causes resistant periradicular dental infections. *E. faecalis* settles in root canals, mainly the apical part and forms biofilm layer in deeper dentin tubules (1,2). The extracellular matrix, which forms the basic structure of the biofilm, is a protective polymer structure that bacteria constantly form. As it forms, it ensures that the bacteria are more isolated from the buried external environment (2,3). Therefore, the biofilm layer is considered a small community with three-dimensional signal transport, nutrition, and waste channels that microorganisms create as a suitable habitat for themselves. Because of these channels, microorganisms can protect themselves from changes in the external environment and neutralize the effects of agents such as disinfectants and antiseptics used to destroy them (2).

E. faecalis forms a typical biofilm in which the physicochemical properties are modified to dominate according to the environment. In a typical biofilm, living cells are located on the surface of the biofilm, and irregular growth of adjacent cell clusters is observed in aerobic or anaerobic and nutrient deprived-medium (1,3-5). Therefore, resistant periradicular dental infection develops because of the complex structure of the biofilm and the first step to eliminate the infection should be the destruction of the biofilm. At this point, sodium hypochlorite at a concentration of 0.5-6% is routinely applied in dental treatments as it is a cost-effective irrigation solution to eliminate *E. faecalis* biofilm (5). However, the search for alternative irrigation agents continues due to their toxic and caustic nature to the periradicular tissues (5-7).

When considering agents with antibacterial solid activity as well as minimal tissue irritation, herbal-based agents can be thought. Therefore, natural products with herbal ingredients are used in the development of many oral hygiene products because they have rich antimicrobial components (7,8). *Thymus vulgaris* (thyme) essential oils (TEO) and *Origanum onites* essential oils (OEO) are herbal agents with antibacterial activities, but there is insufficient information in the literature regarding the effectiveness of these essential oils (EOs) on *E. faecalis* biofilms. So, this study aimed to determine the antibacterial activity of TEO and OEO on *E. faecalis* biofilm by evaluating minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and antibiofilm activity at different concentrations.

Methods

This microbiological research study was conducted in accordance with the principles of the Declaration of Helsinki. This study was approved by Başkent University Medical and Health Sciences Research Board Institutional Review Board (approval number: KA22/187, date: 28.04.2022). A series of microbiological tests were conducted to calculate MIC, MBC, biofilm formation inhibition percentage, and biofilm viability effect of different concentrations of TEO and OEO to evaluate their effectiveness on *E. faecalis* biofilm within the scope of this study. *E. faecalis* ATCC 29212 was used for all biofilm and antimicrobial activity tests. TEO and OEO were provided by the manufacturer (Caliskan Agriculture, Türkiye). All the EOs were obtained by the hydro-distillation method.

MIC and MBCs for TEO and EOS

For determining MIC values, broth microdilution method was used as recommended by the Clinical and Laboratory Standards Institute (9). The EOs in combination with 1% dimethyl sulfoxide, were diluted twofold in Mueller Hinton Broth (BD Difco™, France). After 24h of incubation at 37 °C, absorbance was measured at 570 nm (BioTek Instruments, ELX 800, USA). All studies were performed in triplicate. The TEO and OEO concentrations ranged from 0.039-10 µL/mL (10). The wells containing only bacterial suspension were used for bacterial growth control, and sterile Mueller Hinton Broth without bacteria or antibacterial agent was used for sterilization control. Plates were incubated at 37 °C for 24h and each experiment was repeated 3 times. MBC was determined by inoculating 10 µL of suspension from each well in Tryptic Soy Agar (Condalab, Spain) after the incubation period of MIC assay (11). MBC was considered the lowest concentration of the test substance in which no microbial growth was observed after the incubation period at 37 °C for 24h.

Biofilm Formation Assay

The biofilm formation assay was performed as described previously (12). Briefly, flat-bottom polystyrene microtiter plates

containing 180 µL of tryptic soy broth (Condalab, Spain) were inoculated with 20 µL of bacterial culture adjusted to $1x10^6$ CFU/mL. After 48h of incubation, biofilm formation was detected by the crystal violet staining method (CVS) (13). The absorbance was measured at 570 nm (11), and biofilm formation ability was evaluated as negative, weak, intermediate and strong for tested strain (14). *Staphylococcus aureus* ATCC 6538 and *S. aureus* ATCC 29213 were used as a positive and negative controls, respectively.

Calculation of Biofilm Formation Inhibition Percentage of EOs

The effect of TEO and OEO against *E. faecalis* biofilm were tested in 96-well plates at MIC and MBC concentrations and evaluated by comparing untreated wells. Briefly, 100 µL of bacterial suspension for each isolate were added to each well containing different concentration of EOs at MIC, MBC concentrations. After 24h of incubation, CVS was applied, and the OD values were quantified as described above. Wells with PBS were used as a control to calculate the percentage of biofilm inhibition (PI) as follows (11):

PI = [(Control OD570 nm-Sample OD570 nm)/Control OD570 nm] x 100

Effects of TEO and OEO on Biofilm Viability

Biofilm formation was carried out without antimicrobial agent as described above for prolonged time. In this study, the medium was refreshed every 48h and biofilm formation was provided in 2 weeks. At the end of the process, the wells containing enterococcal biofilm were washed twice with saline and 200 µL of each agent was added to each well at different concentrations. Then the sonication procedure (Daihan, Korea) for 10 seconds at 30k Hz was performed, and wells were mixed to remove excess biomass after sonication. To calculate viable microorganisms in the biofilm, serially diluted suspensions were cultured and the plates were incubated for 24h at 37 °C. The colonies were counted and the number of viable cells within the biofilm was calculated as CFU/mL. The number of viable microorganisms was compared with those in the control well with PBS (11).

Statistical Analysis

The IBM SPSS for Windows, version 22.0 was used for the statistical analysis (IBM Corp., USA). MIC and MBC were given as average values of repeated tests while calculation of biofilm formation inhibition was given as percentages. Biofilm viability data of TEO and OEO were analyzed by using one-sample t-test and statistical significance was sat at $p<0.05$.

Results

The MIC and MBC values of TEO and OEO were given in Table 1. MIC values of TEO and OEO were same at 0.078 µL/mL; however, TEO had higher MBC (0.156 µL/mL) than OEO (0.078 µL/mL). Biofilm formation inhibition percentages of TEO and OEO were calculated as 53.9% and 47.9% at 0.078 µL/mL concentrations while 55.6% and 44.8% at 0.156 µL/mL concentration for TEO and OEO, respectively. Antibiofilm activity of TEO and OEO on E. faecalis was given in Figure 1 indicating the OD values at 570 nm.

The effects of TEO and OEO on biofilm viability were shown in Table 2 and Figure 2. It was observed that both TEO and OEO has a significant effect on biofilm viability as both EOs caused sharp decrease in the number of viable cell within the biofilm at 0.078 µL/mL concentration. There was not statistical difference between TEO ($p=0.483$; $p\geq0.05$) and OEO ($p=0.169$; $p\geq0.05$) activities on biofilm viability.

Table 1. Antimicrobial efficacy of TEO and OEO

Essential oils	MIC (µL/mL)	MBC (µL/mL)
TEO	0.078	0.156
OEO	0.078	0.078

TEO: Thyme essential oil, OEO: Oregano essential oil, MIC: Minimum inhibitory concentration, MBC: Minimum bactericidal concentration

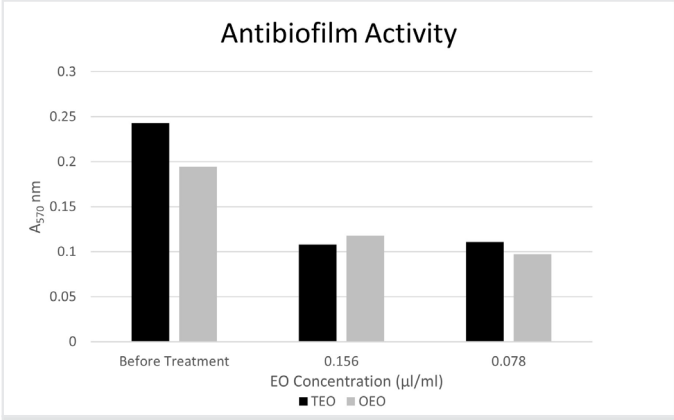

Figure 1. Antibiofilm activity of TEO and OEO on *E. faecalis*
TEO: Thyme essential oil, OEO: Oregano essential oil

Table 2. Effects of TEO and OEO on biofilm viability

EOs	PK	0.019	0.039	0.078	0.156	0.312	0.625	1.25	2.5	5	10	p-value (%95 CI)
TEO	8×10^8	49×10^8	392×10^6	0	0	0	0	0	0	0	0	0.483
OEO	3×10^9	43×10^8	115×10^3	0	0	0	0	0	0	0	0	0.169

TEO: Thyme essential oil, OEO: Oregano essential oil. Concentrations were in CFU/mL units; $p<0.05$ refers significant difference with One Sample t-test

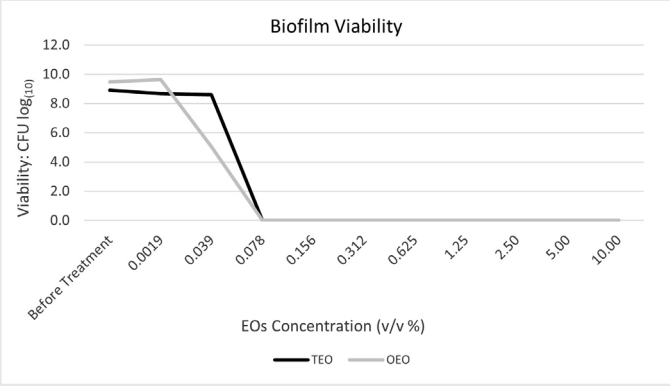

Figure 2. Effects of TEO and OEO on biofilm viability
TEO: Thyme essential oil, OEO: Oregano essential oil

Discussion

Sodium hypochlorite is considered the gold standard for root canal antisepsis and in routine clinical applications, chlorhexidine and sodium hypochlorite are the most frequently used irrigation materials in endodontic treatments to eliminate residual microorganisms (1,8,12). According to the literature on the elimination of endodontic pathogens, chemo-mechanical treatment did not show sufficient success due to the microbial adhesion to the dentin surface because of the specific anatomic structure of the root canal system and the adhesion ability of these endodontic pathogens.

Other than sodium hypochlorite, calcium hydroxide and triple antibiotic paste are the intracanal medicaments that can penetrate deep dentin tubules to eliminate biofilm (6,15). Ethylenediaminetetraacetic acid (EDTA) is one of the most used agents to remove the smear layer from root canals. Since *E. faecalis* biofilm is embedded in deep dentin tubules, EDTA application is assumed to provide an antibacterial effect during the removal of the smear layer. However, its antimicrobial activity against biofilms is still controversial (2). According to the previous *in vitro* studies, irrigation with silver nanoparticles (16), photodynamic therapy with lasers (16,17), or ozone application (18) during the irrigation might increase the antibacterial efficacy of sodium hypochlorite or EDTA.

Tissue-friendly herbal products have been started to be preferred in intracanal irrigation to prevent the exposure of healthy tissues and stem cells around the root from the toxic effects of irrigation solutions or medicaments that overflow from the root apex. Therefore, natural products derived from plants are rich in antimicrobial compounds and are often used to make oral hygiene products. EOs obtained from medicinal plants have been used in the treatment of some diseases due to their antimicrobial effects (18-20). However, there are few studies on their success in root canal treatments (8). According to the improvements in dental materials, the reason for resistant root canal infections seemed to be the presence of some resistant bacteria inside the roots such as *E. faecalis* and its important virulence factor biofilm formation (21,22). Therefore, the aim of this study was to evaluate MIC, MBC, and antibiofilm activity at different concentrations of TEO and OEO to evaluate their efficacy and record them for further laboratory, *in vitro*, and *in vivo* studies.

In the present study, TEO and OEO were preferred to determine antibacterial and antibiofilm efficacies in different concentrations. Thosar et al. (20) reported an *in vitro* study evaluating the antimicrobial activity of TEO and, according to the results zinc oxide cement mixed with TEO showed higher antimicrobial activity than zinc oxide and eugenol group against *Staphylococcus aureus*, *Escherichia coli*, *E. faecalis*, and *Pseudomonas aeruginosa*. Furthermore, Janani et al. (23) reported another *in vitro* study about the antimicrobial efficacy of OEO by evaluating MIC and MBC values amongst *E. faecalis*. According to the results, MIC was found to be 25 µg/mL while MBC was found to be 50 µg/mL. So, OEO was reported to be an effective antimicrobial agent against *E. faecalis*. According to the results of another study (24) comparing the antimicrobial efficacy and removal of the smear layer in dentin tubules, 1% OEO and 5.25% NaOCl had the same antimicrobial activity against *E. faecalis* and 2% or 5% OES had more effective antibacterial action than 5.25% NaOCl. According to these articles, the high antibacterial efficacy of OEO and TEO against *E. faecalis* can be seen. However, within the scope of this study, not only the antimicrobial activity of *E. faecalis* but also the antibiofilm activity of the biofilm it formed was investigated. According to the antibacterial results of these EOs in the present study, TEO and OEO had the same MIC values but, different MBC values. MBC values of TEO were higher than MIC values so, it can be said that TEO might be more unstable and suspicious than OEO according to higher MBC than MIC.

Biofilm formation is considered an important virulence factor of resistant root canal infections. So, in this study, antibiofilm efficacy was evaluated to determine the correct answers for resistant clinical root canal infections. According to the obtained results of biofilm formation inhibition, TEO showed higher inhibition on biofilm formation compared to OEO at both MIC and MBC concentrations. This antibiofilm feature might be a clue for the higher efficacy of TEO rather than OEO at the same MIC value.

According to the results, there were controversial results amongst MBC and biofilm formation inhibition values. TEO had bactericidal efficacy at higher MBC results than OEO. This means suspicious behavior has a toxic effect on tissues while showing higher antibiofilm activity than OEO by biofilm formation inhibition percentages at the MIC level. The results of biofilm viability showed that OEO caused a significant decrease rather than TEO and this result means that it was more effective against the formation of biofilm which is the most important virulence factor of *E. faecalis*. Although, these results showed that both EOs might be used due to higher antimicrobial and antibiofilm activity on *E. faecalis* biofilm even at low concentrations; OEO might be accepted as a more stable EO than TEO due to the same MBC than MIC values and similar antibiofilm activity results before and after the biofilm formation. However, these controversial results should be

evaluated with more *in vitro* studies. A significant cost is spent every year for the treatment of resistant root canal infections. Despite this, patients still need to get the teeth with the high clinical and radiographic success they deserve. Therefore, biocompatible structure and lower costs are important causes besides good antibacterial and antibiofilm effects against *E. faecalis* biofilm to prefer TEO and OEO.

Study Limitation

The strong point of this study was the penetration ability of EOs into dentin tubules. However, to the best of our knowledge, this is the first study comparing the antibacterial and antibiofilm efficacy of TEO and OEO against the *E. faecalis* biofilm. Studying a single bacterial species can be considered as the limitation of the study. The results of the present study should be evaluated for future *in vitro* and clinical studies including *E. faecalis* biofilm.

Conclusion

High antimicrobial effects of different concentrations of TEO and OEO were determined as MIC, MBC, biofilm formation inhibition percentage, and biofilm viability on *E. faecalis* biofilm. Therefore, it can be concluded that TEO and OEO might be used in resistant and recurrent endodontic lesions to destroy and remove the biofilm layer from root canals. However, further microbiological, and clinical studies should be done to evaluate the preliminary results of TEO and OEO and compare them with other irrigation agents such as sodium hypochlorite or chlorhexidine.

Ethics

Ethics Committee Approval: This study was approved by Başkent University Medical and Health Sciences Research Board Institutional Review Board (project no: KA22/187, date: 28.04.2022).

Informed Consent: Informed consent is not required.

Footnotes

Authorship Contributions

Surgical and Medical Practices: D.S.U., A.Ü.G., Concept: D.S.U., A.Ü.G., Design: D.S.U., A.Ü.G., Data Collection or Processing: D.S.U., A.Ü.G., Analysis or Interpretation: D.S.U., A.Ü.G., Literature Search: D.S.U., A.Ü.G., Writing: D.S.U., A.Ü.G.

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Original Article

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Evaluation of the Effects of Preeclampsia and Gestational Diabetes Mellitus on Endothelial Function

Preeklamsi ve Gestasyonel Diyabet Öyküsünün Endotel Fonksiyona Etkisinin Değerlendirilmesi

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ABSTRACT

Objective: Gestational diabetes mellitus (GDM) and preeclampsia are the most common medical complications of pregnancy. Both GDM and preeclampsia are risk factors for cardiovascular diseases (CVD) and atherosclerosis. Flow-mediated dilatation (FMD) is a good ultrasonographic marker of early atherosclerotic changes used to measure endothelial function. In this study; we evaluated FMD, an indicator of endothelial function, and investigated whether there was an increased risk of CVD in patients with a history of preeclampsia or GDM.

Methods: The study was carried out with 104 patients who gave birth in the Obstetrics and Gynecology Clinic of Bolu Abant İzzet Baysal University Training and Research Hospital between January 2016 and January 2017. Thirty four patients with a history of preeclampsia, 37 patients with a history of GDM, and 33 patients with uncomplicated deliveries were included in the study. All patients in the study had only one live birth and their age range was between 20 and 30 years. Demographic data, cardiovascular risk markers, obstetric data, laboratory tests and FMD change (%) measurements of all patients were compared. Mean and standard deviation values of the obtained data were calculated.

Results: The mean FMD change (%) in the group of patients with a history of preeclampsia was 9.8±3.1. It was 10.32±2.50 in the group of patients with a history of GDM and it was 13.19±3.03 in the control group. A statistically significant difference was found between the control group and GDM and preeclampsia groups in terms of FMD change (%) (<0.001). There was a significant negative correlation between FMD change (%) and systolic blood pressure, diastolic blood pressure, the amount of proteinuria, and glucose, low-density lipoprotein and total cholesterol levels.

ÖZ

Amaç: Gestasyonel diabetes mellitus (GDM) ve preeklampsi gebeliğin en sık medikal komplikasyonlarındandır. Gestasyonel diabet ve preeklampsi hem kardiyovasküler hastalıklar (KVH) hem de ateroskleroz için risk faktörüdür. Akım aracılı dilatasyon (FMD), endotel fonksiyonunu ölçmek için kullanılan, erken aterosklerotik değişikliklerin iyi bir ultrasonografik belirtecidir. Bu çalışmada; endotel fonksiyonlarının bir göstergesi olan FMD değerlendirilerek preeklampsi veya GDM öyküsü olan hastalarda artmış bir KVH riskinin olup olmadığını araştırdık.

Yöntemler: Çalışmamız Ocak 2016 ile Ocak 2017 tarihleri arasında Bolu Abant İzzet Baysal Üniversitesi Eğitim ve Araştırma Hastanesi, Kadın Hastalıkları ve Doğum Kliniği'nde doğum yapmış olan 104 hasta ile gerçekleştirildi. Preeklampsi öyküsü olan 34 hasta, GDM öyküsü olan 37 hasta ve komplikasyonsuz doğum yapmış 33 hasta çalışmaya dahil edildi. Olgu kontrol çalışması olarak dizayn edildi. Çalışmadaki tüm hastalar sadece 1 canlı doğum yapmış, yaş aralıkları 20 ile 30 arasında olacak şekilde belirlendi. Tüm hastaların demografik verileri, kardiovasküler risk belirteçleri, obstetrik verileri, laboratuvar tetkikleri ve FMD değişim (%) ölçümleri karşılaştırıldı.

Bulgular: Her üç grup FMD değişim (%) açısından karşılaştırıldığında preeklampsi öyküsü olan hasta grubu ortalaması 9,8±3,1, GDM öyküsü olan hasta grubu ortalaması 10,32±2,50, kontrol grubu hasta ortalaması 13,19±3,03 saptandı. Kontrol grubu ile GDM ve preeklampsi grupları arasında FMD değişimi (%) açısından istatistiksel olarak anlamlı farklılık saptandı (p<0,001). FMD değişim (%) değerleri ile sistolik tansiyon, diastolik tansiyon, glukoz, düşük dansiteli lipoprotein ve total kolesterol düzeyleri ve proteinüri miktarı ile anlamlı negatif korelasyon mevcuttu.

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ABSTRACT

Conclusion: The use of FMD change (%) measurement in patients with a history of GDM and preeclampsia can be used as a predictive marker for CVD, and early detection of risk can be time-consuming in terms of prevention. Demonstrating that GDM and preeclampsia cause increased cardiovascular risk in women's lives will raise awareness of taking measures to reduce the risk in this group of patients.

