MEANDROS
MEDICAL AND DENTAL JOURNAL

ADNAN MENDERES
UNIVERSITY
1992

Suat Doğancı
University of Health Sciences Turkey, Gülhane Training and Research Hospital, Clinic of Cardiovascular Surgery, Ankara, Turkey
E-mail: suat_doganci@yahoo.com

Kadri Murat Gürses
Aydın Adnan Menderes University Faculty of Medicine, Department of Histology and Embryology, Aydın, Turkey
ORCID ID: orcid.org/0000-0003-3904-0985

Statistical Editors

İmran Kurt Ömürlü
Aydın Adnan Menderes University Faculty of Medicine, Department of Biostatistics, Aydın, Turkey

English Language Editor

Teoman Akçay

Editorial Board

Sakari Kellokumpu
Oulu University Faculty of Medicine, Department of Biochemistry, Oulu, Finland

Domingo Martin
Clinica de Ortodoncia, Private Practice, Donostia, Spain

Ivana Milanovic
Belgrade University Faculty of Dentistry, Department of Endodontics, Belgrade, Sırbia

Jose Antonio Pariente Llanos
Extremadura University Faculty of Science, Department of Physiology, Badajoz, Spain

M. Alparslan Turan
Cleveland Clinic Main Campus, Department of General Anesthesiology, Cleveland, OHIO, USA

Duygu Karakış
Gazi University Faculty of Dentistry, Department of Prosthodontics, Ankara, Turkey
ORCID ID: orcid.org/0000-0001-9976-8797

Tuğrul Arslan
Erciyes University Faculty of Dentistry, Department of Endodontics, Kayseri, Turkey
ORCID ID: orcid.org/0000-0002-5055-1551

Zeynep Pınar Keleş
Giresun University Faculty of Dentistry, Department of Periodontology, Giresun, Turkey
ORCID ID: orcid.org/0000-0001-9139-8752

Didem Öner Özdaş
İstanbul Aydın University Faculty of Dentistry, Department of Pediatric Dentistry, İstanbul, Turkey

Olgun Topal
Afyonkarahisar University of Health Sciences, Faculty of Dentistry, Oral and Maxillofacial Surgery Department, Afyon, Turkey
ORCID ID: orcid.org/0000-0003-3550-8739

Başak Bıyıkoğlu
Altınbaş University Faculty of Dentistry, Department of Periodontology, İstanbul, Turkey
ORCID ID: orcid.org/0000-0001-8830-9835

Berza Yılmaz
Bezmialem Foundation University Faculty of Dentistry, Orthodontics Department, İstanbul, Turkey
ORCID ID: orcid.org/0000-0002-7961-0535

Meltem Mert Eren
Altınbaş University Faculty of Dentistry, Department of Restorative Dentistry, İstanbul, Turkey
ORCID ID: orcid.org/0000-0002-5903-6636

Gülsüm Sayın Özel
İstanbul Medipol University Faculty of Dentistry, Department of Prosthodontics, İstanbul, Turkey
ORCID ID: orcid.org/0000-0001-8833-5259

AIMS AND SCOPE

Meandros Medical and Dental Journal is the official, scientific, open access publication organ of the Adnan Menderes University, Faculty of Medicine and Faculty of Dentistry that is published in accordance with independent, nonbiased, double blind peer review principles.

The publication language of the journal is English must be in accordance with the international publication standards. The journal is published four times in a year. The aim of the Meandros Medical and Dental Journal is to publish original, high quality clinical and experimental researches conducted in all fields of medicine, dentistry, case reports, review articles on current topics, and letters to the editors. The target audience of the journal includes specialists in general surgery, students and all specialists and medical professionals who are interested in surgery.

The editorial policies and publication process are implemented in accordance with rules set by the International Committee of Medical Journal Editors (ICMJE), World Association of Medical Editors (WAME), Council of Science Editors (CSE), European Association of Science Editors (EASE), Committee on Publication Ethics (COPE), and the Heart Group. Information on the publication process and manuscript preparation guidelines are available online at http://meandrosmedicaljournal.org/

Meandros Medical and Dental Journal is indexed in **TUBITAK/ULAKBIM Turkish Medical Database, Emerging Sources Citation Index (ESCI), Index Copernicus, EMBASE, CINAHL Complete Database, Gale, British Library, ProQuest, J-Gate, Turkiye Citation Index, EBSCO, Hinari, GOALI, ARDI, OARE, AGORA, IdealOnline** and **Turk Medline.**

Open Access Policy

This journal provides immediate open access to its content on the principle that making research freely available to the public supports a greater global exchange of knowledge.

Open Access Policy is based on rules of Budapest Open Access Initiative (BOAI) http://www.budapestopenaccessinitiative.org/.

http://www.budapestopenaccessinitiative.org/ By "open access" to [peer-reviewed research literature], we mean its free availability on the public internet, permitting any users to read, download, copy, distribute, print, search, or link to the full texts of these articles, crawl them for indexing, pass them as data to software, or use them for any other lawful purpose, without financial, legal, or technical barriers other than those inseparable from gaining access to the internet itself. The only constraint on reproduction and distribution, and the only role for copyright in this domain, should be to give authors control over the integrity of their work and the right to be properly acknowledged and cited.