Keywords: Preeclamsia, gestational diabetes mellitus, endothelial function, flow-mediated dilatation

ÖZ

Sonuç: Gestasyonel diabetes mellitus öyküsü ve preeklampsi öyküsü olan hasta grubunda FMD değişiminin (%) ölçümü KVH açısından prediktif bir markır olarak kullanılabilir ve erken yaşta risk tespiti yapılabilmesi önlem alma açısından zaman kazandırıcı olabilir. GDM ve preeklampsinin kadınların yaşamlarında ilerleyen dönemde artmış kardiyovasküler riske neden olduğunun gösterilmesi, bu grup hastalarda riski azaltma yönünde önlemlerin alınması konusunda farkındalık oluşturacaktır.

Anahtar Sözcükler: Preeklamsi, gestasyonel diyabetes mellitus, endotel fonksiyon, akım aracılı dilatasyon

Introduction

Gestational diabetes mellitus (GDM) is a varying degree of carbohydrate intolerance that begins during pregnancy or is diagnosed during pregnancy (1). GDM causes short- and long-term complications for both mother and fetus. It should be advised to educate patients for symptoms of hyperglycemia and patients should come to control if they experience such symptoms. Macrovascular and microvascular complications of diabetes are known (2). It is obvious that both type 1 DM and type 2 DM increase the risk of cardiovascular diseases (CVD) (3). Insulin resistance in patients with GDM decreases nitric oxide (NO) activity in the vascular endothelium, leading to endothelial dysfunction (4). However, endothelial dysfunction and accelerated atherosclerosis in diabetic patients are thought to play a key role in the formation of cardiovascular complications (5).

Preeclampsia is defined as hypertension (HT) (systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg) that occurs after the middle of the second trimester of pregnancy and is accompaniedby newly emerging proteinuria (≥300 mg/24 hours) (6). Preeclampsia is not only a hypertensive condition, but a complex and multisystemic syndrome that concerns all systems of the body (7). The incidence of preeclampsia increases due to increase in maternal age, obesity, diabetes, HT and renal diseases. Although its etiology is not known precisely, it is anticipated to be caused by inadequate trophoblast invasion and widespread endothelial damage caused by vasospasm in the uteroplacental vascular bed. The cause of vascular endothelial dysfunction is unknown (8). There is increasing evidence that women with a history of preeclampsia during pregnancy are more likely to develop CVD later in life (9).

Endothelial dysfunction is by definition functional and reversible changes of endothelial cells due to the availability of NO in the endothelial cells and the oxidative stress disorder. It is seen in various pathological conditions such as endothelial dysfunction, atherosclerosis, hypercholesterolemia, diabetes, HT, heart failure, smoking, aging and obesity. Obesity is closely associated with the risk of CVD. Inflammation and impaired endothelial function can improve with weight loss. It functions to keep the endothelial vessel relatively dilated in basal conditions. However, the endothelium has the capacity to react to various physical stimuli such as shear stress (10). Blood vessels dilate in response to tearing stress, which is called flow-mediated dilation (FMD) (11). FMD is a good ultrasonographic marker of early atherosclerotic changes used to measure endothelial function, showing the vasodilation response of peripheral arteries against physical stimuli (12). The endothelium-dependent response is mainly regulated by the release of NO from the endothelium.

In this study; by evaluating FMD, which is an indicator of endothelial functions, we investigated whether there was an increased risk of CVD in patients with a history of preeclampsia or GDM.

Methods

Our study started with the approval of Bolu Abant İzzet Baysal University Ethics Committee (decision number: 2019/85, date: 11.04.2019). Written informed consents were obtained from all patients included. A total of 104 patients who were followed up at Obstetrics and Gynecology Clinic of Bolu Abant İzzet Baysal University Training and Research Hospital were included in the study. The study consisted of three groups. Thirty-four patients with a pre-history and at least 24 months from birth, 37 patients with GDM history, 33 patients with normal pregnancy follow-up and no pregnancy complications and additional diseases. Patients with a prior history of chronic disease (DM, HT), patients with a history of smoking, patients with a history of myocardial infarction, patients with chronic obstructive pulmonary disease, asthma, cor pulmonale, those with systemic disease and pregnant women using drugs for systemic disease, pregnants with multiple pregnancy, pregnancy cholestasis, gestational dermatosis, polyhydramnios, placenta previa, or detachment of the placenta, patients with thyroid dysfunction during pregnancy, pregnant women with rheumatological or autoimmune disease, and patients with the presence of peripheral or coronary artery disease were not included. The diagnostic criteria for preeclampsia are; systolic blood pressure ≥140 mmHg occurring after 20 weeks of gestation; diastolic blood pressure ≥90 mmHg and presence of proteinuria (≥300 mg/24h, urinary protein creatinine ratio ≥0.3 or 1+ protein in spot urine) or thrombocytopenia (<100,000/mm^3), renal dysfunction (creatinine ≥1.2 mg/dL and serum creatinine level increased by at least 2 times), liver dysfunction (AST-ALT >2 times of normal level), pulmonary edema or cyanosis, headache, blurred vision. The 24-hour protein level of all patients in the preeclampsia group was recorded. In all

pregnant women, oral glucose tolerance tests (OGTT) was performed with 50 grams of glucose for GDM screening at 24-28 weeks. In the screening test, women whose blood glucose was found to be 140-180 mg/dL at the first hour after drinking 50 grams of glucose were subjected to a 3-hour OGTT with 100 grams of glucose to make a definitive diagnosis of GDM.

The patient files were scanned retrospectively and pregnant women with a diagnosis of preeclampsia and GDM and pregnant women with normal follow-up were called by phone. GDM screening of all patients, history of preterm birth, abortion or in utero fetal loss, history of preeclampsia, smoking, drug use, dieting, hyperandrogenemia findings, obstetric, gynecological, medical and surgical resumes and family history (DM, HL in family, HT, CAD etc.) were questioned and detailed anamnesis was obtained. Height, weight and weight gain during pregnancy were recorded in all three groups. The pregnants were weighed without shoes and with light clothes on them (kg), and their height was measured without shoes and a hat, and their hair was measured without a flat bun (cm). Body mass indexes (BMI) of the patients were calculated according to the formula (BMI = body weight (kg)/height (m^2) = kg/m^2). All these data were noted on the patient evaluation form. The blood pressure of each patient in the control and study groups was measured in the clinic using the standard measurement technique. The measurement of the tension was assessed with brachial artery blood pressure after 5 minutes of rest while sitting, while the cuff was at the level of the heart. If there was a difference in systolic or diastolic blood pressure >5 mmHg at least twice in the measurements, it was deemed appropriate to repeat with two additional measurements. Each repeat was made by giving 30 minutes of rest and recorded.

All blood samples were taken on an empty stomach, using vacutaine from the antecubital area. HbA1c, glucose, low-density lipoprotein (LDL), very low density lipoprotein (VLDL), high-density lipoprotein (HDL), total cholesterol, and triglyceride levels were measured in maternal serum.

Ultrasonographic Evaluation of The Brachial Artery

Brachial artery Doppler ultrasonography examination was performed in the cardiology clinic of Bolu Abant İzzet Baysal University Training and Research Hospital, Faculty of Medicine, Cardiology Clinic for all patients in the study and control group. For brachial artery doppler ultrasonography examination, Philips EPIQ 7 device (Philips Medical Systems, Bothell, WA, USA) and a 12 L Doppler ultrasonography probe in the echocardiography laboratory were used in all patients and the control group. All echocardiographic procedures were performed by a single processor. The study was performed in a quiet and controlled environment, followed by a 8-12 hour fasting period, and the patients were placed in a supine position. Patients were advised to avoid exercise and not to take antioxidants such as caffeine, high-fat foods, and vitamin C in the last 4-6 hours. The transducer was placed on the right brachial artery tract 4-5 cm above the elbow, and the image was taken longitudinally in the region where the best image could be taken and enlarged. In this position, the projection of the transducer edge was marked on the skin with a ballpoint pen. The brachial artery diameter (intima to intima) was measured three times, and the average of these three measurements was recorded as the basal diameter. The cuff of the sphygmomanometer was connected to the forearm and inflated at least 50 mmHg above the systolic artery pressure, and after holding it for 5 minutes, the cuff was suddenly lowered, and the transducer was placed at the point marked with the pen, and the artery diameter at 60 sec (endothelium dependent vasodilator response) was recorded. The difference between the diameter measured after the reactive hyperemia and the basal diameter was taken as FMD. [FMD = 100x (radial diameter after reactive hyperemia)/basal diameter].

Statistical Analysis

The data were evaluated in the statistical package program IBM SPSS Statistics for Windows, Version 20.0 (Chicago, IL, USA: IBM Corp.). Number (n), percentage (%), average, median (minimum-maximum) values were given. Data were presented as mean arithmetic tools and standard deviations were calculated for each group. Kolmogorov-Smirnov test was used to evaluate the distribution of numerical variables. One-way analysis of variance (ANOVA) was used for comparing multiple groups with homogeneous distribution, and post-hoc Tukey test was used for comparisons between subgroups.

Kruskal-Wallis test was used for comparisons of nonparametric data belonging to multiple groups, and Bonferroni corrected Mann-Whitney U test was used for post-hoc analysis. The degree of relationship between continuous variables was calculated using the correlation analysis of Pearson or Spearman where appropriate.

Results

Our study was conducted with a total of 104 patients, including 37 patients with a history of of GDM, 34 patients with preeclampsia and 35 controls.

Demographic characteristics of the participants are shown in Table 1. The mean gestational age was found as 25.41±2.53 (year) in pregnant women with a history of GDM and 25.35±2.67 (year) in pregnant women with preeclampsia, while the mean age was 26±3.01 (year) in the control group (Table 1). There was a significant difference between the groups in terms of weight and BMI. This is because insulin resistance and obesity are more common in the GDM group. Although this affects endothelial function, the fact that the FMD value is lower than the control group may also be related to this situation. There was no statistically significant difference between the three groups in terms of average age, average height, family history, occupation, gravida, parity, number of abortions, time after delivery, and type of delivery ($p<0.05$) (Table 1).

Obstetric data of the previous pregnancies of all three patient groups are given in Table 2. All patients in the study were selected as having had 1 live birth. Patients who had given birth more than one or had a history of still birth were excluded from the study. The mean time elapsed after birth was 28.50±3.03

Table 1. GDM History, preeclampsia history and demographic findings of the control group

		Patients with a history of preeclamsia	Patients with a history of GDM	Control group	p-value
Age		25.35±2.67	25.41±2.53	26±3.01	0.561
Height		162.88±5.08	163.65±5.82	162.7±6.7	0.771
Weight		63.29±7.67	81.30±12.38	70.39±10.06	**<0.001**
BMI		23.86±2.81	30.25±3.72	26.58±3.50	**<0.001**
DM in the family (+)		3 (8.8%)	8 (21.6%)	3 (9.1%)	
HT in the family (+)		9 (26.5%)	1 (2.7%)	0 (0%)	
DM + HT + in the family		1 (2.9%)	4 (10.9%)	2 (6.1%)	0.082
No family history		21 (61.8%)	24 (64.9%)	28 (84.8%)	
Profession	**Housewife**	14 (41.2%)	17 (45.9%)	15 (45.5%)	
	Officer	10 (29.4%)	10 (27%)	8 (24.2%)	0.889
	Worker	10 (29.4%)	10 (27%)	10 (30.3%)	

GDM: Gestational diabetes mellitus, BMI: Body mass index, DM: Diabetes mellitus, HT: Hypertension

Table 2. GDM history, preeclampsia history, and obstetric data from previous pregnancies of the control group

		Patients with a history of preeclampsia	Patients with a history of GDM	Control group	p-value
Gravida		1.79±1.09	1.35±0.58	1.36±0.69	0.142
Number of abortions		0.79±1.09	0.35±0.58	0.36±0.69	0.142
The time elapsed after childbirth		28.50±3.03	28.62±3.51	28.66±3.38	0.927
Week of birth		35.15±3.15	37.3±5.18	39.39±1.34	**<0.001****
Baby birth weight		2494.32±760	3676.59±684	3315.30±238	**<0.001****
Mode of birth	**Normal birth**	15 (44.1%)	20 (51.1%)	23 (69.7%)	
					0.107
	Cesarean	19 (55.9%)	17 (45.9%)	10 (30.3%)	

GDM: Gestational diabetes mellitus, **: <0.01 compared to controls

months in the preeclampsia group and 28.62±3.51 months in the GDM group. It was found to be 28.66±3.38 in the control group. The postpartum periods in each of the 3 groups were selected as between 24 months and 36 months. The average postpartum time between the groups was found to be close to each other. The mean birth week of the patients in the patient group with a history of preeclampsia was 35.15±3.15, the mean birth week of the patients in the patient group with a history of GDM was 37.3±5.18, and the mean birth week of the patients in the control group was 39.39±1.34. There was a statistically significant difference between the weeks of birth in all three groups (p<0.001). When the groups were compared in terms of birth forms, 44.1% of the preeclampsia group, 51.1% of the GDM group and 69.7% of the control group had a normal birth (Table 2).

As seen in Table 3; the mean systolic blood pressure and diastolic blood pressure in patients with a history of GDM was 116.16±6.48/76.95±5.50, 121.3±8.8/78.6±5.9 in patients with preeclampsia and 111.97±7.63/71.67±7.04 in the control group. There was a statistically significant difference in terms of systolic and diastolic blood pressure averages in all three groups (p<0.001). Glucose and HbA1c values of all three patient groups were; 79.5±7.5, 5.1±0.3, 84.78±7.20, 5.62±0.27 in the GD group, 79.94±8.34, 5.01±0.36 in the control group. A statistically significant difference was found between the group with a history of GDM and the other two groups in terms of glucose and HbA1c values (p=0.007). LDL, HDL, VLDL, total cholesterol level averages were 138±41.5, 49.7±10.2, 138±41.5, 219.8±48 in the preeclampsia group; 126.49±36.57, 50.48±16.51, 33.78±15.69, 207.86±48.15 in the GDM group; and 117.09±37.31, 53.03±14.14, 32.48±17.71, 198.64±50.90 in the control group, respectively. There was no statistically significant difference between the groups in terms of the levels of those 4 parameters. The mean triglyceride levels were 208±110, 164.35±86.08 and 141.00±74.24 in the preeclampsia, GDM and control groups, respectively. There was a statistically significant difference between the three groups (p=0.005) (Table 3).

The mean FMD changes (%) were 9.8±3.1, 10.32±2.50 and 13.19±3.03 in the preeclampsia, GDM and control groups, respectively (Table 3). A statistically significant difference was found between the control group and GDM and preeclampsia groups in terms of FMD change (%) (p<0.001) (Table 3).

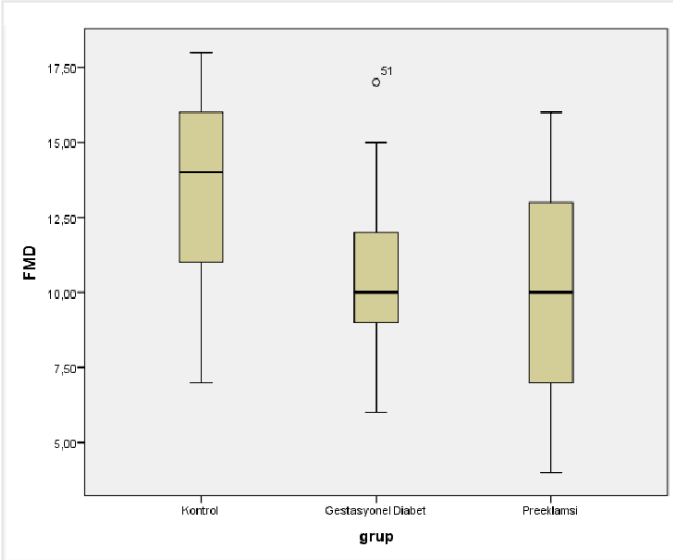
Table 3. Cardiovascular risk markers by groups (physical examination, laboratory, ultrasound parameters)

	Patients with a history of preeclampsia	Patients with a history of GDM	Control group	p-value
Systolic blood pressure	121.3±8.8	116.16±6.48	111.97±7.63	**<0.001****
Diastolic blood pressure	78.6±5.9	76.95±5.50	71.67±7.04	**<0.001****
Glucose	79.5±7.5	84.78±7.20	79.94±8.34	**0.007***
HbA1c	5.1±0.3	5.62±0.27	5.01±0.36	**<0.001****
LDL	138±41.5	126.49±36.57	117.09±37.31	0.087
HDL	49.7±10.2	50.48±16.51	53.03±14.14	0.59
VLDL	39±17.5	33.78±15.69	32.48±17.71	0.168
Total cholesterol	219.8±48	207.86±48.15	198.64±50.90	0.213
Triglyceride	208±110	164.35±86.08	141.00±74.24	**0.005***
FMD change (%)	9.8±3.1	10.32±2.50	13.19±3.03	**<0.001****

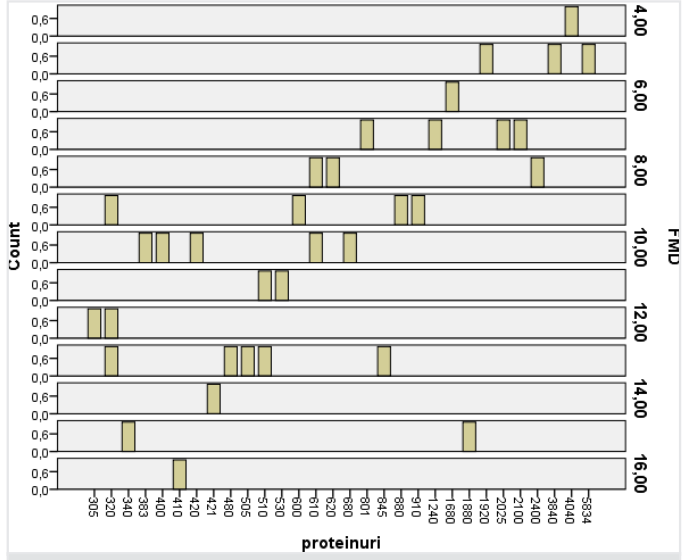
GDM: Gestational diabetes mellitus, FMD: Flow-mediated dilatation, LDL: Low-density lipoprotein, HDL: High-density lipoprotein, VLDL: Very low density lipoproteins

As seen in Graphic 1, among all groups, the lowest was in the preeclampsia group and the highest was in thecontrol group. The lowest and highest FMD change (%) values were 4 and 16 in the preeclampsia group, 7 and 18 in the control group, and lowest value was 6 in the GDM group (Graphic 1).

As seen in Graphic 2, it was observed that FMD change (%) rate gradually decreased as the amount of proteinuria increased in the patient group with a preeclampsia history.



Graphic 1. FMD change (%) of case and control groups
FMD: Flow-mediated dilatation



Graphic 2. Relationship between proteinuria amount and FMD change (%)
FMD: Flow-mediated dilatation

Discussion

Endothelial dysfunction is an important predictor of future CVD development. In this study; FMD, which is an indicator of endothelial functions, was evaluated and it was investigated whether there was an increased risk of CVD in patients with a history of preeclampsia or GDM. Investigating the effects of having a history of GDM or preeclampsia on the endothelium is important in order to predict and prevent macrovascular events that may result in mortality. In our study, the demographic data, obstetric data, laboratory parameters, and endothelial structure by using FMD of the preeclampsia, GDM and control groups were compared.

The FMD measurement has been studied many times before in HT, preeclampsia, metabolic and CVD groups. The decrease in this value, which is accepted as a marker of subclinical atherosclerosis, is considered significant. In many studies conducted in patients with preeclampsia and GDM, FMD measurements were found to be significantly lower compared to normotensive and normoglycemic pregnant women (13). The originality of our study was the careful selection of patient groups. Since FMD measurement was affected by the age factor, the age range was limited and healthy women between the ages of 20 and 30 were included in the study. However, only patients who had a live birth were included in the study. When the literature was reviewed, the same number of births and no study of the patient population with a similar age range were seen. In our study, women with similar age group who had a history of preeclampsia, GDM history and who did not experience these two conditions during pregnancy were compared in terms of laboratory and FMD change (%) in terms of showing cardiovascular risk ratio. FMD in the group with a history of preeclampsia was significantly lower than the group without a history of preeclampsia. When all three groups were compared in terms of FMD change (%), the mean of the patient group with a preeclampsia history was 9.8±3.1, the mean of the patient group with a history of GDM was 10.32±2.50, and the mean of the control group was 13.19±3.03. A statistically significant difference was found between the control group and GDM and preeclampsia groups in terms of FMD change (%) (<0.001). Among all groups, the lowest was in the preeclampsia group and the highest was in the control group.