All published content is available online free of charge at http://meandrosmedicaljournal.org/

Editors in Chief

Doç. Dr. Mehtap Kılıç Eren
Aydın Adnan Menderes University
Faculty of Medicine
Department of Medical Biology, Aydın, Turkey
E-mail: mkilic@adu.edu.tr

Web page: www.meandrosmedicaljournal.org

Doç. Dr. V Özgen ÖZTÜRK
Aydın Adnan Menderes University
Faculty of Dentistry
Department of Periodontology, Aydın, Turkey
E-mail: ozgen.ozturk@adu.edu.tr

OPEN ACCESS

INSTRUCTION FOR AUTHORS

The Meandros Medical and Dental Journal (Formerly Adnan Menderes Üniversitesi Dergisi), is the official, scientific, open access publication organ of the Adnan Menderes University Faculty of Medicine and Dentistry that is published four times in a year in accordance with independent, unbiased, double blind peer review principles. The aim of the Meandros Medical and Dental Journal publishes high quality clinical and experimental research, case presentations, reviews and letters to the editor conducted in all fields of medicine and dentistry.

Originality, high scientific quality and citation potential are the most important criteria for a manuscript to be accepted for publication.

The aim of the Meandros Medical and Dental Journal is to publish original, high quality clinical and experimental researches conducted in all fields of medicine, case reports, review articles on current topics, and letters to the editors.The target audience of the journal includes specialists in general surgery, dentistry, students and all specialist, medical and dental professionals who are interested in surgery.

Instructions for Authors

The Meandros Medical and Dental Journal is a publication that publishes manuscripts prepared in English. IMPORTANTLY publications are being accepted ONLY in English since December 2015. Therefore, accepted manuscripts before December 2015 may be in English and Turkish. This is an important issue that the authors SHOULD consider. Submission of a Turkish title, abstract and keywords is not compulsory for international submissions. If accepted, translation services will be provided by the journal for international submissions.

Meandros Medical and Dental Journal does not charge any article submission or processing charges.

Manuscripts can only be submitted through the journal's online manuscript submission and evaluation system, available at www.http://meandrosmedicaljournal.org/. Manuscripts submitted via any other medium will not be evaluated.

The ORCID (Open Researcher and Contributor ID) number of the correspondence author should be provided while sending the manuscript. A free registration can be done at http://orcid.org

Manuscripts submitted for evaluation should not be previously presented or published in an electronic or printed medium. Editorial board should be informed of manuscripts that have been submitted to another journal for evaluation and rejected for publication. Manuscripts that have been presented in a meeting should be submitted with detailed information on the organization including the name, date and location of the organization.

Statements or opinions expressed in the manuscripts published in the journal reflect the views of the author(s) and not the opinions of the editors, the editorial board and/or the publisher; the editors, the editorial board and the publisher disclaim any responsibility or liability for such materials.

The authors transfer all copyrights of their manuscripts within the scope of local and international laws to the journal as of submission. For this purpose, a copyright transfer form should be signed by all contributing authors and a scanned version of the form should be submitted with the manuscript. The wet signed version of the form should be posted to the Editorial Office. The financial and legal responsibilities of a manuscript, including the text, tables, images and any other content that may be subject to international or local copyrights belong to the authors.

Authorship contribution form should be filled in the corresponding author and a signed and scanned version should be submitted during manuscript submission process in order to act appropriately to authorship rights and prevent ghost or honorary authorship.

Any financial grants or other support received for the study from individuals or institutions should be disclosed to the Editorial Board and to disclose potential conflicts of interest ICMJE Potential Conflict of Interest Disclosure From should be filled in and submitted.

The manuscripts should be prepared in accordance with ICMJE-Recommendations for the Conduct, Reporting, Editing and Publication of Scholarly Work in Medical Journals (updated in August 2013 - http://www.icmje.org/icmje-recommendations.pdf). Authors are required to prepare manuscripts in accordance with CONSORT guidelines for randomized research studies, STROBE guidelines for observational original research studies, STARD guidelines for studies on diagnostic accuracy, PRISMA guidelines for systematic reviews and meta-analysis, ARRIVE guidelines for experimental animal studies and TREND guidelines for non-randomized public behaviour.

An approval of research protocols by Ethics Committee in accordance with international agreements (Helsinki Declaration of 197, revised 2008, "Guide for the care and use of laboratory animals - www.nap.edu/ catalog/5140.html/) is required for experimental, clinical and drug studies and some case reports. If required ethics committee reports or an equivalent official document may be requested from the authors. In manuscripts reporting the results of an experimental study, it should be stated within the main text that the patients were informed in detail about the treatment technique and that the patient's consent was obtained. For studies carried out on animals, the measures taken to prevent pain and suffering of the animals should be stated clearly. Information on patient consent, name of the ethics committee and the ethics committee approval number should also be stated in the Materials and Methods section of the manuscript.