In the study of Tarim et al. (14), there was no difference between the groups with and without GDM interms of total cholesterol, HDL, LDL and insulin levels. Triglyceride and fasting glucose levels were significantly higher in the group with a history of GDM (14). In our study, a significant difference was found between the patient group with a history of GDM and the control group in terms of glucose, HbA1c and FMD change (%) values. In our study, there was a statistically significant difference between the group of patients with preeclampsia and the control group in terms of systolic blood pressure, diastolic blood pressure, triglyceride level and FMD change (%).

There are studies in the literature that have different results from our study. Women with a history of preeclampsia and gestational HT in previous pregnancies and healthy women who had a healthy pregnancy were compared in terms of endothelial dysfunction 2-12 years after their pregnancy in the study by Mangos et al. (15). FMD values were similar in women with a history of preeclampsia or gestational HT. However, in this study, the periods after delivery were accepted for 10 years and could not beabstracted from other parameters that might affect the FMD measurement of the patients after birth.

In our study, we found a significant negative correlation between FMD change (%) and systolic and diastolic blood pressure, glucose, LDL, total cholesterol, and triglyceride levels and amount of proteinuria in the preeclampsia and GDM groups. In particular, we found that FMD change (%) decreased more as the proteinuria amount increased in patients with pre-eclampsia. While there was no difference in terms of LDL, HDL, VLDL, and total cholesterol levels; glucose and triglyceride levels were significantly higher in the group with a history of GDM. In addition, in our study, HbA1c was found tobe significantly higher in the GDM group. This is important in terms of showing the relationship between glucose level and HbA1c and increased cardiovascular risk. The distribution of factors such as age, height, family history of DM or HT, CAD, gravida, parity, abortion number in GDM, preeclampsia and control groups were determined. This suggests that the resulting endothelial dysfunctional directly related to a history of GDM.

Low FMD change detected in patients complicated with GDM or preeclampsia during their pregnancy indicate that having GDM or preeclampsia carries a risk for CVD and that these patients should be followed up. Although many studies conducted in patients with a history of GDM or with a preeclampsia history have shown low FMD values; there are also studies in the literature that show opposite results. It would be appropriate to conduct prospective studies covering large patient series or meta-analysis with current studies. In addition to FMD measure's advantages such as being cheap, simple and easy to apply, repeatability, no risk for the patient, it should not be forgotten that it has difficulties such as difficult to display, the necessity of keeping the probe fixed throughout the measurement, the measurement requires experience, and there is still no consensus on the measurement technique.

Study Limitations

This study has potential limitations. In our study, it is a negative situation that the number of patients is small and there are patients who have given birth in a single hospital. In addition, we believe that the fact that the patients do not have the same weight may affect the FMD change. A study with a larger sample group and patients with similar demographic data will be more meaningful.

Conclusion

The FMD measurement was significantly lower in women with a preeclampsia or GDM history during pregnancy than in the control group. Significant results can be interpreted that FMD measurement increases the risk of developing CVD in women with a history of preeclampsia or GDM. The FMD change in the premenopausal patient group can be used as a predictive marker for CVD, and early risk detection can be time-saving. If an increased cardiovascular risk is detected in these patients, we can ensure that the patient takes measures to reduce this risk. Counseling can be given to this patient group on lifestyle changes, diet, weight control, blood pressure monitoring.

Demonstrating that GDM - causes increased cardiovascular risk in women in the future will create awareness in taking measures to reduce risk in this group of patients.

Ethics

Ethics Committee Approval: Our study started with the approval of Bolu Abant İzzet Baysal University Ethics Committee (decision number: 2019/85, date: 11.04.2019).

Informed Consent: Written informed consents were obtained from all patients included.

Footnotes

Authorship Contributions

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

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Original Article

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Efficacy Comparison of Ibuprofen 400 Mg and 800 mg in the Treatment of Renal Colic: Prospective Randomized Double-blind Clinical Study

Renal Kolik Tedavisinde İbuprofen 400 Mg ve 800 Mg'nin Etkinlik Karşılaştırması: Prospektif Randomize Çift-kör Klinik Çalışma

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ABSTRACT

Objective: Renal colic, predominantly due to ureteral stones, constitutes a significant portion of urinary system-related hospital admissions. Non-steroidal anti-inflammatory drugs, notably ibuprofen, are favored over opioids for pain management. However, the optimal intravenous (IV) ibuprofen dosage for renal colic remains unclear. This study aimed to compare the analgesic efficacy of IV ibuprofen at doses of 400 mg and 800 mg in moderate to severe renal colic patients.

Methods: A multicenter, prospective, randomized, double-blind controlled clinical trial was conducted on patients with moderate to severe renal colic. Patients meeting inclusion criteria were randomly assigned to receive either 400 mg or 800 mg IV ibuprofen. Pain scores were assessed using numeric rating scale at baseline, 15, 30, 60, and 120 minutes. The need for rescue analgesics and occurrence of side effects were recorded.

Results: Out of 150 initially enrolled patients, 126 completed the study. Pain reduction was more significant in the 800 mg group compared to the 400 mg group, especially at the 120-minute mark ($p<0.05$). The need for rescue analgesics and occurrence of side effects did not significantly differ between the two groups.

ÖZ

Amaç: Üreter taşlarına bağlı olarak ortaya çıkan renal kolik, üriner sistemine ilişkin acil servis başvurularının önemli bir kısmını oluşturur. Bu durumda ağrı yönetimi, non-steroidal anti-enflamatuvar ilaçlar arasında yer alan ibuprofen gibi ilaçların opioidlere tercih edilmesiyle gerçekleştirilir. Ancak, renal kolik için en etkili intravenöz (IV) ibuprofen dozu hala netlik kazanmamıştır. Bu çalışmanın amacı, orta ila şiddetli renal koliği olan hastalarda IV ibuprofenin 400 mg ve 800 mg dozlarının analjezik etkinliğini karşılaştırmaktır.

Yöntemler: Orta ila şiddetli renal koliği olan hastalarda çok merkezli, prospektif, randomize, çift-kör kontrollü klinik bir çalışma gerçekleştirilmiştir. Dahil edilme kriterlerini karşılayan hastalar, rastgele olarak ya 400 mg ya da 800 mg IV ibuprofen almışlardır. Ağrı skorları, başlangıçta ve 15, 30, 60 ve 120 dakika sonra sayısal değerlendirme skalası kullanılarak değerlendirilmiştir. Kurtarıcı analjezik kullanımı ve yan etki görülme durumları kaydedilmiştir.

Sonuç: Başlangıçta 150 hasta dahil edilmiş olup, çalışmayı 126 hasta tamamlamıştır. Ağrı azalması, özellikle 120. dakikada, 800 mg grubunda 400 mg grubuna göre daha belirgin olmuştur ($p<0,05$). Kurtarıcı analjezik kullanımı ve yan etki görülme durumları ise iki grup arasında anlamlı bir fark göstermemiştir.

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ABSTRACT

Conclusion: This study suggests that IV ibuprofen at a dosage of 800 mg provides more effective analgesia for renal colic compared to 400 mg. Therefore, the higher dosage may be preferred in clinical practice due to its superior efficacy and safety profile. Further research could explore the long-term effects and optimal dosing regimens of IV ibuprofen in renal colic management.

Keywords: Renal colic, ibuprofen, pain management

ÖZ

Sonuç: Bu çalışma, orta ila şiddetli renal koliği olan hastalarda IV ibuprofenin 400 mg ve 800 mg dozlarının karşılaştırılmasını içermekte olup, 800 mg dozunun daha etkili bir analjezi sağladığını göstermektedir. Bu nedenle, klinik uygulamada daha yüksek dozun tercih edilmesi, üstün etkinlik ve güvenlik profili nedeniyle düşünülebilir. Gelecekteki çalışmalar IV ibuprofenin uzun vadeli etkilerini ve optimal dozaj rejimlerini daha ayrıntılı olarak araştırmalıdır.

Anahtar Kelimeler: Renal kolik, ibuprofen, ağrı yönetimi

Introduction

Pain originating from the renal tract accounts for approximately 75% of hospital admissions related to the urinary system (1). Among the most commonly identifiable causes of obstruction, ureteral stones are prominent, with more than 75% of them spontaneously passing without the need for any intervention (1,2). These complaints are observed in men at a rate of approximately 12%, whereas among women, it shows an equal distribution. Additionally, the most common age range for occurrence is between the 4th and 6th decades for men, while for women, it occurs toward the end of the 2nd decade and the beginning of the 3rd decade. Presentation rates vary according to geographical regions and seasonal periods, with an increased frequency noted in hot climates and during the summer months (3). In the pathophysiology, it is believed that the increase in prostaglandin synthesis plays a role. In this case, there is an increase in arterial vasodilation, vascular permeability, and consequently, edema and spasmodic pain occur in the ureter (1). It has been noted that paracetamol and nonsteroidal anti-inflammatory drugs (NSAIDs) are more effective in the treatment of renal colic compared to opioids (4). The mechanisms of pain relief by NSAIDs in renal colic are thought to involve the suppression of increased prostaglandin synthesis, reducing glomerular filtration and pressure, and decreasing stimulation of stretch receptors in that region (5,6). Additionally, there is evidence of direct effects of NSAIDs on smooth muscles (5).

In renal colic patients, a commonly used stone scoring system is frequently employed to establish the diagnosis of obstructive renal stones (7). This scoring system includes 5 main factors: gender, time, race, nausea/vomiting, and the presence of red blood cells in the urine, resulting in a total of 13 possible points. Patients' likelihood of having a stone is calculated based on the total score on a 13-point scoring system, categorized as low (0-5), moderate (6-9), and high risk (10-13) (2). Although there have been pain studies related to renal colic, there are few studies in the literature conducted using ibuprofen. Specifically, due to the routine use of two doses of ibuprofen based on physician's or patient's discretion, it was planned to compare these doses and evaluate whether there were differences in terms of potential side effects, efficacy, and safety. If there was no difference in analgesic efficacy between the two doses, we hypothesized that 400 mg might be more appropriate, and we planned to compare the 400 mg and 800 mg preparations from this perspective.

Methods

Study Design and Setting

The research was designed as a multicenter, prospective, randomized, double-blind controlled clinical study. The study was conducted by including patients with moderate and severe renal colic complaints [numeric rating scale (NRS) >4] and a stone scoring of >5 points who presented to the emergency departments of two tertiary level hospitals. Approval was obtained from the Atatürk University Faculty of Medicine Clinical Research Ethics Committee (decision number: 2, date: 27.10.2022). All patients participating in the study were informed, and written informed consent was obtained in accordance with the principles of the Helsinki Declaration for Good Clinical Practice. This study adheres to the CONSORT guidelines.

Patient Selection

Patients who presented to both emergency departments with complaints of renal colic, NRS >4, and stone score >5 were included in the study. The study included patients aged 18-65 who were diagnosed as having stones on computed tomography, hemodynamically stable, without any comorbidities, without a history of adverse reactions to the drugs to be used in the study, conscious, fully oriented and cooperative, with no differential diagnoses considered, planned for discharge to home, and willing to participate in the study.

Patients who were not willing to participate in the study, patients in whom NRS-15 and subsequent pain scores could not be obtained and patients who were pregnant or breastfeeding, allergic to any of the drug groups under study, had any contraindications related to the use of these drugs, had other comorbidities, were not planned for discharge, had any additional diseases (hypertension, kidney failure, liver disease, chronic obstructive pulmonary disease, heart failure, diabetes, etc.), had used analgesic drugs within the last 6 hours, were mentally retarded and uncooperative, had hearing impairments, and had underlying organic neurological and psychiatric disorders were excluded from the study.

Sample Size

The sample size was determined using G*Power 3.1 software. According to the sample size calculation based on the study by Cenker et al. (8), with a power of 80% and a type-1 error rate of 1%, a minimum sample size of 23 patients per group was calculated. Taking into consideration potential data losses and patients who might drop out during follow-up, we planned to enroll 75 patients in each group, resulting in a total of 150 patients (n=150).

Intervention

Randomization was performed by the principal investigator. After eligible patients were included according to the criteria by the researchers, comprehensive medical histories were taken, and vital signs at the time of admission were examined and recorded. The randomization table was created using the website https://www.randomizer.org/. Patients were assigned to groups in a 1:1 ratio. Fifteen block randomizations were conducted for each medication dose group, with 10 patients in each group. Each code and the corresponding ibuprofen dose (400 mg, 800 mg) were written on paper and placed in opaque envelopes. The envelopes were sequentially numbered to indicate the order in which they should be opened. Blinded nurses prepared the ibuprofen doses by opening the envelopes. The study nurse added the ibuprofen dose from the envelope to 100 mL of saline solution and then handed the prepared treatment to the attending nurse. The treatment was administered to the patient as a rapid infusion, not exceeding 5 minutes. In this way, the patient, the physician, and the administering nurse remained unaware of the treatment dose. Case report forms and the NRS scores contained within were filled out by these blinded physicians. The remaining 120-minute study period was completed. Patients were monitored for 120 minutes, and if there was no improvement in pain score by the 30th minute or if NRS >4 was observed at the 60th minute, the rescue treatment protocol was initiated, adding 100 mg of tramadol hydrochloride to 500 mL of saline solution. Parameters such as vital signs, NRS scores, stone scores, and the occurrence of side effects were recorded at baseline (0 minutes), 15, 30, 60, and 120 minutes.

Outcomes

The primary outcomes of the study included pain scores at the following time points: baseline (0 minutes), 15 minutes, 30 minutes, 60 minutes, and 120 minutes. Additionally, the degree of decrease in pain scores was assessed at the following time intervals: 0-15 minutes, 0-30 minutes, 0-60 minutes, and 0-120 minutes. Secondary outcomes encompassed the need for rescue analgesics and the occurrence of drug-related side effects. These data were analyzed comparatively between the two groups.

Statistical Analysis

The study data were recorded on preprepared case report forms and subsequently entered into IBM Statistics for macOs, Version 28.0 (Armonk, NY: IBM Corp) software for blind analysis. The Shapiro-Wilk test was employed to assess the normality of continuous data. Normally distributed parameters were presented as mean values, standard deviations, and 95% confidence intervals, while non-normally distributed parameters were represented as medians and interquartile ranges.

For non-normally distributed parameters, the Mann-Whitney U test was utilized to compare medians between the two groups. On the other hand, the independent samples t-test was employed to evaluate mean differences between the two groups for normally distributed parameters. The Pearson chi-square test was used to compare the ratios of categorical data between the main groups.

The data of patients whose NRS data could not be obtained were excluded from the study, and the analysis was concluded using a per-protocol analysis. A significance level of $p<0.05$ was considered for statistical significance.

Results

A total of 150 patients who met the inclusion criteria were initially enrolled in the study. However, due to 24 patients expressing a desire to withdraw from the study after enrollment, the study was completed with 126 patients via per-protocol analysis. The consort flow diagram of the study was provided as Figure 1. Of these patients, 38.1% were women, and the mean age was 42.6±13.2 years. The patients were divided into two groups: 61 (48.4%) in the 400 mg group and 65 (51.6%) in the 800 mg group. Among the participants, 75 (59.5%) had a history of urinary stones, and 44 (34.9%) had a positive family history of the condition.

The distribution of parameters, including demographic data, characteristics of symptoms (pain), urinary stone history, analgesic use, and macroscopic hematuria, among the groups, is presented in Table 1. Although there were differences in some of these parameters, none of these differences were statistically significant (Table 1).

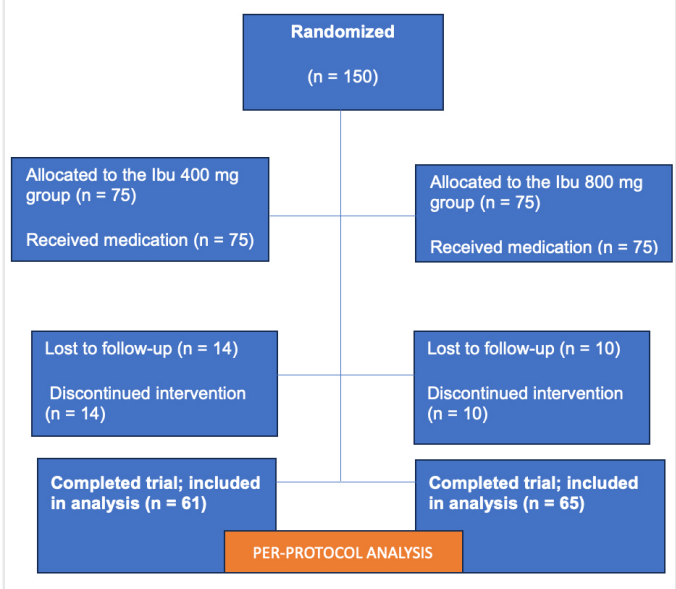

Figure 1. CONSORT flow diagram of the study

Pain measurements using the numerical rating scale at 0, 15, 30, 60, and 120 minutes, as well as the differences between these time intervals, are presented in Table 2. These parameters were compared between the two groups. In this analysis, a statistically significant difference was observed only in the "Diff 0-120" parameter. It was found that the pain score decreased more in the 800 mg group compared to the 400 mg group (Table 2, Figure 2).

Table 1. Demographic, symptom and background characteristics in 400/800 mg groups

Variables		Treatment group 400 mg			800 mg			p-value
		n (%)	Mean ± SD or median	25-75% or 95% CI	n (%)	Mean ± SD or median	25-75% or 95% CI	
Gender	Male	34 (55.7)			44 (67.7)			0.167*
	Female	27 (44.3)			21 (32.3)			
Age			42.6±12.3	39.5-45.7		42.6±14.1	39.1-46.0	0.982†
Height			168.4±9.3	166.0-171.0		169.9±7.7	168.0-172.0	0.332†
Weight			77.4±15.7	73.4-81.4		78.0±12.4	74.9-81.1	0.810†
Symptom time			13.0	4.0-22.0		16.0	5.0-48.0	0.182‡
Stone history		33 (54.1)			42 (64.6)			0.229*
Family stone history		22 (36.1)			22 (33.8)			0.794*
Pain localization	Flank	46 (75.4)			59 (90.8)			-
	Upper	6 (9.8)			3 (4.6)			
	Lower	8 (13.1)			1 (1.5)			
	Inguinal	1 (1.6)			2 (3.1)			
Pain radiation	Flank	35 (57.4)			39 (60.0)			-
	Upper	6 (9.8)			7 (10.8)			
	Lower	12 (19.7)			14 (21.5)			
	Inguinal	3 (4.9)			5 (7.7)			
	Ureter	5 (8.2)			0 (0.0)			
Agitation		17 (27.9)			21 (32.3)			0.587*
Analgesic use		12 (19.7)			14 (21.5)			0.796*
Analgesic using time			8.0	7.5-11.0		8.5	7.0-12.0	0.855‡
Stone score			8.0	6.0-9.0		7.0	6.0-8.0	0.206‡
Macroscopic haematuria		19 (31.1)			19 (29.2)			0.815*

*Pearson chi-square test, †Independent samples-t test; mean ± SD, 95% CI, ‡Mann-Whitney U test; median, interquartile range, SD: Standard deviation, CI: Confidence interval

Table 2. Numerical rating scale and differences between time periods

Variables	Treatment group 400 mg		800 mg		p-value
	Median (25-75%)	Mean ± SD	Median (25-75%)	Mean ± SD	
NRS-0	8.0 (6.0-9.0)	7.7±1.7	8.0 (7.0-9.0)	7.7±1.4	0.982
NRS-15	6.0 (4.0-7.0)	5.7±2.4	5.0 (4.0-7.0)	5.7±2.1	0.853
NRS-30	4.0 (2.0-5.0)	3.8±2.6	4.0 (2.0-5.0)	3.9±2.2	0.735
NRS-60	2.0 (0.0-4.0)	2.9±2.7	2.0 (1.0-3.0)	2.2±1.9	0.339
NRS-120	1.0 (0.0-3.0)	1.7±1.8	1.0 (0.0-2.0)	1.2±1.4	0.132
Diff0.15	2.0 (1.0-3.0)	2.0±1.7	2.0 (1.0-3.0)	2.0±1.4	0.707
Diff0.30	4.0 (3.0-5.0)	3.9±2.0	4.0 (3.0-5.0)	3.9±1.7	0.924
Diff0.60	5.0 (4.0-6.0)	4.8±2.1	6.0 (5.0-7.0)	5.5±1.5	0.050
Diff0.120	6.0 (5.0-7.0)	6.0±1.7	7.0 (6.0-7.0)	6.5±1.3	0.008

Mann-Whitney U test, SD: Standard deviation, NRS: Numeric rating scale

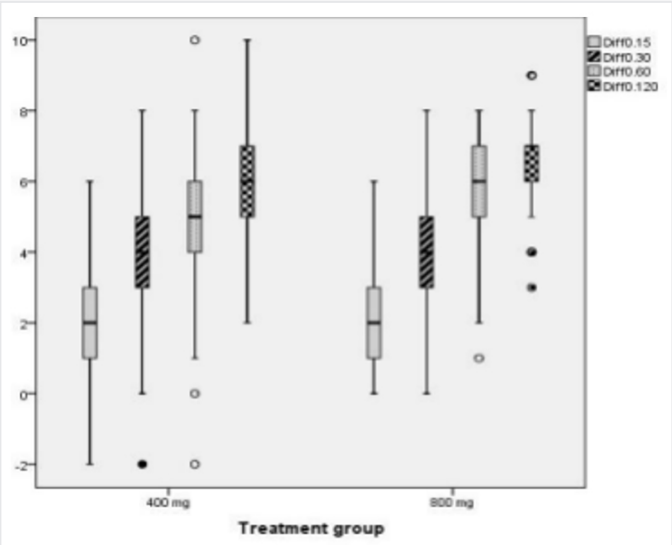

Figure 2. NRS differences in two groups
NRS: Numeric rating scale

Rescue analgesics were administered to 12 (19.7%) patients in the 400 mg group and 8 (12.3%) patients in the 800 mg group. However, this difference was not found to be statistically significant (p=0.258; Pearson chi-square test). Additionally, no drug-related side effects were observed in either group. No major or minor side effects were observed in the patients.

Discussion

The results of this study have provided us with the opportunity to compare the doses of ibuprofen and their analgesic efficacy in the treatment of patients presenting to the emergency department with suspected renal colic.

While renal colic itself is not a life-threatening condition, the pain induced by kidney stones necessitates treatment to provide comfort to the patients (9). Several factors such as male gender, advanced age, and obesity have been reported to influence the prevalence and frequency of renal colic (10). In accordance with these available data, the findings of this study exhibit similarities and concordance with the existing literature.

The pain experienced by patients suffering from renal colic demands effective analgesic treatment, and various analgesic agents, including NSAIDs, opioids, and drugs from the paracetamol group, have been employed for this purpose. Furthermore, the efficacy of the chosen analgesic plays a critical role in the treatment (11). In a clinical study comparing the intravenous (IV) forms of ibuprofen (800 mg) and paracetamol (1 gram) in the treatment of renal colic pain, it was demonstrated that the 800 mg IV ibuprofen was more effective than the 1 gram IV paracetamol (8). Another clinical study on renal colic compared the doses of 800 mg ibuprofen and 30 mg ketorolac, concluding that the ibuprofen group provided faster and more effective pain relief (9). Shaker and Borghei (12) also conducted a study with 70 patients experiencing renal colic pain, comparing 800 mg IV ibuprofen and 30 mg ketorolac. Their study showed that the change in pain scores was not statistically significant between the two groups. In another study conducted by Safaie et al. (13), it was demonstrated that combinations of ibuprofen (800 mg) with morphine (5 mg) and ketorolac (30 mg) with morphine (5 mg) were more effective than morphine alone, and both combinations exhibited similar efficacy. These findings suggest that ibuprofen either provides faster and more effective pain control than other analgesics or enhances the analgesic efficacy when used in combination treatments.

This current study supports the notion that the 800 mg form of ibuprofen is more effective. In previous studies related to renal colic, the 800 mg dose of ibuprofen has been considered an effective dose, and successful results have been obtained (11-13). There are also studies in the literature that investigate different doses of ibuprofen for the same conditions. For instance, in a study conducted with patients suffering from migraine-type headaches, both 200 mg and 400 mg IV doses of ibuprofen were found to be superior to placebo and exhibited similar efficacy (14). In a placebo-controlled study aimed at postoperative pain control, both 400 mg and 800 mg IV doses of ibuprofen were found to be significantly effective compared to placebo and had similar efficacy (15). When examining the time curve of this study, it became evident that the 800 mg dose of ibuprofen provided more effective pain relief in the second hour. Although the results of this study and other studies in the literature may differ across disease groups, it is suggested that the pain relief property of ibuprofen yields more efficient results with increasing doses. A review by Derry et al. (16) reported a parallel relationship between the use of ibuprofen at increasing doses in the treatment of post-dental operation pain control, with increased effectiveness as the dose increased.

Additionally, in a meta-analysis conducted on renal colic cases, it was reported that ibuprofen and ketorolac exhibited similar analgesic efficacy up to the 120th minute (17). Furthermore, in a study conducted by Özdemir et al. (18), where they compared paracetamol, ibuprofen, and dexketoprofen, they compared all three drug groups and reported no statistically significant differences among them. In the same study, they also compared the 400 mg and 800 mg doses of ibuprofen and found no statistical difference between them. Therefore, the presence of a statistical difference in favor of the 800 mg dose of ibuprofen in our results does not align with the literature.

In conclusion, the superior or similar efficacy of ibuprofen observed in studies involving different drug groups has prompted the need for a dose comparison evaluation of ibuprofen. This study, considering the number of patients, demographic data, patient height and weight ratios, NRS and stone scoring, demonstrated that the groups were homogeneously distributed. This homogeneous and balanced distribution of the groups enhanced the reliability of the study. Although the NRS difference values were similar up to the 60th minute, the difference became more significant in the group using 800 mg of ibuprofen at the 120th minute. Additionally, the lower requirement for rescue medication and the absence of a difference between the two

groups in terms of side effects suggest that 800 mg of ibuprofen is a more preferable option for controlling renal colic pain.

Study Limitation

The use of the NRS for pain grading, while advantageous for its ease of use, may not provide as sensitive results as the visual analog scale. Not monitoring pain beyond the 120th minute can also be considered a separate limitation of this study. Furthermore, not conducting the study with a larger sample size is among the limitations of the study. The absence of other treatment doses also represents another limitation. Given that emergency departments are environments characterized by rapid patient turnover and circulation, the inability to monitor patients for longer periods, even for study purposes, can be considered a significant limitation and weakness. Since the study concluded at the 120th minute, it is unknown whether patients experienced recurrent pain within the 24-hour timeframe or required additional medical treatment. However, future studies can be designed considering these factors.

Conclusion

Based on the findings of this study, it can be concluded that the IV formulation of ibuprofen at a dose of 800 mg has more effective analgesic activity in the treatment of renal colic. Therefore, it may be more rational to use the 800 mg IV dose of ibuprofen, especially since there were no serious side effects associated with its use. Given the widespread use of ibuprofen in this context, it is likely that these results can be readily applied in clinical practice.

Ethics

Ethics Committee Approval: Approval was obtained from the Atatürk University Faculty of Medicine Clinical Research Ethics Committee (decision number: 2, date: 27.10.2022).

Informed Consent: All patients participating in the study were informed, and written informed consent was obtained.

Footnotes

Authorship Contributions

Surgical and Medical Practices: S.F., E.T., A.B.E., A.Ş., Concept: S.F., E.T., A.B.E., A.Ş., Design: S.F., E.T., A.B.E., A.Ş., Data Collection or Processing: S.F., A.B.E., A.Ş., M.Y., E.Ü., Analysis or Interpretation: S.F., A.Ş., Literature Search: S.F., E.T., A.B.E., A.Ş., M.Y., E.Ü., Writing: S.F., E.T., A.B.E., A.Ş., M.Y., E.Ü.

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Original Article

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Evaluation of Toxicity Associated with CAR-T Cell Therapy and Nursing Interventions

CAR-T Hücre Tedavisi ile İlişkili Toksisite Değerlendirme ve Yönetiminde Hemşirelik Girişimleri

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ABSTRACT

Objective: Chimeric antigen receptor (CAR)-T cell therapy is a new immunotherapy approach that has started to been used in recent years and is developing rapidly. CAR-T cells, which are used as an immunotherapy treatment, destroy the tumor cell both directly and by increasing the release of cytokines. Our aim in this study is to evaluate inpatient CAR-T cell therapy patients in our clinic in line with cytokine release syndrome (CRS) and CAR-T related encephalopathy syndrome (CRES) management and to guide clinical practices by sharing the nursing interventions we apply in the management of CAR-T cell post-treatment toxicities.

Methods: Thirteen patients who received CAR-T cell therapy between 2020 and 2023 were included in this descriptive study. Following CAR-T cell infusion, the following nine-day period was retrospectively examined from the patients' files. CRS toxicity findings that might occur after CAR-T cell infusion, CRES toxicity findings, cognitive findings recorded in the CARTOX-10 neurological evaluation form, as well as treatment methods and nursing interventions applied, were evaluated and recorded.

Results: When we evaluated the CAR-T cell infusion toxicity findings, 38.46% of the patients had CRS stage 1, 30.79% had CRES stage 2, 15.38% had dysgraphia, 23.07% had cognitive impairment, 7.69% had somnolence and contraction in the arm and shoulder muscles were detected in 7.69%. It was determined that two patients were transferred to the intensive care unit due to both CRS and CRES toxicity findings.

Conclusion: The role of nurses is important in monitoring and managing toxicity after CAR-T cell infusion, which is a new

ÖZ

Amaç: Kimerik antijen reseptörü (CAR)-T hücre tedavisi son yıllarda kullanılmaya başlanan ve hızlı gelişim gösteren yeni bir immünoterapi yaklaşımıdır. Bir immünoterapi tedavisi olarak kullanılan CAR-T hücreleri ise tümör hücresini hem direkt olarak hem de sitokin salınımını artırma yoluyla yok etmektedirler. Bu çalışmada amacımız, sitokin salınım sendromu (CRS) ve CAR-T ilişkili ensefelopati sendromu (CRES) yönetimi doğrultusunda, kliniğimizde yatarak CAR-T hücre tedavisi yapılan hastaları değerlendirmek ve ortaya çıkan CAR-T hücre tedavi sonrası toksisitelerinin yönetiminde uyguladığımız hemşirelik girişimlerini paylaşarak klinik uygulamalara rehberlik edebilmektir.

Yöntemler: Tanımlayıcı nitelikte olan bu çalışmaya 2020-2023 yılları arasında CAR-T hücre tedavisi yapılan 13 hasta dahil edildi. CAR-T hücre infüzyonunu takip eden dokuz günlük takip süreci hastaların dosyasından retrospektif olarak incelendi. CAR-T hücre infüzyonu sonrası ortaya çıkabilen CRS toksisite bulguları, CRES toksisite bulguları ve CARTOX-10 nörolojik değerlendirme formuna kayıt edilen bilişsel bulgular ile uygulanan tedavi yöntemleri değerlendirilerek kayıt edildi.

Bulgular: CAR-T hücre infüzyonu toksisite bulgularını değerlendirdiğimizde hastaların %38,46'sında CRS evre 1, %30,79'unda CRES evre 2, %15,38'inde disgrafi, %23,07'sinde bilişsel durumda bozulma, %7,69'unda somnolans, %7,69'unda kol ve omuz kaslarında kasılma geliştiği saptandı. İki hastanın ise hem CRS hem de CRES toksisite bulguları nedeniyle yoğun bakım ünitesine transfer edildiği belirlendi.

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ABSTRACT

treatment option, in determining the patient's clinical status changes, in the care of the patient, and in expanding the knowledge base on this subject.

Keywords: CAR-T cell therapy, hematology, immunotherapy, nursing

ÖZ

Sonuç: Yeni bir tedavi seçeneği olan CAR-T hücre infüzyonu sonrası toksisite takibi ve yönetiminde, hastanın klinik durum değişikliklerini belirlemede, hastanın bakımında ve bu konuda bilgi tabanını genişletmede hemşirelerin rolü önemlidir.

Anahtar Kelimeler: CAR-T hücre tedavisi, hematoloji, immünoterapi, hemşirelik

Introduction

Chimeric antigen receptors (CAR) are receptor proteins designed to give T-cells a new ability to target a specific antigen. CAR-T cell therapy is a new immunotherapy approach that has started to been used in recent years and is developing rapidly (1). While immunotherapies boost the immune system, some immunotherapies directly target cancer cells (2). CAR-T cells used as immunotherapy destroy the tumor cell both directly and through an increased release of cytokines (3).

In CAR-T cell therapy, the patient's T-cells are equipped with the ability to seek out and destroy cancer cells by combining the specificity of a monoclonal antibody with the cytotoxic and memory capabilities of T-cells (4). T lymphocytes are genetically engineered to express these artificial receptors to fight cancer cells; it may be called immunotherapy, gene therapy or cancer treatment (5). Immunotherapy is also known as biotherapy because the immune system can naturally recognize pathogens and cancer cells (6).

CAR-T cell therapy is used to reduce tumor burden by lymphodepletion, reduce the number of regulatory T-cells that may negatively affect CAR-T cell functions, and make the cytokine profile suitable for immunotherapy (7). The treatment is carried out by modifying T-cells with CAR under laboratory conditions, targeting them to any surface-expressed antigen and infusing the cells back into the patient after their numbers have been increased (8). The number of T-cells infused into the patient varies depending on the patient's condition, but the average number of CAR-T cells to be infused is between 1-5x10^6 and these cells can remain in the bloodstream for 30 to 300 days (9,10).

Today, CAR-T cell therapy is approved in trials targeting CD19 in hematologic and non-hematologic cancers and is used as a promising treatment for cancer patients whose cancer is resistant and recurrent or whose cancer does not respond to other treatments (11). CD19 is a surface glycoprotein that is expressed from the earliest stages of B-cell development until terminal differentiation of plasma cells, where its expression is lost (12). However, the efficacy of CAR-T cell therapy in non-hematologic malignancies that do not exhibit CD19 positivity has not yet been fully established (13,14). In hematologic cancers, their efficacy has been demonstrated in diseases such as acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia, multiple myeloma and non-Hodking's lymphoma (NHL) (15). In particular, the use of CAR-T cell therapy in children and young adult patients for the treatment of relapsed/refractory CD19-positive B-cell ALL and B-cell NHL is now widespread (16).

Many factors play a role in the success of CAR-T cell therapy. The success of the treatment is determined by the treatment plan, management of cytopenia, antibiotic treatment, infusion management of T-cells, toxicity management of cytokine release syndrome (CRS), toxicity management of immune effector cell-related encephalopathy syndrome (CRES), and management of other potential short-term complications that may arise, as well as appropriate nursing interventions that impact patient psychological support and long-term follow-up (17-19).

Despite the successes of CAR-T cell therapy, the toxicities associated with CRS and CRES, which can be fatal during treatment, are serious conditions that must be managed during the treatment process. The chimeric antigen receptor toxicity assessment form (CARTOX-10) and neurological assessment are crucial for early intervention in monitoring CRS symptoms and especially for early diagnosis of CRES-related toxicity (20). Therefore, a multidisciplinary approach and specific nursing education and intervention are required to successfully implement CAR-T cell therapy.

Our aim in this study is to evaluate inpatient CAR-T cell therapy patients in our clinic in line with CRS and CRES management and to guide clinical practices by sharing the nursing interventions we apply in the management of CAR-T cell post-treatment toxicities.

Methods

This descriptive study included 13 patients who received CAR-T cell therapy at a private hospital in İstanbul between 2021 and 2023. Ethics committee approval for the study was given by the Ethics Committee of Acıbadem University (decision number: 15/20, date: 12.08.2021). The period of nine days after CAR-T cell infusion was retrospectively examined in the patient records. Personal characteristics of the patients (age, gender, marital status), clinical characteristics (disease diagnosis, stage, presence of concomitant diseases, central nervous system involvement, hematopoietic stem cell transplantation), vital signs (body temperature, pulse, blood pressure, oxygen saturation), the number of CAR-T cells administered, CRS toxicity findings that may occur after CAR-T cell infusion, CRES toxicity findings, cognitive findings recorded in the CARTOX-10 neurological assessment form, and the treatment methods and care measures used were evaluated and recorded.

During the follow-up of the patients, the body temperature was measured via the armpit using an electronic thermometer (with digital display). When the patients' body temperature was evaluated, 38 °C and above was considered a high fever (21). A digital vital signs monitor was used to measure heart rate. When evaluating patients' heart rate, a heart rate of 100/min or more was considered tachycardia (22). A digital vital signs monitor was used to assess the patients' blood pressure. It was assessed as systolic blood pressure >140 mm/Hg hypertension and diastolic blood pressure <90 mm/Hg hypotension (23). When assessing patients' oxygen saturation, an O_2 value of <80% in arterial blood was considered hypoxemia (24). In assessing the presence of nausea and vomiting in the patients, the Baxter Retching Faces scale was used to determine nausea, and the scores obtained were 1-4: mild, 4-7: moderate, and 7-10: severe (25). The Visual analog scale (VAS) was used to assess the presence of headache and muscle pain in the patients. The scores obtained were rated as follows: 1-4: mild, 4-7: moderate, 7-10: severe (26). The CARTOX-10 score sheet was used to determine the cognitive status of the subjects (orientation, attention span, concentration, naming, following commands, writing). In the CARTOX-10 score sheet, which contains a total of 10 points, 1 point was awarded for each question and the total score achieved by the patient was calculated and recorded. For example, a full score of 10 means that the patient is not affected by toxicity findings, while a score of 9 means that they are negatively affected by toxicity findings (27). The CRES form was used to recognize neurological toxicity findings of the cases, and the CRS form was used to recognize toxicity findings of CRS. The CRES form was used to assess and record the symptoms of cases that may develop with the toxic encephalopathic state, such as confusion, delirium symptoms, seizures, sleep disturbances, incontinence, and cerebral edema. The CRS form was used to record possible symptoms such as high fever, hypotension, tachycardia and hypoxia.

Statistical Analysis

The IBM SPSS Statistics 18 program was used for the statistical analysis of the study data. The distribution of results was reported using descriptive statistics such as percentage, mean, median and standard deviation. Chi-square test, t-test, Mann-Whitney U test, ANOVA and Kruskal-Wallis test were used to compare the values of the numerical data. Spearman correlation analysis was performed to determine the relationship between the values of the numerical data. In the statistical analyzes, the significance level was accepted as $p<0.05$.

Results

Most of the patients included in the study were male (76.9%) and married (53.8%). Most patients were treated with a diagnosis of NHL (61.5%), 100% had resistant disease and 92.3% had no central nervous system (CNS) involvement. Most patients had previously received hematopoietic stem cells (69.3%) and 46.2% had a Karnofsky Performance score of 80 (good) (Table 1).

Evaluation of toxicity findings after CAR T-cell infusion revealed that the cognitive impairment developed in 23.01% of patients, CRS stage 2 in 23.1%, CRES stage 2 in 15.4%, somnolence in 7.7%, dysgraphia in 15.4%, high fever in 38.5%, tachycardia in 53.8%, hypotension in 30.8%, headache in 15.4%, and myalgia in 15.4%. Only 15.4% of patients were transferred to the intensive care unit (ICU) due to both CRS and CRES toxicity findings (Table 2).

When we examined the distribution of toxicity findings after CAR T-cell infusion among the patients, it was found that the CARTOX-10 score could not be determined in case 1 due to the development of somnolence on the seventh day of treatment, it was scored as CRES stage 1, and CRS did not develop in this case. It was determined that case 2 was assessed as CRS stage 2 due to the occurrence of high fever, tachycardia, hypotension, and muscle pain with a VAS score of 6 on the fifth day of treatment. It was noted that case 5 was assessed as CRS stage 2 due to the occurrence of high fever and tachycardia on the fourth day of treatment. It was noted that case 7 was assessed as CRS stage 2 due to the development of hypotension on the first day of treatment, high fever and tachycardia on the third day and CRES stage 2 due to the development of impaired handwriting in addition to CRS on the seventh day. For case 8, it was determined that the high fever and tachycardia that occurred on the second day of treatment were not considered signs of toxicity because they were due to a viral infection. It was noted that case 9 was assessed as CRS stage 2 due to the occurrence of

Table 1. Personal and clinical characteristics of patients receiving CAR-T cell infusion (n=13)

		n	%
Gender	Male	10	76.9
	Female	3	23.1
Marital status	Single	6	46.2
	Married	7	53.8
Diagnosis	NHL	8	61.5
	ALL	5	38.5
Disease stage	Relaps	13	100.0
Chronic disease	No	10	76.9
	Yes	3	23.1
CNS involvement	No	12	92.3
	Yes	1	7.7
HSCT	No	4	30.8
	Allogeneik	5	38.5
	Autologous	4	30.8
Karnofsky score	50	1	7.7
	60	2	15.4
	70	3	23.1
	80	6	46.2
	90	1	7.7

NHL: Non-Hodking lymphoma, ALL: Akut Lymphoblastic lymphoma, CNS: Central nervous system, HSCT: Hematopoietic stem cell transplantation, CAR: Chimeric antigen receptors

high fever, tachycardia and headache with a severity score of VAS 4 on the second day of treatment, and on the eighth day he was assessed as CRES stage 2 due to the development of impaired handwriting in addition to CRS. Case 13 was assessed as CRS stage 1 because oxygen saturation fell below 80% on the fifth day of treatment, and the patient who developed contractions in the shoulders and arms in addition to CRS on the ninth day of treatment was assessed as CRES stage 2. It was determined that the patient whose hypoxemia progressed on the ninth day of treatment was transferred to the general ICU to receive biphasic positive airway pressure support (Table 3).