All manuscripts submitted to the The Meandros Medical and Dental Journal are screened for plagiarism using the Crossref Similarity Check powered by iThenticate software. Results indicating plagiarism may result in manuscripts being returned or rejected.

Pre-evaluation checks of each submission are carried out by the Editorial Board. Manuscripts are scanned for plagiarism and duplication at this stage. If an ethical problem detected regarding plagiarism and duplication the Editorial Board will act in accordance with the Committee on Publication Ethics (COPE). Manuscript that pass this stage are assigned to at least two double blind peer-reviewers. Reviewers are selected among independent experts who has published publications in the international literature on the submission subject and received considerable amount of citations. Research articles, systematic reviews and meta-analysis manuscripts are also reviewed by a biostatistician. By submitting a manuscript to the journal authors accept that editor may implement changes on their manuscripts as long as the main idea of the manuscript is not interfered with.

Once a manuscript is accepted for publication, the author list of the manuscript can't be altered with.

Manuscripts should be prepared using Microsoft Word software and should be structured in accordance with the rules below depending on their type.

Research Article

The abstract should be submitted in both English and Turkish; should be structured with Objective, Materials and Methods, Results and Conclusion subheadings and should not be longer that 300 words. Key words should be concordant with National Library of Medicine (NLM) Medical Subject Headings (MeSH) vocabulary terms and there should be at least 3 and the number should be limited with 6. Key words should be listed below the abstract both in Turkish and English. The main text should be structured with Introduction, Materials and Methods, Results subheadings and should be limited to 5000 words. Number of cited references should be limited with 50.

Statistical analysis should be performed in accordance with guidelines on reporting statistics in medical journals (Altman DG, Gore SM, Gardner MJ, Pocock SJ. Statistical guidelines for contributors to medical journals. Br Med J 1983: 7; 1489-93). Software used for analysis should be described. For parametric tests represent continuous variables as Mean±Standard Deviation, while for nonparametric tests represent data as Median and range (Minimum-Maximum) or Median and interquartile range (25th and 75th percentiles). Whenever complex analyses are used support the relative risk, odds or hazard ratios values by providing confidence intervals and p values.

INSTRUCTION FOR AUTHORS

Review Article

Review articles are solicited by the Editorial Board from authors who are experts in their field of study. The abstract should be submitted in both English and Turkish; should be unstructured and should not be longer that 300 words. Key words should be concordant with National Library of Medicine (NLM) Medical Subject Headings (MeSH) vocabulary terms and there should be at least 3 and the number should be limited with 6. Key words should be listed below the abstract both in Turkish and English. The main text should include a title, abstract, key words, main topics and references and should belimited to 5000 words. Number of cited references should be limited with 150.

If a previously published image is used its original version (both printed and online) should be cited properly and the permission obtained from the copyright holder (publisher, journal or author) to reproduce the material should be submitted to the journal.

Case Report

The journal allocates a limited space for case reports in each issue. Only case reports that make an original contribution to the literature, have an educative purpose, or offer a new method of treating rare clinical diseases which are difficult to diagnose and treat are considered for publication. The abstract should be unstructured and should not be longer than 150 words. Key words should be concordant with National Library of Medicine (NLM) Medical Subject Headings (MeSH) vocabulary terms and there should be at least 3 and the number should be limited with 10. The main text should be structured with Introduction, Case and Discussion subheadings. References and tables should be presented in the main document and the images should be submitted through the submission system in .TIFF or .JPEG formats. The main text should not be longer than 1500 words and the number of references cited should be limited to 20.

Letters to the Editor

These type of manuscripts discuss the importance, an overlooked detail or a missing point of a previously published manuscript. In addition to these, letters to the editor can be prepared on a subject within the scope of the journal that may draw the readers' attention, especially on educative cases. Readers can also submit their opinions on published material in letter to the editor format. An abstract, keywords, tables and figures are not required with this type of manuscripts. The main text should not be longer than 500 words, and must be limited to 3 authors and 5 references. Proper citation of the study that the letter is about including the authors' names, title, publication year, volume and page numbers is required.

Title Page / Cover Letter

Title: The title should be concise and informative.

Running Title: A running title (not more than 40 characters including spaces should be entired.

Author names and affiliations: Please clearly indicate the given name(s) and family name(s) of each author. Present the authors' affiliation addresses below the names. Indicate all affiliations with a lower-case superscript number immediately after the author's name and in front of the appropriate address. Provide the full postal address of each affiliation, including the country name and, if available, the e-mail address of each author.

Corresponding author. Clearly indicate who will handle correspondence at all stages of refereeing and publication, also post-publication. Ensure that the e-mail address is given and that contact details are kept up to date by the corresponding author.

Tables / Graphs / Illustrations / Photograph

Tables and figures should be located at the end of the main document, images should be submitted in.JPG and.TIFF formats.Tables must be prepared in a Microsoft Office Word document using "Insert Table" command and be placed at the end of the references section in the main document. Decimal points in the text, tables and figures should be separated by comma in Turkish sections and by dots in English sections. Particularly, tables should be explanatory for the text and should not duplicate the data given in the text.

Each table and figure should have a self-explanatory title and be numbered in order of their citation in the text. Arabic numbers should be prefered for tables, graphs, figures and photographs. In the case of the use of a previously published table, figure or illustration, written permission from the publisher should be submitted with the manuscript. Information or illustrations must not permit identification of patients, and written informed consent for publication must be sought for any photograph.

In microscopic images, magnification and staining techniques must be specified in addition to figure captions. All images should be in high resolution with minimum 300 dpi. It would be more appropriate if the drawings are prepared by the professionals. Gray color should be avoided. 3D graphs should be avoided.

References

References should be numbered in the order they are cited. Only published data or manuscripts accepted for publication and recent data should be included. Inaccessible data sources and those not indexed in any database should be omitted. Titles of journals should be abbreviated in accordance with Index Medicus-NLM Style (Patrias K. Citing medicine: the NLM style guide for authors, editors, and publishers [Internet]. 2nd ed. Wendling DL, technical editor. Bethesda (MD): National Library of Medicine (US); 2007 - [updated 2011 Sep 15; cited Year Month Day] (http://www.nlm.nih.gov/citingmedicine). All authors should be listed if an article has six or less authors; if an article has more than six authors, first six authors are listed and the rest is represented by "ve ark." in Turkish articles and by "et al." in English articles. Reference format and punctuation should be as in the following examples.

Standard Journal Article

Özhan MÖ, Süzer MA, Çomak İ, Çaparlar CÖ, Aydın GB, Eşkin MB, et al. Do the patients read the informed consent? Balkan Med J 2012; 29: 252-60.

Book Section

Sherry S. Detection of thrombi. In: Strauss HE, Pitt B, James AE, editors. Cardiovascular Medicine. St Louis: Mosby, 1974: 273-85.

Books with Single Author

Cohn PF. Silent myocardial ischemia and infarction. 3rd ed. New York: Marcel Dekker; 1993.

Editor(s) as author

Norman IJ, Redfern SJ, editors. Mental health care for elderly people. New York: Churchill Livingstone; 1996.

Conference Proceedings

Bengisson S. Sothemin BG. Enforcement of data protection, privacy and security in medical informatics. In: Lun KC, Degoulet P, Piemme TE, Rienhoff O, editors. MEDINFO 92. Proceedings of the 7th World Congress on Medical Informatics; 1992 Sept 6-10; Geneva, Switzerland. Amsterdam: North-Holland; 1992.p.1561-5.

Scientific or Technical Report

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

Thesis

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

Manuscripts published in electronic format

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

CONTENTS

CONTENTS

Original Article / Özgün Araştırma

Radiographic and Histopathologic Evaluation of Radiolucent Lesions Involving Impacted Teeth: A Multicenter Study

Gömülü Diş İçeren Radyolüsent Lezyonların Radyografik ve Histopatolojik Değerlendirilmesi: Çok Merkezli Bir Çalışma

◉ Zeynep Fatma Zor[1], ◉ Zühre Akarslan[2], ◉ Nur Hacıosmanoğlu[3], ◉ Yavuz Tolga Korkmaz[4], ◉ Cenk Durmuşlar[5],
◉ Emre Barış[6], ◉ Saadettin Kayıpmaz[7], ◉ İsmail Saygın[8], ◉ Murat İçen[9], ◉ Doğan Gün[10], ◉ Nuray Bağcı[2], ◉ İlkay Peker[2]

[1]Gazi University Faculty of Dentistry, Department of Oral and Maxillofacial Surgery, Ankara, Turkey
[2]Gazi University Faculty of Dentistry, Department of Oral and Maxillofacial Radiology, Ankara, Turkey
[3]Ankara University Faculty of Dentistry, Department of Oral and Radiology, Ankara, Turkey
[4]Karadeniz Technical University Faculty of Dentistry, Department of Oral and Maxillofacial Surgery, Trabzon, Turkey
[5]Bülent Ecevit University Faculty of Dentistry, Department of Oral and Maxillofacial Surgery, Zonguldak, Turkey
[6]Gazi University Faculty of Dentistry, Department of Oral Pathology, Ankara, Turkey
[7]Karadeniz Technical University Faculty of Dentistry, Department of Oral and Maxillofacial Radiology, Trabzon, Turkey
[8]Karadeniz Technical University Faculty of Medicine, Department of Pathology, Trabzon, Turkey
[9]Bülent Ecevit University Faculty of Dentistry, Department of Oral and Maxillofacial Radiology, Zonguldak, Turkey
[10]Bülent Ecevit University Faculty of Medicine, Department of Pathology, Zonguldak, Turkey

Abstract

Objective: The purpose of this retrospective study was to evaluate the radiographic and histopathologic features of the pathologic lesions associated with an impacted tooth in the maxilla and mandible of patients who were admitted to three different university hospitals located in different cities.