Table 2. Number of patients with toxicity findings occurring after CAR-T cell infusion (n=13)

	n	%
Impairment in cognitive status	3	23.1
CRS stage 1	2	15.4
CRS stage 2	3	23.1
CRES stage 1	2	15.4
CRES stage 2	2	15.4
Somnolence	1	7.7
Dysgraphia	2	15.4
Muscle contraction	1	7.7
ICU transfer	2	15.4
High fever	5	38.5
Tachycardia	7	53.8
Hypotension	4	30.8
Hypoxia	1	7.7
Nausea	1	7.7
Vomiting	1	7.7
Headache	2	15.4
Muscle pain	2	15.4

CRS: Cytokine release syndrome, CRES: CAR-T related encephalopathy syndrome, ICU: Intensive care unit, CAR: Chimeric antigen receptors

When we evaluated the treatment response status of the cases after CAR-T cell infusion, there was a partial response in ten cases (76.9%) and a complete response in two cases (15.4%). If we look at the overall survival time, the average survival time was 210 days and the cases relapsed after an average of 86 days. The CAR-T cells were administered to the cases on two different days on average, and the average number of CAR-T cells infused was $3.60x10^6$ (Table 4).

When examining the factors that influenced toxicity findings after CAR T-cell infusion, gender, response rate, age, number of CAR T-cells administered and SpO_2 had no effect on toxicity findings after CAR T-cell infusion ($p>0.05$). Disease diagnosis, Karnofsky score and elevated body temperature during treatment were found to be important variables.

The CRES findings were more frequent in patients with low Karnofsky performance score (Spearman r=-0.64 p=0.02). Karnofsky score was found to have no effect on CRS toxicity findings (Spearman r=-0.09 p=0.77). Body temperature was found to increase with increasing CRS toxicity findings (Spearman r=0.73 p=0.05), but there was no increase in CRES toxicity findings (Spearman r=0.16 p=0.61). The relationship between CRS and CRES scores was found to be statistically borderline (Spearman r=-0.54 p=0.055).

Table 3. Toxicity findings after CAR-T cell infusion and distribution by cases (μ)

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8	Case 9	Case 10	Case 11	Case 12	Case 13
Cartox 10 score	0	10	10	10	10	10	9	10	9	10	10	10	8
CRS	0	2	0	0	2	0	2	0	2	0	0	0	1
CRES	1	0	0	0	0	0	2	0	2	0	0	0	2
Fever °C	37.2	40.5	37.5	36.9	38.8	37.5	40	38.7	39.04	36.3	36	36.6	36.9
Pulse /mn	102	126	96	88	117	110	140	120	106	68	86	76	96
Blood pressure/ mmHg	114/86	95/56	120/70	135/78	120/70	130/70	90/50	98/58	90/44	115/70	95/60	115/60	120/70
SpO_2	91.5	98.7	96.7	97	97.1	95.3	98.4	96.8	96.3	99.4	96.6	98.8	<90
Nausea (BARF)	0	0	0	0	0	0	8	0	0	0	0	0	0
Vomiting (BARF)	0	0	0	0	0	0	6	0	0	0	0	0	0
Headache (VAS)	0	0	0	0	0	0	8	0	4	0	0	0	0
Stomachache (VAS)	0	6	0	0	0	0	8	0	0	0	0	0	0
Somnolans	1	0	0	0	0	0	0	0	0	0	0	0	0
Dysgraphia	0	0	0	0	0	0	1	0	1	0	0	0	0
Muscle contraction	0	0	0	0	0	0	0	0	0	0	0	0	1

CRS: Cytokyn release syndrome, CRES: CAR-T related encephalopathy, BARF: Baxter retching faces scale, VAS: Visual analog scale, CAR: Chimeric antigen receptors

Table 4. Treatment response status of cases after CAR-T cell infusion (n=13)

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8	Case 9	Case 10	Case 11	Case 12	Case 13
Treatment response	PR	PR	NR	PR	PR	PR	PR	PR	PR	CR	PR	CR	PR
General survival days	254	437	377	449	219	45	209	131	90	310	90	82	40
Day of relaps	63	365	15	59	171	45	209	115	45	0	30	0	1
Infused CAR-T cell number x10^6	5	6.4	4.5	4.8	4	7.3	2.48	4.75	2.2	1.3	2	1	1.18
Days with CAR T cells infusion	1-3	1-3	1-3.	1-3-6	1-3	1-3-7	1-3	1-3-7	1-3	1-3	1-3-7	1-3	1-3

PR: Partial remission, NR: No response, CR: Complete Remission, CAR: Chimeric antigen receptors

Discussion

Cytokine release syndrome and CRES toxicity symptoms observed in patients during CAR-T cell therapy are quite common (19). As a result of appropriate supportive treatment of toxicity symptoms, patients' symptoms may resolve within weeks, but evidence-based standard approaches for the management of CAR T-cell toxicity are still unclear. Therefore, monitoring, management and treatment of toxicity symptoms in patients receiving CAR-T cell therapy are crucial (28,29).

While high fever, tachycardia and hypotension, which are CRS symptoms, were frequently observed in our data, muscle pain, headache and hypoxemia occurred less frequently. Among the CRES toxicity symptoms, impaired handwriting, somnolence and stiffness in the arms and shoulders were less frequently observed. The fact that CRS findings were more frequent and CRES findings less frequent was similar to the studies on recent developments in CAR-T cell toxicity and another study on CRS and CRES neurotoxicity findings after CAR-T cell therapy (30,31).

When we examined the toxicity classification after CAR-T cell infusion, patients who developed high fever, hypotension, tachycardia, and hypoxemia as CRS toxicity symptoms were classified as CRS stage 1, whereas patients with organ toxicity findings such as muscle pain, headache, nausea, and vomiting in addition to CRS stage 1 symptoms were classified as CRS stage 2. Our CRS and CRES grading criteria are similar to the studies on grading toxicity after CAR-T cell infusion (8,9,17,19,32).

While the normal score on the CARTOX-10 cognitive assessment, which contributes to the early detection of CRES, should be 10, patients who develop impaired handwriting (dysgraphia) have a score of 9, a mild CRES stage 1, and patients who additionally develop muscle pain, headache, vomiting and nausea have a score of 9 and have been assessed as having CRES stage 2. The patient who developed drowsiness was categorized as CRES stage 1 with only one finding. In a study that comprehensively reviewed the staging models for CRS and CRES toxicity findings emerging in the literature, it was emphasized that despite the use of the CRS, CRES and CARTOX-10 staging models, the staging models should be updated in the future due to the emergence of unique toxicity profiles of CAR-T products (33).

When we examine the correlation analysis of the data, although there is no direct study in the literature showing that CRES toxicity findings are more common in patients with low Karnofski scores, the patients' ability to perform daily living activities and their low dependency status may explain the higher incidence of neurological findings. For this reason, in studies on CAR-T cell therapy, patients with a Karnofski score of 60% and above are generally included in the treatment, supporting our findings. The increase in body temperature in patients who develop CRS can be explained by the fact that high fever is the most common toxicity symptom among CRS toxicity findings (34).

When we evaluate all of our data, as in the CAR-T cell therapy toxicity management studies in the literature, the recommendations and practices, management of findings, and interventions for the CRS and CRES toxicity symptoms experienced by our patients parallel the practices in the literature and include supportive care. However, the literature emphasizes that the evidence-based standard approaches for toxicity management are still not clear and that the monitoring, management and care of toxicity symptoms in patients receiving CAR-T cell therapy are crucial (28,29).

Toxicity profiles for our group of patients with previously treated/refractory disease who received an average of 5.38x10^6 CAR-T cells were acceptable and similar to those reported in the literature (8,9).

Nursing Intervention Algorithm

In accordance with CRS and CRES management, we have created an algorithm that includes nursing measures for the assessment of CAR-T cell therapy patients in our clinic and that we use in the management of toxicities following CAR-T cell therapy. We used this algorithm as a guide for patient follow-up and toxicity management and intended it to guide our colleagues (Table 5, 6).

In nursing practice, patients and their families were initially informed about CRS and CRES toxicity findings that developed after CAR-T cell infusion, their awareness was raised, and collaboration was achieved.

In nursing practice, the vital signs of patients classified as CRS stage 1 were monitored every 30 minutes and the vital signs of patients classified as CRS stage 2 were monitored every 15 minutes. Oxygen supplementation was initiated in patients who developed hypoxemia, and fall prevention measures were

Table 5. Nursing algorithm for CRS toxicity findings that may occur after CAR-T cell infusion

CRS nursing intervention algorithm			
General symptoms	**CRS stage 1**	**CRS stage 2**	**CRS stage 3 or 4**
Fever **Fatigue** **Loss of appetite** **Nausea/vomiting** **diarrhea** **Head/body pains** **Rashes on the skin**	Patient/relative education Vital signs follow-up every 30 minutes Assistance with activities of daily living Antipyretic according to physician order, Administration of analgesics	Patient/relative education Vital sign monitoring every 15 minutes Evaluation with Glasgow coma scale Supportive care Elevating the head of the bed if there is aspiration risk Oral medications switch to intra venous Administration of antipyretic, analgesic	Transfer to intensive care unit
If cardiac symptoms are added to the general symptoms, the following nursing interventions should be applied in addition to the general nursing interventions in the CRS stages			
Heart and blood vessels			
Tachycardia **Arrhythmia** **Hypotension** **Edema**	Cardiac monitoring Liquid support	IL-6 inhibitor Tocilizumab (Aktembra), IL-1 inhibitor Kineret (Anakinra), inotrope and corticosteroid administration Cardiac monitoring	
If neurogical symptoms are added to the general symptoms, the following nursing interventions should be applied in addition to the general nursing interventions in the CRS stages			
Brain and nervous system			
Confusion **Dizziness** **Coordination and movement problems** **Difficulty swallowing** **Epileptic seizures** **Hallucinations**	Fall precautions CARTOX-10 rating 24st one Switch treatments to Intra Venous	Fall precautions CARTOX-10 rating 12st one Switch treatments to Intra Venous	
If pulmonary symptoms are added to the general symptoms, the following nursing interventions should be applied in addition to the general nursing interventions in the CRS stages			
Pulmonary system			
Cough **Decrease in lung function** **Shortness of breath** **Difficulty breathing**	O_2 support Highly concentrated O_2 support Position (semi fowler)	O_2 support Highly concentrated O_2 support Position (semi fowler)	

CRS: Cytokyn release syndrome, CAR: Chimeric antigen receptors, IL: Interleukin

taken in patients who developed hypotension to avoid the risk of falling. In addition, patients who developed CRS symptoms were treated with analgesics, antipyretics, antibiotics, fluid therapy, interleukin (IL) 6 and IL-1 inhibitors as directed by the physician. Patients who developed CRS symptoms were monitored closely until symptoms improved. After the symptoms had completely disappeared, the patients were closely monitored for a further 4 hours. After vital signs were monitored at 2 hour intervals for the next 24 hours, standard treatment was started.

The cognitive status evaluation form, which was evaluated every twenty-four hours in patients with CRES stage 1 and CRES stage 2, was now performed every twelve hours. Neurological assessment was performed every 4 hours using the Glasgow Coma scale.

The patient who developed drowsiness slept for more than twenty hours and the drowsiness disappeared spontaneously without medical intervention. The patient's CRES neurological assessment, previously performed every twenty-four hours, was

Table 6. Nursing algorithm for CRES toxicity findings that may occur after CAR-T cell infusion

CRES nursing intervention algorithm			
General symptoms	**CRES stage 1**	**CRES stage 2**	**CRES stage 3 or 4**
Headache **Seizures** **Delirium** **Anxiety** **Tremor** **Disgraphia** **Aphasia** **Decreased consciousness** **Coma with cerebral edema**	Fall precautions Cartox 10 rating 24st one Switch treatments to IV	Fall precautions Cartox 10 rating 12st one Switch treatments to IV[1] Evaluate with Glasgow coma scale Supportive care Raise the head of the bed if there is aspiration risk Oral medications switch to IV Administration of antipyretic, analgesic (IL-6 inhibitor Tocilizumab (Aktembra), IL-1 inhibitor Kineret (Anakinra), inotrope and corticosteroid administration Cardiac monitoring	Transfer to intensive care unit

CRES: CAR-T related encephalopathy syndrome, CAR: Chimeric antigen receptors, IV: Intravenous, IL: Interleukins

now performed every twelve hours and supportive treatment was scheduled. Oral treatments and nutrition were administered intravenously. To minimize the risk of aspiration, the head of the bed was kept elevated by 30 degrees. The patient was repositioned every two hours to prevent the development of pressure ulcers. Care was taken not to use drugs that could cause CNS depression. While the patient slept, he/she was monitored with a cardiac monitor to avoid missing any signs of his health condition. As the patient's basal SpO_2 was low at 91% due to the presence of the cervical mass, the SpO_2 was maintained at 96% by oxygenation.

The problem of patients who developed dysgraphia (impaired handwriting) persisted for 15 days. The dysgraphia resolved spontaneously without any special treatment or intervention. During this process, the patients received nursing support based on their needs in daily life.

Study Limitations

This research had sample limitations because it was applied only to inpatients in a private hospital and CAR-T cell therapy, a new treatment option, was performed in very small numbers. For this reason, the results of the study cannot be generalized to all patients receiving CAR-T therapy.

Conclusion

In our nursing experience and literature, CAR-T cell therapy presents unique challenges and responsibilities. Nurses play a critical role in the multidisciplinary care team, particularly in monitoring, managing potential complications, and providing information and support to patients and their families. The six key aspects of the nursing perspective in CAR-T cell therapy are; pre-treatment preparation, monitoring and management, toxicity management, patient and family support, ongoing information, collaboration and teamwork. In detail all of the six key aspects are listed below:

Pre-treatment Preparation

- **Patient education:** Educate patients and their families about the CAR-T cell therapy process, potential side effects, and the importance of reporting symptoms promptly.

- **Protocol familiarity:** Nurses should be well-versed in protocols for managing common side effects, such as CRS and CRES.

Monitoring and management

- **Vital signs and symptom monitoring:** Regularly monitor the patient's vital signs and be vigilant for early signs of CRS, CRES, and other complications. Use tools like the CARTOX-10 for cognitive assessments.

- **Laboratory testing:** Perform routine lab tests to monitor renal and liver function, blood coagulation factors, electrolytes, and C-reactive protein levels, as well as immunoglobulins and viral tests.

Toxicity management

- **Medication administration:** Be prepared to administer medications such as tocilizumab, anakinra, and corticosteroids for managing toxicity, under the guidance of the treatment team.

- **Emergency preparedness:** Have emergency equipment and supplies, including an emergency cart, cardiac monitor, and oxygen mask, readily available.

Patient and family support

- **Communication:** Maintain open lines of communication with the patient and their family, providing updates and reassurance as needed.

- **Emotional support:** Offer emotional and psychological support to help patients and families cope with the stress and uncertainty associated with CAR-T cell therapy.

Ongoing information

- **Continual learning:** Stay updated with the latest research and best practices related to CAR-T cell therapy.

- **Knowledge sharing:** Contribute to the collective knowledge by documenting and sharing experiences and outcomes, which can help refine and improve care protocols.

Collaboration and teamwork

- **Interdisciplinary collaboration:** Work closely with other healthcare professionals, including physicians, pharmacists, and support staff, to provide comprehensive care.

- **Clinical coordination:** Ensure seamless coordination of care, including scheduling and preparing for follow-up appointments and tests.

In conclusion, nurses are integral to the successful implementation of CAR T-cell therapy, providing essential care, monitoring, and support. Their role in recognizing and responding to complications, informing patients and families, and contributing to clinical research and education is vital for optimizing patient outcomes and advancing the field of CAR-T cell therapy.

Ethics

Ethics Committee Approval: This study was granted ethical approval by the Ethics Committee of Acıbadem University (decision no: 15/20, date: 12.08.2021).

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

Footnotes

Authorship Contributions

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

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Case Report

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A Rare Case: Scrotal Hemangioma

Nadir Bir Olgu: Skrotal Hemanjiom

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ABSTRACT

Hemangioma is a common soft tissue tumor. Scrotal hemangioma is a very rare benign vascular lesion. There are approximately 55 cases reported so far in the literature. It can cause bleeding, ulceration, pain and aesthetic complaints. Some authors have reported that it may even cause infertility. We presented a case of a 30-year-old male patient who was admitted to the urology clinic with the complaint of a testicular mass which he stated had been present since birth. On physical examination, vascular structures with a mass of approximately 10 cm were observed, filling more than half of the left hemiscrotum. These structures were painless on palpation and externally ecchymotic. On magnetic resonance imaging, as the primary diagnosis, cavernous hemangioma originating from the scrotum wall was considered. Then, the patient underwent excisional surgery under general anesthesia. The pathology result was reported as venous hemangioma. No complications were observed in the follow-ups. After a successful surgical approach, it was observed that the sexual performance of the patient also increased.

Keywords: Cavernous hemangioma, testicular mass, scrotal hemangioma

ÖZ

Hemanjiom, yaygın bir yumuşak doku tümörüdür. Skrotal hemanjiom ise çok nadir gözlenen benign bir vasküler lezyondur. Literatürde şimdiye kadar bildirilen yaklaşık 55 olgu bulunmaktadır. Skrotal hemanjiom estetik şikayetlerin yanı sıra kanama, ülserasyon ve ağrı gibi şikayetlere de neden olabilir. Bazı yazarlar, infertiliteye bile sebep olabileceğini bildirmiştir. Olgumuz, 30 yaşında bir erkek hastaydı. Kliniğimize doğumdan itibaren mevcut olduğunu ifade ettiği testiste kitle şikayeti ile başvurdu. Yapılan fizik muayenede sol hemiskrotumun yarıdan fazlasını dolduran, palpasyonla ağrısız, haricen ekimotik, yaklaşık 10 cm'lik vasküler yapılar gözlendi. Manyetik rezonans görüntülemede ön planda skrotum duvar kaynaklı kavernöz hemanjiom düşünüldü. Ardından hastaya genel anestezi altında eksizyonel cerrahi uygulandı. Patoloji sonucu, venöz hemanjiom ile uyumlu olarak neticelendi. Takiplerinde herhangi bir komplikasyon gözlenmedi. Başarılı bir cerrahi yaklaşım ardından hastanın cinsel performansının da yükseldiği gözlendi.

Anahtar Kelimeler: Kavernoz hemanjiom, testiste kitle, skrotal hemanjiom

Introduction

Hemangioma is a common soft tissue tumor. Testicular cavernous hemangioma is a very rare benign vascular lesion (1,2). There are approximately 55 cases reported so far in the literature (3). In this case report, a patient with scrotal hemangioma is discussed.

Case Report

A 30-year-old male patient was admitted to the urology clinic with a testicular mass, which had been present since birth. However, he stated that he was disturbed recently due to the lack of visual aesthetics and scrotal bleeding. No significant feature was found in the patient's history and family history. On physical examination, vascular structures with a mass of approximately 10 cm were observed, filling more than half of the left hemiscrotum, crossing the raphe and extending to a part of the right hemiscrotum. These structures were painless on palpation and externally ecchymotic (Figure 1). Routine urinalysis, complete blood count, coagulation parameters, alpha-fetoprotein level, beta-human choriogonadotropic hormone level and kidney function

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tests were normal. Scrotal magnetic resonance imaging (MRI) was performed to exclude possible testicular tumors. On MRI, a lobulated contoured lesion that completely involved the left hemiscrotum wall was observed with the largest dimensions of 120x77x78 mm. Cavernous hemangioma originating from the scrotum wall was considered as the primary diagnosis (Figure 2). Then, the patient underwent excisional surgery under general anesthesia. As a result of pathology; different sized, dilated, thick-walled, valve-containing vascular proliferation was observed in the entire dermis. In these vessels, erythrocytes and proteinous material were observed, and some of them had organized and calcified thrombi. Venous hemangioma was considered in the foreground. In the post-op 3rd month control, the wound healed well and the patient did not have any complaints (Figure 3). Informed consent was obtained.