Materials and Methods: One hundred one patients (36 females and 65 males) aged between 8 and 67 and who have radiolucent lesions associated with the impacted teeth were included in this study. Data related to the age and gender of the patients, and the findings of cone-beam computed tomography, and histopathologic diagnosis of the lesions were recorded and analyzed.

Results: Majority of the lesions were in the posterior region of the mandible (62.4%), related to the mandibular third molars (59.4%), and were diagnosed as a dentigerous cyst. The most common features of the lesions were unilocular radiolucency (91.1%), well-circumscribed (90.1%), and expansive (85.1%). A statistically significant relationship was found between the migration of the impacted tooth/teeth related to the lesion (p<0.05) and the expansion of the lesion (p<0.01) according to gender. A statistically significant relationship was found between the migration of the impacted tooth/teeth related to the lesion (p<0.05) and the histopathological diagnosis of the lesion (p<0.01) according to age groups.

Conclusion: Knowing all of the clinical, radiological and histopathological features of the lesions provide the surgeon to reach the correct diagnosis. Thus, the doctors achieve high success in treatment with the right treatment plan.

Öz

Amaç: Bu retrospektif çalışmanın amacı, farklı şehirlerde bulunan üç farklı üniversite hastanesine başvuran hastaların üst ve alt çenesindeki gömülü diş ile ilişkili patolojik lezyonların radyografik ve histopatolojik özelliklerini değerlendirmektir.

Gereç ve Yöntemler: Bu çalışmaya gömülü diş ile ilişkili radyolüsent lezyonu olan 8-67 yaş aralığındaki 101 hasta (36 kadın ve 65 erkek) dahil edildi. Hastaların

yaşı ve cinsiyeti, lezyonların konik–ışınlı bilgisayarlı tomografi bulguları ve histopatolojik tanısı ile ilgili veriler kaydedildi ve analiz edildi.

Bulgular: Lezyonların çoğu mandibula posterior bölgede (%62,4), mandibular üçüncü molar dişlerle (%59,4) ilişkiliydi ve dentigeröz kist tanısı aldı. Lezyonlarda en fazla görülen özellikler uniloküler radyolüsensi (%91,1), iyi sınırlı (%90,1) ve ekspansif (%85,1) olması idi. Cinsiyete göre lezyon ile ilişkili gömülü diş/dişlerin migrasyonu (p<0,05) ve lezyonun ekspansiyonu (p<0,01) arasında istatiksel olarak anlamlı bir ilişki bulundu. Yaş gruplarına göre lezyon ile ilişkili diş/dişlerin migrasyonu (p<0,05) ve lezyonun histopatolojik tanısı (p<0,01) arasında istatiksel olarak anlamlı bir ilişki bulundu.

Sonuç: Lezyonların tüm klinik, radyolojik ve histopatolojik özelliklerinin bilinmesi cerrahın doğru tanıya ulaşmasını sağlar. Böylece hekimler doğru tedavi planı ile tedavide yüksek başarı elde ederler.

Introduction

Impacted teeth are one of the most common situations in dentistry. Insufficient space in the dental arch, malposition, and absence of eruption force are some etiologic factors for the impaction (1). Impacted teeth can be extracted because of inflammation and infection, cyst and tumors, destruction of adjacent teeth, periodontitis, caries, and etcetera (2). The most common impacted teeth are mandibular third molars followed by maxillary third molars, maxillary canines, and mandibular premolars, respectively. Approximately, one in every five mandibular third molars and maxillary third molars are impacted. Also, radiolucency around the crowns in radiographic examination are present in 37% of impacted mandibular third molars and 15% of maxillary third molars (1,2).

The crowns of impacted teeth are surrounded by a soft tissue called dental follicle. Radiographically, the dental follicle is a radiolucent space with a thin radiopaque border (1). Immune-histochemical studies showed that the dental follicle cells have a great potential for growth and proliferation (3). Thus, pathologic changes can occur from the dental follicle and it can be detected as an enlargement of the pericoronal space. Dentigerous cyst (DC) is the most common pathology associated with the dental follicle. Odontogenic keratocyst (OKC) and ameloblastoma are the other pathologies associated with impacted molars. Bifurcation cyst, glandular odontogenic cyst, calcifying epithelial odontogenic tumor, adenomatoid odontogenic tumor, ameloblastic fibroma, and ameloblastic fibro-odontoma are rarely seen in radiolucent lesions associated with impacted teeth (4).