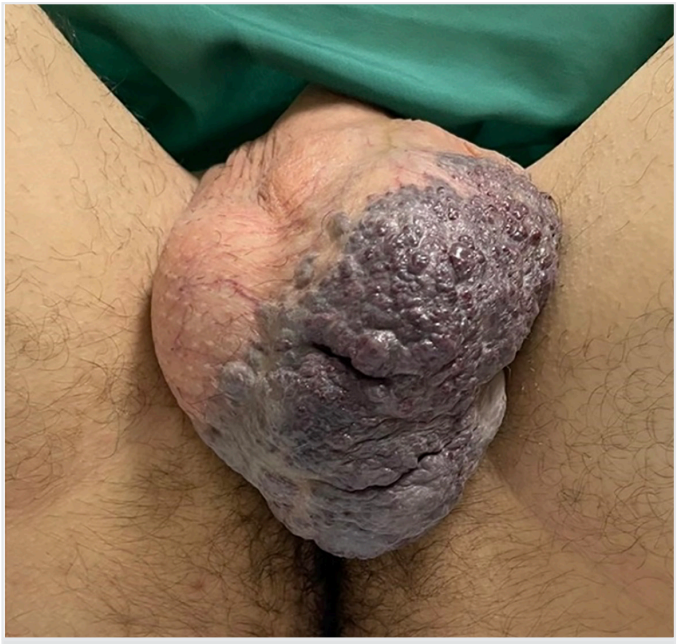

Figure 1. Preoperative image

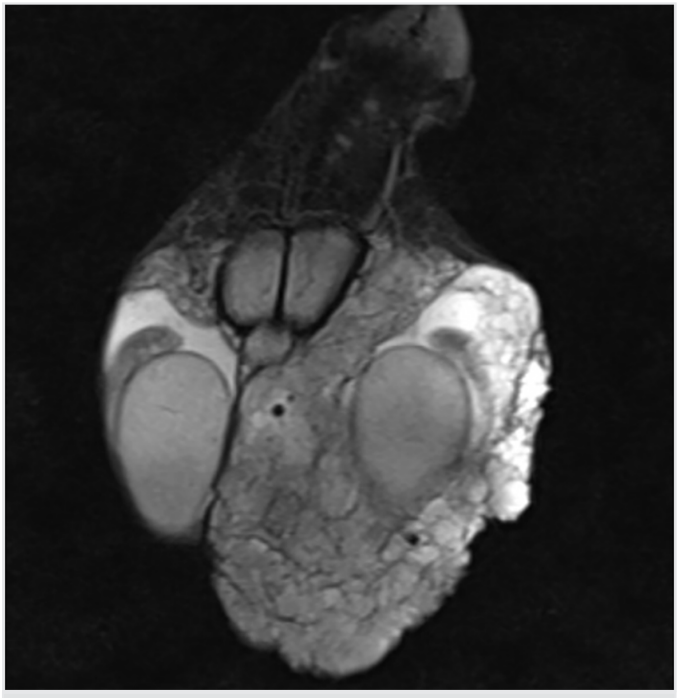

Figure 2. Scrotal MRI image
MRI: Magnetic resonance imaging

Discussion

Scrotal hemangiomas are congenital and usually painless benign vascular lesions (4). There are approximately 55 cases reported so far in the literature (3). Complications can be listed as hemorrhage especially in deep scrotal hemangioma, rectal bleeding and hematuria due to the spread of the lesion to surrounding tissues such as rectum or bladder, ulceration, infection, pain, and negative effect on spermatogenesis due to increased intrascrotal temperature (5-7). Since its complications are not observed frequently, it does not attract much attention unless it causes aesthetic problems until adolescence (8). Although the etiology of hemangiomas is not clear, they may cause discomfort due to local compression. Therefore, treatment is important after diagnosis (3).

In the diagnosis of scrotal hemangioma, MRI is important (3). Because, it is non-invasive and gives information about the dimensions, borders and the characteristics of the lesion.

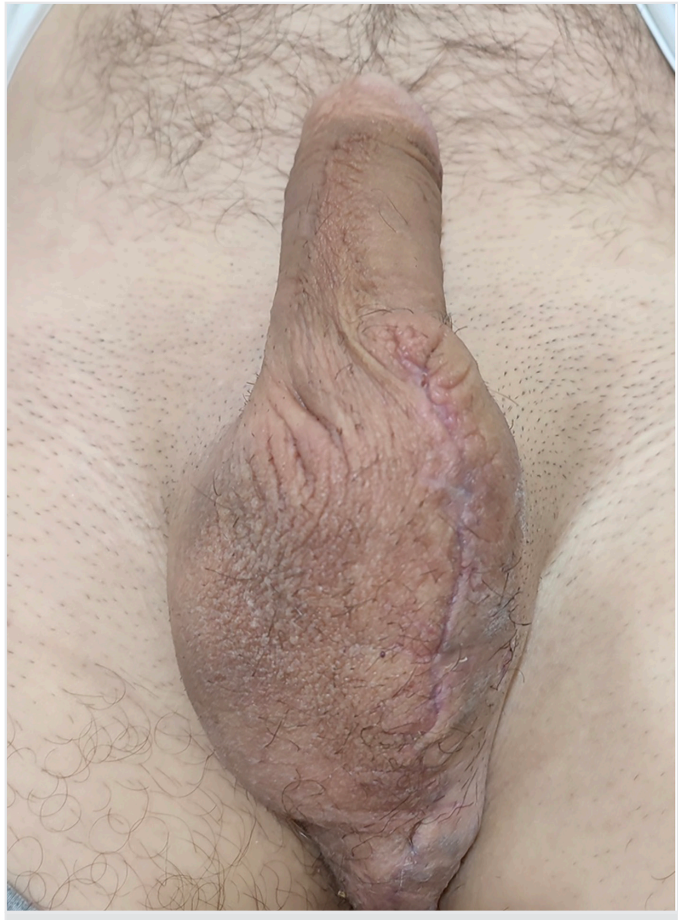

Figure 3. Postoperative image

Hemangiomas are important in the differential diagnosis of testicular tumors. Besides testicular tumors; inguinal hernia, cord lesions, and epididymal lesions should not be ignored in the differential diagnosis (4). The general approach in treatment is surgical removal of the lesion, especially in large lesions. Vascular lesions should not be excised before reaching the clean surgical margin and attention should be paid to vascular rupture and hemorrhage during surgery (4). Other treatment options include; intralesional sclerotherapy, laser fulguration, and cryotherapy (9). There is no consensus on the effect of scrotal hemangioma on infertility. Although it is accepted by some authors, that it does not pose a risk in terms of infertility due to the testis and epididymis are not being affected (4), it has been stated by some authors that it may cause infertility in the long term (10). According to Gotoh et al. (5) and Stahl et al. (6), testicular damage and azoospermia can be observed due to increased temperature as a result of scrotal hemangioma. Sexual health is crucial in sexually active individuals with scrotal hemangioma, as it can cause bleeding, pain and aesthetic discomfort. There are not many studies in the literature that investigate the effects of scrotal hemangioma on sexual health. Our patient was married and sexually active. He had no complaints about erection and ejaculation. Preoperative International Index of Erectile Function (IIEF) score was found to be 22. In the postoperative third month controls, the IIEF score was calculated as 26. The patient's wife was also interviewed. She stated that she was more satisfied with her sexual life because her husband had a more aesthetic appearance after the operation and scrotal bleeding stopped.

Ethics

Informed Consent: Informed consent was obtained.

Footnotes

Authorship Contributions

Surgical and Medical Practices: E.C.C., M.A., Concept: E.C.C., M.A., A.İ., Y.Ö.İ., Design: M.A., A.İ., Y.Ö.İ., Data Collection or Processing: E.C.C., M.A., F.G., Analysis or Interpretation M.A., A.İ., F.G., Y.Ö.İ., Literature Search: E.C.C., A.İ., F.G., Writing: E.C.C., A.İ.

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Review

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Evaluation of Academic Dissertations via Mindfulness in Children Using Content Analysis Method

Çocuklarda Bilinçli Farkındalığa Yönelik Gerçekleştirilen Akademik Tezlerin İçerik Analizi Yöntemiyle Değerlendirilmesi

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ABSTRACT

Mindfulness, which means being aware of the moment and what is happening around and accepting it unconditionally, is especially valuable for children who are in the age of discovery and development. Research question: In the recent years Council of Higher Education (CoHE) Thesis Center publications, how has the mindfulness been studied in Turkish children? Searches were conducted of CoHE Thesis Center until October 2023 to identify dissertations (master of science, doctorate, specialization in medicine). Using the keywords "mindfulness" and "child", "mindfulness" and "children" were scanned. Studies which did not have full text access and did not include assessment and intervention on mindfulness with children were excluded. Eighty-nine dissertations met the study's inclusion criteria. Once duplicates were removed and were screened for eligibility, totally 27 studies between 2018 and September 2023 were reviewed. Types of these studies were as follows: 22 were master of science, 3 were doctorate and 2 were specialization in medicine. The most common field outcomes were psychology and education and training. Within the scope of this determination, there were two main themes: evaluation methods (n=20) and intervention methods (n=7). While there were 10 studies between 2017 and 2019, 17 studies were found in the last four years. It has been observed that postgraduate dissertations on mindfulness have increased in recent years. Although the researchers on the subject appear to be from different scientific fields and disciplines and that interest in the subject has increased, future studies are needed to create a general consensus.

Keywords: Academic dissertations, theses, mindfulness, child

ÖZ

Yaşanılan anın ve etrafta olan bitenin farkında olma ve koşulsuz kabul etme olarak anlam kazanan bilinçli farkındalık özellikle keşif ve gelişim çağında olan çocuklar için oldukça değerlidir. Araştırma sorusu: Yükseköğretim Kurulu (YÖK) Tez Merkezi yayınlarında son yıllarda Türk çocuklarında farkındalık nasıl araştırılıyor? Ekim 2023 tarihine kadar tezlerin (yüksek lisans, doktora, tıpta uzmanlık) tespiti amacıyla "bilinçli farkındalık" ve "çocuk" ile "bilinçli farkındalık" ve "çocuklar" anahtar kelimeleri kullanılarak YÖK Tez Merkezi'nde taramalar yapıldı. Tam metin erişimi olmayan ve çocuklarla bilinçli farkındalık üzerine değerlendirme ve müdahale içermeyen çalışmalar hariç tutuldu. Kriterleri karşılayan 89 tez ele alındı. Tekrarlayan çalışmalar çıkarıldıktan ve uygunluk açısından tarandıktan sonra 2018 ile Eylül 2023 arasında bulunan toplam 27 çalışma incelendi. Bu çalışmaların 22'si yüksek lisans, 3'ü doktora ve 2'si tıpta uzmanlık tezidir. En yaygın alan sonuçları psikoloji ile eğitim ve öğretim idi. Bu belirleme kapsamında değerlendirme yöntemleri (n=20) ve müdahale yöntemleri (n=7) olmak üzere iki ana tema yer almaktaydı. 2017-2019 yılları arasında 10 çalışma bulunurken son dört yılda 17 çalışmaya ulaşıldı. Son yıllarda bilinçli farkındalık üzerine yapılan lisansüstü tezlerin arttığı görülmektedir. Konuyla ilgili araştırmacıların farklı bilim alanlarından ve disiplinlerden olduğu, ayrıca konuya ilginin artmış olduğu görülse de genel bir fikir birliğinin oluşması için yeni yapılacak çalışmalara ihtiyaç vardır.

Anahtar Sözcükler: Akademik tezler, tezler, bilinçli farkındalık, çocuk

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Introduction

Aware is associated with aspects of individuals such as being open, non-judgmental, friendly, curious, accepting, compassionate and kind. Mindfulness is explained as moment-by-moment awareness of thoughts, emotions, bodily sensations and the environment (1). Mindfulness has recently begun to draw interest in scientific and popular discourses in daily life (2). It is also studied in various disciplines, especially psychology, physiology, health services, neuroscience and art. In this context, mindfulness research shows that mindfulness-based approaches are aimed at improving stress management, cognitive, emotional and interpersonal functions (3).

Mindfulness studies include various age and focus groups. Studies on its effects on children that one of the focus groups within this scope have started to improve. "Child" which includes the 0-18 age group, is the period before adulthood (4). For this reason, mindfulness studies implemented on individuals who have not yet stepped into adulthood provide that individuals in this age group are supported in terms of academic, social and cognitive functions. Additionally, mindfulness practices are emphasized in this critical period to support the development of different skills (5).

Considering that about half of mental disorders occur before the age of 14, it is clear that the period between the ages of 0-18 is especially transit for adulthood (6). It is important to screen child development, evaluations at an early stage, determine possible situations and apply appropriate protocols regularly. Besides all this, it has been shown that practices aimed at evaluating and improving individuals' mindfulness are also successful in the treatment of medical conditions such as depression, anxiety, chronic pain, heart diseases and cancer (7). For this reason, mindfulness-based interventions are accepted as a hopeful approach and are followed with increasing interest in the field of mental health. Mindfulness practices include movement-based practices such as mindfulness meditations, breathing exercises, yoga, and approaches that protect attention and enhance focus by choosing a focus point (7,8). Mindfulness-based intervention results in enhanced mindfulness through the mindfulness regulation process and a more integrated sense of personal accomplishment among key and common goals (7). Mindfulness-based rehabilitation approaches can be an effective keynote for developing self-regulation by facilitating the individual to learn how to be calmer or more relaxed in general and how to calm himself/herself in times of anxiety or potential threat (9).

As long as mindfulness-based assessment, practices and methods become gradually widespread, the concept of mindfulness is becoming a term that is increasingly heard and included in daily life, along with many academic researches, books, projects and trainings, especially in health services (7,10). However, the scientific basis on which this issue is researched has not been clearly revealed to what extent it is addressed both academically and practically. For this reason, postgraduate theses that include the child population on mindfulness are an important data source. In this study, the postgraduate theses were examined according to their years, type, institute, subjects, theses types, methods and findings for children in Türkiye until October 2023. This research aimed to determine what the current situation was so far in theses whereby the focus group was children and to reveal the needs for future studies.

Methods

This research was fulfil through the document analysis, which is one of the qualitative research techniques of postgraduate theses conducted for children in Türkiye. Qualitative research is a type of research in which methods such as observation, interview and documentation are used to collect data. In other words, it is an approach that is based on theory creation and prioritizes research and understanding of social phenomena within the framework to which they depend (11). In this research, postgraduate theses were collected by document analysis. Document analysis consists of a series of processes that occur within the examining and evaluating printed and/or electronic (computer-based and internet-accessible) materials (12). The data obtained were examined by the content analysis method, and similar data were brought together within the framework of categories and interpreted through content analysis (11).

In recent years, it has been observed that the number of mindfulness-based studies has increased with intense interest. For this reason, postgraduate theses published on mindfulness in children in Türkiye were accessed. In accessing theses documents; the keywords "mindfulness", "child" and "children" were used in selecting theses on the subject from the Council of Higher Education (CoHE)-Thesis Center (2023) website (https://tez.yok.gov.tr/UlusalTezMerkezi/). Master of science, doctorate and specialization of medicine postgraduate theses accessed with these keywords were transferred to the computer in pdf format. Published theses fulfilling the following criteria were included: (a) open to full text access, (b) in Turkish or English language, and (c) focus group should be children. Theses on different focus groups were excluded. Twenty-seven postgraduate theses that met the inclusion criteria were reached. The flow chart of the study is given in Figure 1. The aforementioned theses were subsequently collated within the context of main categories through the application of content analysis, and the primary findings were subsequently summarised and interpreted.

Results

Firstly only the word "mindfulness" was scanned in the CoHE National Thesis Center and 494 postgraduate theses were found between May 2011 and October 2023. Then, when the words "mindfulness" and "child" were searched, 60 theses were found between August 2013 and October 2023; when the words "mindfulness" and "children" were searched 29 postgraduate theses were found between June 2018 and October 2023. Duplicate studies (n=20) were excluded, the titles and abstracts of the remaining 69 studies were examined. Forty-two of these studies were excluded because the focus group was not children, and as a result, 27 studies were examined (13-39). Those studies

were published between 2017 and October 2023. Looking at the distribution by years, there was 1 study in 2017, 3 studies in 2018, 6 studies in 2019, 5 studies in 2020, 4 studies in 2021, 3 studies in 2022 and 5 studies in 2023. Twenty-four of the studies were authored in Turkish, while three were written in English.

The most postgraduate theses on mindfulness in children were master of science theses (n=22) with 81.48%, doctorate theses with 11.11% (n=3) and specialization in medicine theses with 7.4% (n=2), respectively. Distribution of these theses according to the institutes and departments they were affiliated with were as follows: education [(n=16); institute of education (n=6), institute of educational sciences (n=9), institute of educational technology (n=1)], social [(n=8); institute of social sciences (n=7), institute of economics and social sciences (n=1)], health [(n=1); institute of health sciences (n=1)], psychiatry [(n=2); department of child and adolescent mental health and diseases].

Of the studies 74.07% that met the inclusion criteria consisted of evaluation studies (n=20). The names of the studies, theses type, methods, main findings and results of these studies are given in Table 1. It was observed that 22 had a quantitative, 1 had a qualitative, and 4 had a mixed (quantitative and qualitative) study design.

Of the studies 25.92% that met the inclusion criteria consisted of intervention (n=7) studies. The names of the studies, theses types, methods, interventions, main findings and results of these studies are given in Table 2.

Discussion

In this study, postgraduate theses were researched through mindfulness in children in Türkiye and it was revealed that it was a multidisciplinary field that attracted attention in recent years. Researchers who study mindfulness emphasize that although the field has gained popularity recently, it is still a developing field (40). It is thought cherish the hope to plan mindfulness-based studies with qualitative, quantitative and mixed methods, as well as to disseminate studies with a high level of evidence and examining with follow-up.

Every human is considered a child until the age of 18, and with the special status provided, the rights of children to life and development are protected (4). For this reason, studies involving children cover the ages of 0-18. In the studies examined, it was seen that the sample groups were determined according to age. However, there is no mention of a standardized categorization. Given as examples: the study by Kıdil (13) titled "Investigation of the Awareness of 5-6 Years Old Children and Their Families on Correct Nutritional Behaviors" and conducted with 5-6 age group; the study by Dursun (14) titled "Investigation of the Effect of Parent Attitudes on the Self-Comfort Levels and Conscious Awareness Based Self-Efficiency of Special Talent

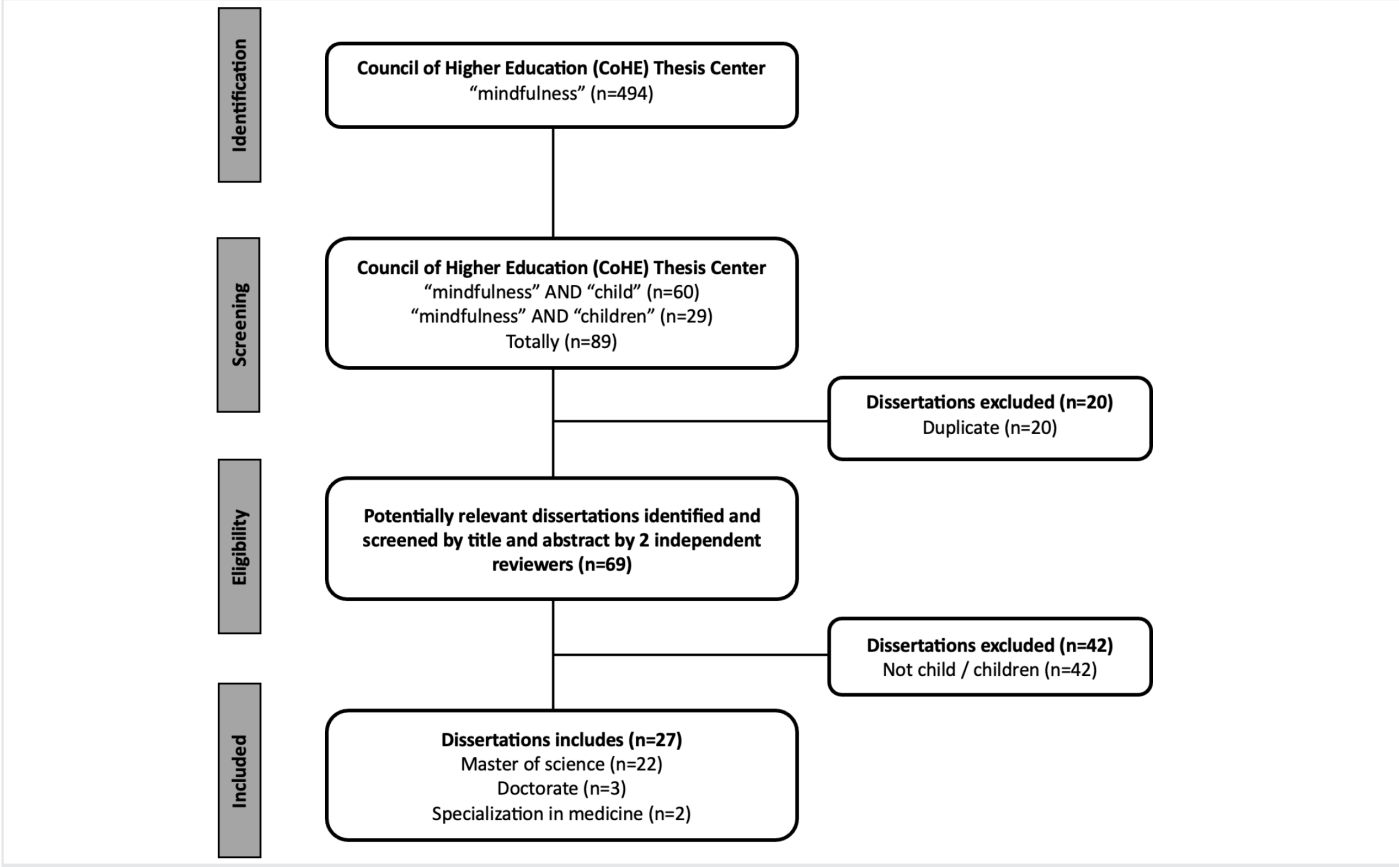

Figure 1. Flow diagram of the study

Table 1. Evaluation studies involving mindfulness in children

Name of the study (researcher and year)	Theses types	Method	Main finding
Investigation of the Awareness of 5-6 Years Old Children and Their Families on Correct Nutritional Behaviors (Kıdil, 2021) (13)	Master of science	Qualitative	It has been revealed that children perceive nutrition by creating different categories and that families have an impact on the formation of children's nutritional knowledge.
Investigation of the Effect of Parent Attitudes on the Self-comfort Levels and Conscious Awareness Based Self-Efficiency of Special Talent Children Aged 9-13 (Dursun, 2023) (14)	Master of science	Quantitative	It has been observed that there is a positive and highly significant relationship between the self-compassion levels of gifted secondary school students and their mindfulness-based self-efficacy levels.
The Examination of Exam Anxiety with Individual and Stability Therapy (ACT) Model in Individuals from 12-15 Years (Önal, 2020) (15)	Master of science	Quantitative	Findings obtained as a result of the research, exam anxiety was found to have an impact on conscious awareness, attention, acceptance, determination, decision making, and values.
The Relationship Among Mindfulness, Digital Game Addiction and Demographic Variables (Karzı, 2020) (16)	Master of science	Quantitative	As a result, an inverse relationship was found between digital game play addiction and mindfulness in the participants.
Investigation of the Relationship Mindfulness-based Self-efficacy, Self-compassion and Happiness Levels in Children (Devrim Kahraman, 2020) (17)	Master of science	Quantitative	There is a positive significant relationship between mindfulness-based self-efficacy, self-compassion and happiness levels in children.
Well-being Children: Parental Attitudes and the Role of Mindfulness (Tohumcu, 2022) (18)	Master of science	Quantitative	The findings showed that responsibility and acceptance from parental attitudes and mindful awareness significantly predicted well-being.
Social Mindfulness in Intergroup Context: The Role of Socio-Cognitive Skills (Koçyiğit, 2023) (19)	Master of science	Quantitative	These findings showed that theory of mind has a critical role in displaying social mindfulness toward both ingroup and outgroup.
Emotional Abuse Awareness of Parents of 4th Grade Primary School Students (Kaya, 2020) (20)	Master of science	Quantitative	No significant correlation was found between the emotional abuse awareness of the parents and the mindfulness-based self-efficacy scores and discouraging family relationships of the children.
Investigation of the Relationship Between Perfectionism, Self-compassion, Mindfulness, Self-esteem, and Perceived Severity of Stuttering in Children who Stutter (Küçükgüzel, 2021) (21)	Master of science	Quantitative	While a negative correlation was expected between the mindfulness subtest scores and the child's level of perception of her own stuttering severity or the stuttering severity of others, no statistically significant relationship was found according to the findings.
Evaluation of Identity Development and Mindfulness in Adolescents with and without Non-suicidal Self-Injurious Behavior (Yazgılı Kahveci, 2020) (22)	Specialization in medicine	Quantitative	Adolescents with non-suicidal self-injurious behavior had more identity confusion, their family relations were more complicated and their mindfulness levels were lower compared to the other two groups.
Evaluation of Emotion Regulation and Mindfulness in Adolescents with and without Identity Confusion (Aydın, 2017) (23)	Specialization in medicine	Quantitative	It has been found that adolescents with identity confusion have more difficulty in regulating emotions and lower levels of mindfulness than those without identity confusion.
Investigation of the Relationship Between COVID-19 Phobia, Metacognitive Problems and Mindfulness Level in High School Students (Yılmaz, 2022) (24)	Master of science	Quantitative	No significant relationship was found between the level of mindfulness and COVID-19 phobia.

Table 1. Continued

Name of the study (researcher and year)	Theses types	Method	Main finding
Investigation the Mediator Role of Mindfulness and Metacognition in the Relationship Between Nomophobia and Alexithymia (Altan, 2019) (25)	Master of science	Quantitative	The metacognition problems and mindfulness had significant mediator effects on the relationship between the alexithymia and mindfulness. The results of the multivariable linear regression analysis revealed that alexithymia personality traits, mindfulness levels, metacognition problems and age significantly predict the nomophobia.
Development of Awareness in Self-Administrative Skills in Preschool Children: Ataşehir Case (Taşkın, 2019) (26)	Master of science	Quantitative	It has been found that children often exhibit and gain self-directed behavior when they are with a parent or a non-parent person, but cannot achieve the same success if they move away from them.
Investigation of the Development of Mindfulness in Middle Childhood in the Context of Mindful Parenting and the Child's Socio-emotional and Socio-cognitive Skills (Karadoğan, 2022) (27)	Master of science	Quantitative	Results of the correlation analyses children's mindfulness was positively to emotion understanding and emotional inhibition and hierarchical regression analysis emotion understanding and emotional inhibition skills predicted children's mindfulness skills.
Investigation of the Relationship Between Digital Game Addiction, Resilience and Mindfulness Levels of Secondary School Students (Keskin, 2019) (28)	Master of science	Quantitative	As a result of the research, digital game addiction and resilience were found to be significantly correlated in the negative direction, digital game addiction and mindfulness were significantly correlated in the negative direction, resilience and mindfulness were significantly correlated in the positive direction.
The Mediator Effect of Mindfulness in the Relationship Between Perceived Stress Level and Quality of Life in Pediatric Asthma (Ayhan, 2020) (29)	Master of science	Quantitative	It was found that perceived stress in asthma had a significant relationship with quality of life, emotional function and symptom sub-dimensions of quality of life; In this context, it has been observed that mindfulness has a partial mediator effect.
Mediating Effects of Emotion Regulation and Creative Problem Solving in the Relationship Between Mindfulness and Foreign Language Learning Anxiety (Gözütok, 2023) (30)	Doctorate	Quantitative	The results of the correlation analysis showed that mindfulness, creative problem-solving attributes and cognitive reappraisal strategy were found to be negatively related with foreign language learning anxiety. Mindfulness was positively associated with creative problem-solving features and cognitive reappraisal strategy.
Assessing Mindfulness in School-Aged Children: Development and Validation of BAU Mindfulness Scale (BAU-MSC) (Taşkın, 2018) (31)	Master of science	Quantitative	The validity evidence has shown that BAU-MSC has promising psychometric properties and it has been recommended for use on Turkish children.
Mindfulness Levels of Gifted and Non-gifted 3 and 4th Grades Students (Barış, 2018) (32)	Master of science	Quantitative	The mindfulness levels of non-gifted and talented girl students concluded a higher degree than boy students. It is also exhibited that the mindfulness levels of non-gifted and talented students indicate no difference depending on class level. The mindfulness levels of 4th grade gifted and talented students result higher than 3rd grade students.

Children Aged 9-13" and conducted with 9-13 age group, and the study by Alpay (36) titled "The Effect of Mindfulness Based Physical Education Lessons on the Mindfulness, Emotional and Psychological Well-Being Levels of Primary School 3rd and 4th Grade Students" and conducted with 3-4 grade students (Tables 1, 2). Studies in the literature on mindfulness include different age groups and life span (41). Mindfulness needs to be evaluated and examined for specific age groups in intervention programs.

Although the concept of mindfulness has its origins in the teachings of Buddha (42), the first scientific publication was found in 1857. The subject of mindfulness in children has been in the literature since 1872. Despite the fact that there has been an increasing interest in the studies examined in our study for years, the most studies were found in 2019. However, the difference in the number of studies compared to other years is negligible. When looking at the literature, it can be seen that the interest in studies on the subject continues to increase. The creation of environments where researchers can present and share their information on the subject will be a source of motivation.

Table 2. Intervention studies involving mindfulness in children

Name of the study (researcher and year)	Theses types	Method	Intervention	Main ginding
The Effects of Mindfulness Based Yoga Intervention on Preschoolers' Self-Regulation Ability (Önoğlu Yıldırım, 2019) (33)	Master of science	Mix (quantitative and qualitative)	The intervention group of children took yoga 2 times a week for 12 weeks for a total of 15 hours of yoga per child.	Results of the child battery showed that children who were in the yoga group performed better on working memory but none of the other aspects of executive function that were measured revealed a difference. Teachers reported no difference between the two groups. Lastly, mothers evaluated that the two groups were different in terms of positive affect such that children in the yoga group were evaluated as higher.
Investigation of the Effect of Mindfulness Education Program on the Emotional Intelligence, Self-Perception and Executive Functioning Level of Pre-School Children (Aydın, 2023) (34)	Doctorate	Mix (quantitative and qualitative)	Mindfulness Education Program was applied 3 days a week for 8 weeks.	It was determined that the mindfulness education program created a statistically significant difference in the experimental group children's emotional intelligence, self-perception and executive functioning levels compared to those of the control group. According to the qualitative analysis results, the teacher of the experimental group stated that the education program applied positively contributed to the children's emotional intelligence, self-perception and executive functioning levels.
Effectiveness of Mindfulness Based Program on Pre-Adolescents (Özen Koç, 2023) (35)	Doctorate	Mix (quantitative and qualitative)	A mindfulness program was implemented for eight weeks.	It was found that there was a positive relationship between mindfulness and psychological well-being, mindfulness predicted psychological well-being, men's mindfulness and psychological well-being scores were higher than women's, and there was no significant difference in terms of class variable.
The Effect of Mindfulness Based Physical Education Lessons on the Mindfulness, Emotional and Psychological Well-Being Levels of Primary School 3rd and 4th Grade Students (Alpay, 2021) (36)	Master of science	Quantitative	Mindfulness practices were carried out in physical education classes, one session per week, for a total of 8 sessions.	As a result, it was determined that 8-week mindfulness based physical education lessons did not have a significant effect on mindfulness, emotional and psychological well-being of primary school 3rd and 4th grade students.
Mindful School Program for Elementary School Children and the Effects of the Program on Children (Yazğan, 2019) (37)	Master of science	Quantitative	The program was named "Mindful Okul" that was implemented for 14 weeks.	Results of the research revealed that "Mindful Okul" program had a positively significant effect on both fully awareness and subscales of mindfulness in 3rd and 4th grade students.
Effect of Mindfulness Based Anger Management Program on Mindfulness and Anger Management Skills on Children (Yıldızhan, 2019) (38)	Master of science	Quantitative	Mindfulness-based anger management program was applied to for 8-weeks.	Results revealed that the 8-week mindfulness psychoeducation program had a significant influence on the anger management skills on children.
The 8 Week Mindfulness Program for Preschool Children and the Effects of the Program on Children (Çollak, 2018) (39)	Master of science	Mix (quantitative and qualitative)	A mindfulness program was implemented twice a week for eight weeks in total.	Results of the data obtained from 1st and 8th month showed that mindfulness program had positive effect on children, especially on children's attention, being calm, self-control, self-acceptance and compassion, being concerned about others and helpfulness.

For all that mindfulness is a field that develops with its roots in psychology, it is seen to be involved in many different scientific fields such as neuroscience, medicine, psychiatry (43). Additionally, mindfulness has been studied on various topics such as addiction, chronic pain, immunity, health behaviors, depression relapse, depression and anxiety symptoms, attention control and focus (41). From this perspective, mindfulness has a rich scope. Similarly, many different areas were included in the studies examined. However, it has been observed that it is not discussed as in the studies examined in scientific fields such as occupational therapy, cardiac rehabilitation, vocational rehabilitation and cognitive rehabilitation, which have an important place in the literature (41,44-46). In this respect, it seems that there is a need for postgraduate theses on the subject of mindfulness in these scientific fields in Türkiye. In addition, studies in the national literature such as "Effect of Mindfulness Based Cognitive Therapy Program on Psychological Symptom Levels of Schizophrenia Patients" (47) and "Mindfulness-Based Cognitive Therapy: A Review" (48) were achieved.

Qualitative research is a subjective, holistic and process-oriented type of study that investigates the nature of real-life phenomena (11). However, in quantitative studies, this is the opposite. Quantitative research comprehends objective, targeted and result-oriented research types that objectify situations, facts and events and reveal them in a way that can be observed, measured and expressed numerically (49). The majority of the studies examined were created with a quantitative design. When the studies on mindfulness in the literature are examined, it is stated that very few studies have qualitative design, but the results are similar to quantitative studies (9). Therefore, findings of this study are similar to the literature. Some of the studies examined included information about their design in the method section, some studies did not. This situation caused the researchers to have difficulty in determining the methods. Besides, study designs need to be clearly stated. The lack of sharp separation of qualitative and quantitative research methods leads to confusion in the evaluation of research results. All qualitative studies emphasize the issues and events that develop in the natural environment, and when examining these facts, the questions of how and why are answered instead of how many or how much. In consequence, instead of collecting numerical data, administering interventions or treatments as in quantitative research, qualitative research helps further explore and understand quantitative data as well as generate hypotheses. A study can be conducted entirely with qualitative or quantitative methods, as well as a mixed-method research combining qualitative and quantitative approaches can also be planned (49).

Study Limitations

This study, which is based on the examination of postgraduate studies on mindfulness for children, draws attention that most of the postgraduate theses are evaluation-oriented. Due to increasing evidence of the positive impact of mindfulness on psychological health, emotional well-being, learning and physical health, assessing mindfulness has become important (31). However, the limitations of this study are that assessment and intervention methods related to mindfulness are not compared with each other, only postgraduate theses are examined, and mindfulness studies related to only children are included.

Conclusion

Researches are needed with professional groups such as occupational therapy, physiotherapy rehabilitation, psychology, and neuroscience in order to develop a further comprehensive understanding of the studies on mindfulness more collaborative/multidisciplinary. Although it seems likely that interest in mindfulness will continue, considering the progress made so far, it is recommended that the impact and development of mindfulness-based studies on human life be examined in populations with different characteristics and with various methods.

Footnotes

Authorship Contributions

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

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Review

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Phytotherapy in Liver Diseases

Karaciğer Hastalıklarında Fitoterapi

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ABSTRACT

The liver is the largest internal organ in the body and has vital functions such as metabolism, elimination of toxins, and bile production. Liver diseases include various conditions that affect the functions of this organ. The most common liver diseases include hepatitis, cirrhosis, fatty liver disease, and liver cancer. These diseases can develop due to genetic factors, alcohol consumption, viral infections, and obesity. A healthy lifestyle is of great importance in preventing liver diseases. Measures such as a balanced diet, regular exercise, limiting alcohol consumption, and protection from viral infections (e.g., vaccinations) help protect liver health. In addition, avoiding exposure to toxins is also important. Interest in phytotherapy has increased considerably in recent years, except for medical applications, in the protection of liver health and the treatment of liver diseases. Among the plants used for this purpose, foods such as artichoke, pomegranate, grapes, and grapefruit, teas such as chamomile, or extracts containing active ingredients such as ellagic acid, resveratrol, and curcumin can be listed. This article will discuss the effects of some plants on liver health. In addition to the plants mentioned here, many other plants are used in this area. While plants are beneficial when used in food format for general health, if they are used for treatment purposes and as an extract, they should be used under the supervision of a physician who has received phytotherapy training. As a result, liver diseases can be prevented, and when they do occur, most of them can be treated. A healthy lifestyle, regulating habits, healthy choices in food intake, and herbal supplements can play an important role in this process.

Keywords: Liver, liver diseases, phytotherapy, herbal treatment, hepatitis

ÖZ

Karaciğer, vücudun en büyük iç organıdır ve metabolizma, toksinlerin atılması, safra üretimi gibi hayati işlevleri vardır. Karaciğer hastalıkları, bu organın işlevlerini etkileyen çeşitli durumları içermektedir. En yaygın karaciğer hastalıkları arasında hepatit, siroz, yağlı karaciğer hastalığı ve karaciğer kanseri bulunmaktadır. Bu hastalıklar, genetik faktörler, alkol tüketimi, viral enfeksiyonlar ve obezite gibi nedenlerle gelişebilmektedir. Karaciğer hastalıklarının önlenmesinde sağlıklı yaşam tarzı büyük önem taşımaktadır. Dengeli beslenme, düzenli egzersiz, alkol tüketiminin sınırlandırılması ve viral enfeksiyonlardan korunma (örn., aşılar) gibi önlemler, karaciğer sağlığını korumaya yardımcı olmaktadır. Ayrıca, toksinlere maruziyetten kaçınmak da önem arz etmektedir. Karaciğer sağlığının önlenmesi ve tedavisinde medikal uygulamalar hariç son yıllarda fitoterapiye olan ilgi oldukça artmıştır. Bu amaçla kullanılan bitkiler arasında; enginar, nar, üzüm ve greyfurt gibi besinler, papatya gibi çaylar ya da ellajik asit, resveratrol ve curcumin gibi etken maddeleri içeren ekstre şeklinde kullanılabilmektedir. Bu yazıda, bazı bitkilerin karaciğer sağlığı üzerindeki etkilerinden bahsedilecektir. Bu alanda burada adı geçen bitkilerin dışında da çok sayıda bitki kullanılmaktadır. Bitkiler genel sağlık için gıda formatında kullanıldığında faydalı olurken tedavi amaçlı ve ekstre olarak kullanılacaksa fitoterapi eğitimi almış bir hekim gözetiminde kullanılması gerekmektedir. Sonuç olarak, karaciğer hastalıkları önlenebilir, hastalık oluştuğunda ise çoğu tedavi edilebilir. Bu süreçte sağlıklı yaşam tarzı, alışkanlıkları düzenlenmesi, gıda alımında sağlıklı seçimler ve bitkisel destekler önemli bir rol oynayabilir.

Anahtar Kelimeler: Karaciğer, karaciğer hastalıkları, fitoterapi, bitkisel tedavi, hepatitis

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Introduction

The liver is a critical center for many physiological processes, weighing approximately 1200-1500 grams, making up 2-3% of the total body weight, making it our heaviest internal organ. This organ, which plays an important role in areas such as metabolism, detoxification, digestion and the immune system, is one of the cornerstones of our health. However, diseases develop in the liver due to various factors. Alcohol consumption, viral infections, obesity and genetic factors are among the causes that threaten liver health.

The gallbladder and bile ducts are among the important structures that support the functions of the liver. While the liver plays a role in bile production, the gallbladder stores this fluid and releases it when necessary during the digestive process. The bile ducts, on the other hand, ensure that bile is transported from the liver to the intestines. Any disorder that may occur in this complex system can affect digestive health and lead to various diseases.

Liver diseases can be listed as hepatitis (viral, alcoholic or toxic hepatitis), fatty liver disease (hepatic steatosis), cirrhosis, liver fibrosis, liver cancer (hepatocellular carcinoma), hemochromatosis, Wilson's disease, cholestasis, and drug-induced hepatotoxicity.

1. Liver Diseases

1.1. Hepatitis

1.1.1. Viral Hepatitis

It is an inflammation of the liver caused by infection with hepatitis viruses (A, B, C, D, E). Each virus can be transmitted in different ways and can cause acute or chronic hepatitis. Among viral hepatitis, hepatitis B and C in particular have the potential to become chronic and can lead to cirrhosis or liver cancer.

1.1.2. Alcoholic Hepatitis

It is characterized by inflammation and damage to liver cells due to long-term and excessive alcohol consumption. Alcoholic hepatitis can progress to liver cirrhosis and liver failure, while symptoms such as jaundice, abdominal pain and fever can be seen in the clinical picture.