Radiographic examination is routinely used to detect the presence or absence of any pathology associated with impacted teeth (5). Different types of cystic and cystic-appearing lesions can be easily detected by periapical, occlusal, and panoramic radiography. Adjunct to these radiographic techniques, cone-beam computed tomography (CBCT), computed tomography (CT), magnetic resonance imaging and scintigraphy are used for detailed three-dimensional imaging of the lesions (6). Especially CBCT provides three-dimensional images with axial, sagittal and coronal sections with a lower radiation dose compared to CT. CBCT is originally designed for the visualization of solid structures in the head and neck region because it produces a lower radiation dose with high spatial resolution. The clinical use of CBCT provides accurate information about the contents and borders of the lesions, their special relations with surrounding structures, and cortical expansion for which the conventional radiographic techniques are usually inadequate (7).

In the literature, there are a lot of studies about impacted teeth and the lesions (8-10). To the best of our knowledge, this paper is one of the very few multicenter studies that evaluate the pathologic lesions related with impacted teeth (11,12). In other multicenter studies, specific lesions have been evaluated. In contrast, in this study, the lesions associated with impacted teeth have been handled more comprehensively. The purpose of this retrospective study was to evaluate the radiographic and histopathologic features of the pathologic lesions associated with impacted tooth in maxilla and mandible of patients who were admitted to three different university hospitals located in different cities.

Materials and Methods

Data Collection

This multicenter study was conducted in three different university hospitals. Before starting the study, ethical approval was received (approval no:

36290600/63, date: 30.11.2015). This study was carried out in accordance with the principles of the Helsinki Declaration. CBCT images belonging to 101 patients (36 females and 65 males) having radiolucent lesions in conjunction with any impacted tooth/teeth were assessed. The CBCT images of patients who had a history of maxillofacial trauma, surgery, and artifacts were excluded from the study.

CBCT images in different centers were obtained by ProMax 3D Mid (Planmeca Oy, Helsinki, Finland), Morita Veraviewepocs 3D R100-CP (J Morita MFG Corp, Kyoto, Japan) and Kodak 9500 3D (Kodak Corp, Carestream Health, Rochester, New York) using parameters of 90 kilovoltage peak, 12-mile ampere, scanning time of 10.8-13.8 seconds and 0.4 mile meter voxel size according to imaging area. Sagittal, coronal, and axial CBCT sections of the radiolucent lesions in conjunction with any impacted tooth/teeth were evaluated in a quiet room with subdued ambient lighting, approximately 50 centimeters away from the screen by dentomaxillofacial radiologists with at least five years of experience. The dentomaxillofacial radiologists held a meeting to detect the evaluation criteria of the lesions before starting the evaluation process (Table 1). Surgical procedures of the lesions were performed by maxillofacial surgeons with at least seven years of experience. Histopathologic validations were carried out by pathologists with at least ten years of experience. Demographic data, CBCT findings and histopathologic validations of the patients were recorded.

Statistical Analysis

Obtained data were statistically analyzed by using SPSS program version 21.0 (SPSS Inc., Chicago, USA). The findings were categorized and statistically analyzed with descriptive statistics, crosstabs, chi-square tests and Fisher's Exact tests where appropriate. Statistical analyses were performed both in 95% and 99% confidence intervals. The patients were categorized into three age groups: 8-30 years old (<30 years old), 31-50 years old and 51 years old and over (51> years old) for the analysis.

Results

CBCT and histopathologic data belonging to 101 patients (36 females; 35.6% and 65 males; 64.4%) aged between 8 and 67 years (mean age ± standard deviation: 38.8±15.7) were evaluated in the study

Table 1. The evaluation criteria of the study
Demographics
1. Age
2. Gender
Features of the lesions
3. Location
a) Anterior region of the jaws
b) Posterior region of the jaws
4. Internal structure
a) Unilocular radiolucent
b) Multilocular radiolucent
5. Periphery
a) Well-defined
b) Scalloped shape
6. Impacted tooth/teeth in conjunction with the lesion
7. The relationship between impacted tooth/teeth and lesion
a) The lesion related with crown of the impacted tooth/teeth
b) The lesion related with root of the impacted tooth/teeth
c) The lesion related with both crown and root of the impacted tooth/teeth
8. Migration of impacted tooth/teeth in conjunction with the lesion
a) No migration
b) Migration towards buccal direction
c) Migration towards lingual/palatal direction
9. Expansion and direction of the lesion
a) Buccal expansion
b) Buccal-lingual or buccal-palatal expansion
c) Lingual or palatal expansion
10. Size of the lesion (mm)
11. Effects on surrounding anatomical structures
a) No effect
b) The lesion is adjacent to cortical boundary of anatomical structures
c) The lesion is related with surrounding anatomical structures
12. Expansion of the lesion
a) Present
b) Absent
13. Cortical bone thinning related with the lesion
a) Present
b) Absent
14. Cortical bone perforation related with the lesion
a) Present
b) Absent
15. Root resorption in adjacent tooth/teeth
a) Present
b) Absent
16. Migration in adjacent tooth/teeth
a) Present
b) Absent
17. Histopathological diagnosis of the lesion

(Table 2). 65 lesions were diagnosed in males, while 36 lesions were diagnosed in females. The lesions consisted of DC (n=72; 71.29%) (Figure 1), OKC (n=15; 14.85%) (Figure 2,3), inflammation (n=7; 6.93%), ameloblastoma (n=5; 4.95%), and glandular odontogenic cyst (n=2; 1.98%) respectively (Table 3).

Majority of the lesions were in the posterior region of the mandible (n=63; 62.4%) and related with the mandibular third molars (n=60; 59.4%). The most common features of the lesions were unilocular radiolucency (n=92, 91.1%), well-circumscribed (n=91, 90.1%), and expansive (n=86, 85.1%). More than half of the lesions did not cause any migration of the related impacted tooth (n=53, 52.5%), resorption of adjacent tooth/teeth (n=52, 51.2%) and migration of adjacent tooth/teeth (n=62, 61.4%). The cortical bone thinning (n=89, 88.1%) and perforation (n=90, 89.1%) were a common finding. The distribution and statistical analysis of the variables were shown in Table 4 in detail according to gender.

A statistically significant relationship was found between the migration of the impacted tooth/teeth

Table 2. Demographic characteristics of the patients

Characteristics	Mean ± SD	n	%
Age	38.8±15.7		
Gender			
Female		36	35.6
Male		65	64.4
Total		101	100
SD: Standard deviation			
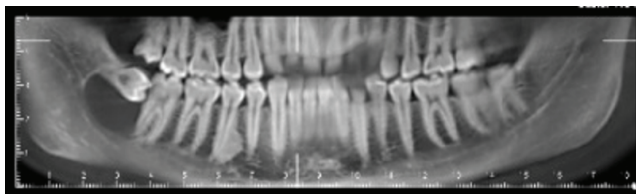
Figure 1. The image of cone-beam computed tomography for dentigerous cyst
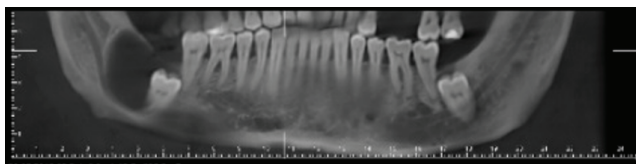
Figure 2. The image of cone-beam computed tomography for odontogenic keratocyst
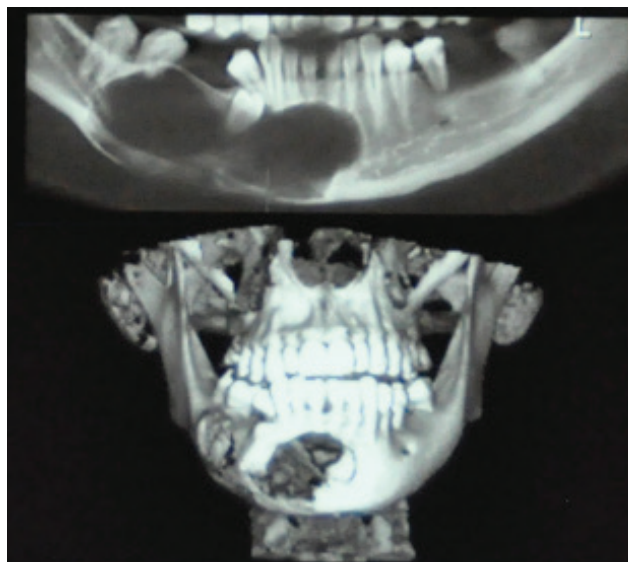
Figure 3. The image of cone-beam computed tomography for odontogenic keratocyst

Table 3. Histopathologic distribution of lesions in total and according to gender

Histopathologic diagnosis	Female	Male	Total n (%)
Dentigerous cyst	23	49	72 (71.3)
Odontogenic keratocyst	7	8	15 (14.85)
Glandular odontogenic cyst	0	2	2 (1.98)
Ameloblastoma	2	3	5 (4.95)
Inflammation	4	3	7 (6.93)
Total	36	65	101 (100)

related to the lesion (p<0.05) and the expansion of the lesion (p<0.01) according to gender. The migration both the buccal and lingual/palatal direction of impacted tooth/teeth was more common in males than in females. Expansion of the lesion was more common in males than in females. No statistically significant difference was found between gender and the other variables (Table 4).

A statistically significant relationship was found between the migration of the impacted tooth/teeth related to the lesion (p<0.05) and the histopathological diagnosis of the lesion (p<0.01) according to age groups. The migration towards buccal direction of impacted tooth/teeth was more common in the patients ≤30 years old, while the migration towards lingual/palatal was more common in the patients 51≥ years old. Most of the patients with DC were 31-50 years old and 51≥ years old, while other lesions were