1.1.3. Toxic Hepatitis

It is an inflammation of the liver caused by drugs, chemicals, toxins or overdosage of some herbal products. Toxic hepatitis can be acute or chronic, depending on the type and dose of the substance taken. Some commonly used painkillers, such as acetaminophen, can also cause toxic hepatitis (1).

1.2. Fatty Liver Disease (Hepatic Steatosis)

It means that the liver contains at least 5% more fat than its own weight. This condition clinically presents itself in two forms: alcohol-related hepatosteatosis and non-alcoholic hepatosteatosis, which we encounter more frequently. Non-alcoholic fatty liver disease (NAFLD) can be seen in all age groups and ethnic groups. Its incidence in the general population is around 14-30%. Basically, any condition accompanied by insulin resistance is a risk factor for non-alcoholic hepatosteatosis. Fatty liver disease is more common in men than in women, and it has been reported that its incidence is related to the waist/hip ratio, independent of body weight (2).

1.3. Cirrhosis

Cirrhosis, also known as chronic liver disease, is the occurrence of damage to the liver at different levels due to various causes. The liver damage caused by cirrhosis is usually irreversible, but if diagnosed early, the damage can be limited. Although cirrhosis does not show symptoms in the early stages, it occurs with severe symptoms as the disease progresses (3).

1.4. Liver Fibrosis

Liver fibrosis is defined as the excessive accumulation of extracellular matrix components that occur as a result of the wound response following chronic injury. Fibrosis initially takes part in the tissue repair mechanism but later becomes pathogenic (4).

1.5. Liver Cancer (Hepatocellular Carcinoma)

It ranks 5th in the world in terms of incidence, and in our country, it most commonly occurs as a result of hepatitis B infection. It is seen 3 times more frequently in men than in women, and the incidence in men is 15 per 100,000 people each year. Of liver cancers 90% develop on the basis of cirrhosis. Liver cancer development rates vary depending on the cause of cirrhosis (5).

1.6. Hemochromatosis

It occurs when iron is absorbed more than necessary and accumulates in excessive amounts in the body. This disease is hereditary and shows an autosomal recessive pattern. Excessive iron accumulation leads to serious health problems such as liver cirrhosis, heart failure, diabetes and joint pain. In the early stages of the disease, symptoms such as fatigue, abdominal pain and tanning of the skin may be observed, but the symptoms usually become apparent in the advanced stages (6).

1.7. Wilson Disease (Copper Metabolism Disorder)

It is an autosomal recessive genetic disease that causes copper to accumulate in the liver, brain and other organs as a result of the body's inability to regulate copper metabolism. Its symptoms include liver disease, neurological disorders and Kaiser-Fleischer ring formation in the eye. Treatment is with drugs that prevent copper accumulation and early diagnosis is important for treatment (7).

1.8. Drug-induced Liver Damage (Hepatotoxicity)

It refers to the damage to the liver by drugs or other chemicals. Hepatotoxicity can result in inflammation, damage or necrosis of liver cells and usually varies depending on the dose, duration and individual sensitivity to the drugs. Symptoms may include jaundice, abdominal pain, nausea, vomiting and elevated liver enzymes. Early diagnosis and treatment are critical to preventing and reversing liver damage (8). Despite major advances in

modern medicine, there are no completely effective drugs that stimulate liver function, protect the organ or help regenerate liver cells. In addition, some drugs may cause adverse or side effects. Therefore, alternative methods need to be determined for the treatment of liver diseases. The aims of these alternatives are to be more effective and less toxic. Phytotherapy in liver diseases aims to support liver function, improve detoxification processes and reduce inflammation. The benefits of this traditional medicine with a long history are also revealed by scientific studies.

2. Plants Used in Liver Diseases

2.1. Grapefruit

Latin name: *Citrus paradisi*

Active ingredient: *Naringin and Naringenin*

Grapefruit juice contains many phytochemicals and nutrients important for a healthy diet. It is used in traditional medicine in many countries due to its antimicrobial, antifungal, anti-inflammatory, antioxidant and antiviral properties. In addition to nutrients such as vitamin C, folic acid, potassium, calcium, iron and various phenolic acids, it is also rich in limonoids, terpenes and D-glucaric acid. The red and pink varieties contain antioxidants such as beta-carotene and lycopene. One of its most important compounds is the flavonoid naringin, which is metabolized into naringenin in the human body. Grapefruit has antioxidant, anti-inflammatory and choleretic features, supports detoxification, and reduces fat in liver diseases. Among the scientific studies conducted to observe its effects, the potential role of grapefruit juice in relieving itching (pruritus) due to cholestatic liver disease was examined in the study by Cadranel et al. (9). The study observed that grapefruit juice could reduce itching caused by this disease. Researchers suggest that the compounds in grapefruit juice can relieve pruritus symptoms due to their effects on improving liver function (9). The study by Nahmias et al. (10) provides an important finding for liver diseases with the ability of naringenin to inhibit the secretion of hepatitis C virus (HCV). Hepatitis C can cause chronic inflammation in the liver, leading to cirrhosis, liver failure and liver cancer. Naringenin has been found to inhibit the secretion of HCV from liver cells in an apolipoprotein B-dependent manner. This flavonoid can limit viral replication in liver cells by reducing the viral load, thus slowing down the progression of the disease. This study shows that the grapefruit compound naringenin may be a potential adjuvant in the treatment of liver diseases and may provide a new perspective on antiviral treatments (10). Although it is known for its liver health-supporting effects, dosage and safety issues should be taken into consideration when consuming this fruit. It should not be forgotten that grapefruit can interact with some drugs and therefore should be used with caution. In particular, it slows down the breakdown of some drugs by inhibiting the CYP3A4 enzyme found in the liver, which can cause an increase in the blood levels of some drugs. As a result, the effects of the drugs may be strengthened and side effects may occur. While grapefruit juice may cause heartburn or digestive problems in some people, allergic reactions to grapefruit may be seen, although rare. In addition to these conditions, it is recommended that those with low blood pressure and liver enzyme problems consume it carefully. In general, 1/2 to 1 fresh grapefruit or a glass of grapefruit juice per day is usually sufficient.

2.2. Grape

Latin name: *Vitis vinifera L.*

Active ingredient: *Catechins, epicatechins, anthocyanidins, proanthocyanidins and resveratrol*

Various preparations used in popular and traditional medicine have been obtained from different parts of this plant, especially its fruit. These preparations stand out with their diuretic, anti-inflammatory and cholesterol-lowering effects, as well as their cancer-preventive activities against cardiovascular diseases and some cancers (especially prostate and colon cancer). The mechanisms of action of grapes are to protect the liver and its enzymes and to show antioxidant properties.

In a study conducted by Khoshbaten et al. (11) to investigate its effects on liver functions, the effect of grape seed extract on liver functions in patients with NAFLD was evaluated. The 50 patients who participated in the study were divided into two groups; one group received grape seed extract and the other group received placebo. After three months of treatment, the levels of liver enzymes (alanine aminotransferase and aspartate aminotransferase) significantly decreased in the group receiving grape seed extract. The study concluded that grape seed extract could improve liver functions in patients with NAFLD (11).

In the study by BedÊ et al. (12), the effects of grape juice, red wine and resveratrol on liver parameters in rats on a high-fat diet were examined. The rats were divided into four groups and received different treatments: grape juice, red wine, resveratrol and the control group. At the end of the study, improvements were observed in parameters indicating liver damage in the groups given grape juice, red wine and resveratrol. In particular, the effect of resveratrol in reducing liver steatosis was noted. As a result, it was found that these components might have mitigating effects on liver damage caused by a high-fat diet (12).

It is rare for grapes to interact with some medications, but it should not be forgotten that wine consumption may interact with some medications. Since it naturally contains high sugar, people with low sugar or glucose tolerance should consume it with caution. Caution should be exercised in its use as it can cause allergic reactions, increase the risk of bleeding, change the levels of liver enzymes and cause stomach and digestive problems. It is generally recommended to consume 1-2 servings of fresh grapes. One serving is approximately composed of 150-200 grams of grapes. This amount can provide sufficient amounts of antioxidants and other nutritional components. Grape seed extract can usually be used as a supplement in doses between 100-300 mg. These dosages are generally recommended ranges in clinical studies and health supplement products.

2.3. Daisy

Latin name: *Matricaria chamomilla or chamomilla recutita*

Active ingredients: *Apigenin, bisabolol, chamazulene, flavonoids, caffeic acid*

Chamomile is an Asteraceae plant native to Europe and is widely found throughout the world except for tropical and polar regions. This plant has been used for its therapeutic properties since ancient Egyptian and Greek civilizations. Pharmacological activities of various components of the plant, such as the modulatory effects and anti-inflammatory capacity of the flavonoids apigenin and quercetin on Hsp, and anti-inflammatory, antioxidant and antiseptic activities detected in α-bisabolol, guargazulene and chamazulene, have been reported. It has antioxidant, anti-inflammatory, hepatoprotective, bile flow regulator, anti-fibrotic, spasmolytic effects and is effective through sedative pathways. Saadh (13) discusses the potential benefits of chamomile for liver health in his article. Due to its anti-inflammatory and antioxidant properties, chamomile helps protect liver cells from damage caused by toxins or oxidative stress, and also it contributes to the improvement of liver functions. In traditional herbal medicine, chamomile is used to reduce inflammation and improve digestion, which plays a supportive role in the management of liver-related disorders (13). Rajaratnam et al. (14) discussed the role of chamomile in the treatment and prevention of liver diseases in their article. Chamomile exhibits protective properties for liver cells with its anti-inflammatory and antioxidant effects and helps reduce cellular damage in liver diseases, especially by providing protection against toxins and oxidative stress. In addition, it supports liver functions with its digestive regulating effects and contributes to the overall healing process. Chamomile, which is frequently used in traditional herbal treatment practices, is emphasized as an important plant for liver health.

Since side effects such as allergic reactions, risk of bleeding, and sedative effects may occur, it should be used with caution in these cases and under the supervision of a doctor during pregnancy and breastfeeding. It is usually consumed in tea or extract form. It is generally recommended to consume 1-2 cups of chamomile tea daily or extract at the dosage specified on the product labels.

2.4. Thistle

Latin name: *Silybum marianum L.*

Active ingredient: *Silymarin*

It is the most researched plant throughout human history. The oldest form of use of the plant is the treatment of snake bites recommended by Dioscorides. Gaius Plinius (23-79 AD) suggested that the plant mixed with honey should be used for bile excretion. Later, in the Middle Ages, it was accepted and used as an antidote for liver toxins. Today, the recommended use by the German Commission E is for toxin-induced liver damage, dyspeptic complaints including cirrhosis, and liver disorders.

The mechanisms of action of thistle in liver disorders are through its anti-inflammatory, immunomodulatory, hepatoprotective, anti-fibrotic, antioxidant properties.

In studies conducted on these properties of thistle, Eren and Şar (15) emphasized the protective and healing effects of the virgin thistle plant, especially in liver diseases. The silymarin compound found in the plant protects the liver from toxins, supports cell renewal and is used in the treatment of liver diseases such as cirrhosis and hepatitis (15). In Turgut's (16) study, the peroxidase enzyme of the thistle (*Silybum marianum*) plant grown in the Sakarya region was characterized. The study examines the biochemical properties of the peroxidase enzyme isolated from this plant and provides a basis supporting the positive effects of the plant on liver health (16). Nausea, abdominal distension and a mild laxative effect may be observed when using this plant. It is contraindicated in those allergic to the daisy family (*Astereceae*) and in pregnants and breastfeeding mothers. The recommended use is 12-15 g of drug/day (200-400 mg of silymarin) or 400-1100 mg of standard extract/day.

2.5. Pomegranate

Latin name: *Punica granatum*

Active ingredient: *Punicalagin and punicic acid*

Pomegranate has strong antioxidant properties and supports mitochondrial functions by reducing oxidative stress in cells. This improves the energy metabolism of liver cells and prevents fat accumulation. The components of pomegranate play an effective role in protecting liver health and preventing mitochondrial dysfunction.

As a result, pomegranate can be defined as an important fruit in the fight against obesity-related liver diseases due to its rich antioxidant content and the protective effects of punicalagin.

In addition to its antioxidant properties, pomegranate has anti-inflammatory properties and plays a role in improving mitochondrial functions, regulating fat metabolism and adjusting cellular energy metabolism.

In the study by Çalışkan et al. (17), the protective effect of pomegranate juice against acute liver toxicity caused by paracetamol in rats was investigated. The study examined the effect of pomegranate juice in reducing liver damage in rats given high doses of paracetamol, which caused liver toxicity. The results showed that pomegranate juice reduced liver damage, improved liver enzyme levels, and reduced oxidative stress due to its powerful antioxidant properties. The study highlights that pomegranate juice may play a potential protective role against paracetamol-induced liver toxicity (17). Zou et al. (18) investigated the role of mitochondrial dysfunction in obesity-related NAFLD and the protective effects of punicalagin, the active ingredient of pomegranate. The study showed that obesity-induced liver damage was associated with mitochondrial dysfunction and was associated with oxidative stress. Punicalagin, the active ingredient of pomegranate, helped prevent the deterioration of mitochondrial functions, reduced oxidative stress, and prevented fat accumulation in the liver. Punicalagin also supported cellular energy metabolism and strengthened antioxidant defense mechanisms. The study concluded that punicalagin provided a strong protective effect against NAFLD and had the potential

to improve mitochondrial health (18). The recommended dose is 3-9 g/day for powder obtained from the root, stem and bark (5% brewing).

2.6. Artichoke

Latin name: *Cynara scolymus*

Active ingredient: *Sinarin*

Cynara scolymus (artichoke) has been consumed as a food in the Mediterranean diet and worldwide for many years. It is used in traditional treatment, especially in the treatment of many diseases, especially liver and gallbladder disorders. In Commission E monographs, the leaves of the plant (Cynarae folium) are recommended in the treatment of digestive system complaints and liver and gallbladder disorders.

In addition, artichoke leaf extracts are used to eliminate digestive problems and lower blood cholesterol levels. We can list the mechanisms of action of artichoke as antioxidant activity, its effectiveness on serum lipid and lipid peroxidation, choleretic effect and hepatoprotective properties. Among the studies conducted on the mechanisms of action of artichoke, the therapeutic effects of milk thistle (Silybum marianum) and artichoke (Cynara scolymus) plants on NAFLD in rats with type 2 diabetes were investigated in the study by Doostkam et al. (19). The potential of these plants to improve liver health was evaluated in the study and it was found that both plants showed antioxidant, anti-inflammatory and fat metabolism regulating effects. Milk thistle improved enzyme levels in the liver, while artichoke was found to help reduce fat accumulation. The results suggest that milk thistle and artichoke may be effective natural treatment options in the management of NAFLD in individuals with type 2 diabetes (19). Panahi et al. (20) studied the effectiveness of artichoke leaf extract on NAFLD. The study was conducted as a pilot double-blind randomized controlled trial. The study evaluated whether artichoke leaf extract improved liver enzyme levels, lipid profiles, and overall liver health in individuals with NAFLD. The results showed that artichoke leaf extract significantly lowered liver enzymes and improved lipid metabolism. The study findings suggest that artichoke leaf extract may be a potential natural treatment option for the treatment of NAFLD (20). Although larger-scale studies are needed, these results support the positive effects of artichoke on liver health, and suggest that the mechanisms of these plants that support liver health may play an important role in the treatment of metabolic disorders. When consuming artichoke, those with allergies should not use it, those with gallbladder problems should consult a physician, and breastfeeding mothers should be careful. The recommended amounts to consume are 5-10 grams of tea made from the base leaves once a day, and 6 mL of 40% concentration per day is predicted to be sufficient for use as a tincture.

2.7. Ellagic Acid

Ellagic acid (EA) is a natural polyphenol found mainly in condensed form in nature as ellagitannins. It is found in many fruits (e.g., pomegranates, raspberries, cloudberries, wild strawberries, blackberries), some nuts (e.g., walnuts, almonds, pecans), various seeds (e.g., nut seeds), vegetables (e.g., radishes), and many medicinal plant species. EA has attracted attention in recent years due to its wide range of biological effects and potential health benefits. It is known for its strong antioxidant properties, anti-inflammatory effects, and antimicrobial and antimutagenic effects. In addition, many studies have reported cardioprotective, neuroprotective, gastroprotective, hepatoprotective, and nephroprotective effects of EA. Moreover, numerous studies indicate that EA has properties that can inhibit cell proliferation and potentially prevent cancer development. EA has antioxidant, anti-hepatotoxic, anti-steatosis, anti-cholestasis, anti-fibrogenic, anti-hepatocarcinogenic and anti-viral properties and supports the structural and functional recovery of the liver against toxic and pathological conditions. As a result of the study conducted by Devipriya et al. (21), it was stated that EA inhibited alcohol-induced liver cell damage, increased antioxidant levels, scavenged free radicals and stabilized cell membranes. This study showed that EA-treated female albino Wistar rats against alcohol-induced damage improved body weight, restored antioxidant status, modulated micronutrients and inhibited alcohol-induced toxicity by reducing circulating lipid levels (21). The administration of EA effectively reduced circulating lipid levels and prevented lipid peroxidation. This was reported by Derosa et al. (22), who confirmed that EA supplementation reduced plasma cholesterol elevations in hyperlipidemic rabbits. It has been stated that EA may reduce the activity of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase or may reduce other lipid levels by increasing hepatic bile acids and neutral sterol in the stool by increasing the speed of the lipid breakdown process (22). There is no specific dosage range for EA to show its protective and therapeutic effects on liver diseases. However, some studies have shown that 30-60 mg of EA per day may be beneficial. However, the effects of EA may vary from person to person and it is important to consult a doctor before use. In addition, consuming foods containing EA (e.g., pomegranate, blackberry and blackcurrant) may also be a natural way. In any case, it is best to get a professional opinion on treatment and dosage.

2.8. Olive Leaf

Latin name: *Olea europaea L.*

Active ingredient: *Oleuropein*

The leaves of the olive tree have been widely used in traditional medicines in Mediterranean countries. Olive leaf extract (OLE) contains high amounts of the phenolic antioxidant oleuropein, which is significantly higher than those found in olive fruit or olive oil. The properties of olive leaves include antioxidant, inflammation reduction, fat metabolism regulation and detoxification mechanisms of action.

OLE has shown protective effects against methotrexate-induced hepatotoxicity and chemically induced liver cirrhosis in rats (23). In addition, OLE has shown a potential hepatoprotective effect against diazinon in male mice and oxytetracycline-induced albino rats (24). Olive oil has also been shown to have protective effects

on hepatotoxicity caused by combined exposure to acrylamide and aluminum in rats and carbon tetrachloride (CCl4)-induced hepatotoxicity in male rats (25,26).

In the study conducted by Elgebaly et al. (27), the effectiveness of olive oil and its extract against fluoxetine-induced liver damage was evaluated. Control rats orally administered physiological saline (1 mL/kg), rats receiving 10 mg/kg/day fluoxetine dissolved in physiological saline, rats receiving 10 mg/kg/day fluoxetine and 1.5 mL/kg extra virgin olive oil orally, and rats receiving 10 mg/kg/day fluoxetine and 100 mg/kg OLE orally were divided into 4 groups and all rats were sacrificed at the end of 7 days and examined biochemically and histopathologically. As a result, it has been shown that olive oil and leaf extract protect against fluoxetine-induced liver damage in rats by reducing oxidative stress, inflammation, and apoptosis (27). OLEs are usually available in capsule or liquid form and are effective when used at lower doses because they are concentrated. It can usually be used in the range of 500-1000 mg per day, but this dosage may vary depending on the concentration of the product. It is recommended to drink 1-3 cups of olive leaf tea per day as tea, while the powder form can be used as 1-2 teaspoons (approximately 5-10 grams) per day. There is a possibility of digestive problems, headaches and skin reactions when consuming olive leaves. It should be used with caution during pregnancy and breastfeeding, in case of allergies, blood clotting problems and low blood pressure, under the supervision of a doctor.

3. Conclusion

This article emphasizes the importance and potential of plants used in liver diseases. These plants, which have been used in traditional medicine since ancient times, are supported by experimental studies today and their effectiveness is proven. The active ingredients of the plants play an important role in protecting liver health and in treatment processes.

However, the dosages and possible side effects that should be taken into consideration when using these plants should also be taken into account. Since each plant has its own mechanism of action and may have side effects, the right choices should be made according to the health conditions of individuals. It should also be remembered that these plants should not be used in some cases.

In conclusion, the role of phytotherapeutics in liver diseases will become clearer with further research in the future. Such studies are of critical importance in ensuring the safe and effective use of herbal treatments. Phytotherapeutics should be considered as a complementary part of medical treatment and scientific knowledge in this field should be increased.

Footnotes

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