Table 4. The distribution and statistical analysis of the variables according to gender					
Variables	**Items**	**Female n (%)**	**Male n (%)**	**Total n (%)**	**p-value**
Location of the lesion	Anterior region of the jaws	13 (36.1)	25 (38.5)	38 (37.6)	0.815
	Posterior region of the jaws	23 (63.9)	40 (61.5)	63 (62.4)	
Internal structure of the lesion	Unilocular	32 (88.9)	60 (92.3)	92 (91.1)	0.405[a]
	Multilocular	4 (11.1)	5 (7.7)	9 (8.9)	
Periphery of the lesion	Well-defined	31 (86.1)	60 (92.3)	91 (90.1)	0.253[a]
	Scalloped shape	5 (13.9)	5 (7.7)	10 (9.9)	
Impacted tooth/teeth in conjunction with the lesion	38	13 (36.1)	15 (23.1)	28 (27.7)	0.363
	48	9 (25.0)	23 (35.4)	32 (31.7)	
	13, 23 or 43	9 (25.0)	13 (20.0)	22 (21.8)	
	Other teeth	5 (13.9)	14 (21.5)	19 (18.8)	
The relationship between impacted tooth/teeth and lesion	The lesion related with crown of the impacted tooth/teeth	19 (52.8)	22 (33.8)	41 (40.6)	0.177
	The lesion related with root of the impacted tooth/teeth	4 (11.1)	11 (16.9)	15 (14.9)	
	The lesion related with both crown and root of the impacted tooth/teeth	13 (36.1)	32 (49.2)	45 (44.6)	
Migration presence of impacted tooth/teeth in conjunction with the lesion	No migration	20 (55.6)	33 (50.8)	53 (52.5)	0.047*
	Migration towards buccal direction	13 (36.1)	14 (21.5)	27 (26.7)	
	Migration towards lingual/palatal direction	3 (8.3)	18 (27.7)	21 (20.8)	
Expansion presence and direction of the lesion	Buccal expansion	11 (30.6)	17 (26.2)	28 (27.7)	0.478
	Buccal-lingual or buccal-palatal expansion	20 (55.6)	43 (66.2)	63 (62.4)	
	Lingual or palatal expansion	5 (13.9)	5 (7.7)	10 (9.9)	
Effects of surrounding anatomical structures	No effect	7 (19.4)	14 (21.5)	21 (20.8)	0.805
	The lesion is adjacent to cortical boundary of anatomical structures	8 (22.2)	11 (16.9)	19 (18.8)	
	The lesion is related with surrounding anatomical structures	21 (58.3)	40 (61.5)	61 (60.4)	
Expansion of the lesion	Present	26 (72.2)	60 (92.3)	86 (85.1)	0.007**
	Absent	10 (27.8)	5 (7.7)	15 (14.9)	
Cortical bone thinning related with the lesion	Present	32 (88.9)	57 (87.7)	89 (88.1)	0.566[a]
	Absent	4 (11.1)	8 (12.3)	12 (11.9)	
Cortical bone perforation related with the lesion	Present	31 (86.1)	59 (90.8)	90 (89.1)	0.342[a]
	Absent	5 (13.9)	6 (9.2)	11 (10.9)	
Root resorption in adjacent tooth/teeth	Present	14 (38.9)	35 (53.8)	49 (48.5)	0.150
	Absent	22 (61.1)	30 (46.2)	52 (51.5)	
Migration in adjacent tooth/teeth	Present	12 (33.3)	27 (41.5)	39 (38.6)	0.417
	Absent	24 (66.7)	38 (58.5)	62 (61.4)	
Histopathological diagnosis of the lesion	Dentigerous cyst	23 (63.9)	49 (75.4)	72 (71.3)	0.221
	Other lesions	13 (36.1)	16 (24.6)	29 (28.7)	
Total		36 (35.6)	65 (64.4)	101 (100)	
[a]Statistical analysis result according to Fisher Exact test. *p<0.05; **p<0.01 Teeth were numbered according to FDI system					

more common among patients ≤30 years old. No statistically significant difference was found between the age groups and the other variables (Table 5).

Discussion

The multicenter studies are very important and valuable to determine the real prevalence of lesions. Actually it is very difficult to collect the data in these types of studies but obtained results are so valuable. Essentially, in the literature some studies which were evaluated the prevalence and characteristics of jaw lesions (8,13,14) can be found and there are a lot of studies about impacted teeth and the lesions (8-10). But the number of the multicenter studies about the impacted teeth and the lesions related with them is limited (11,12). Our study is one of the few studies with these properties.

According to our results, majority of the lesions were in the posterior region of the mandible and related with mandibular third molars. This result is compatible with previous studies (10-12). Additionally, in this study, the most frequently impacted teeth were found as the third molar, canine and other teeth, respectively. Because the third molars account for 98% of all impacted teeth. Generally, impaction of mandibular molars is seen more frequently compared with maxillary molars (7,14).

According to CBCT findings, the most common features of the lesions were unilocular radiolucency, well-circumscribed and expansive. More than a half of the lesions did not cause any migration of the related impacted tooth, resorption of adjacent tooth/teeth and migration of adjacent tooth/teeth. The cortical bone thinning and perforation were a common finding in general. These radiographic findings are common in benign lesions. In general, cysts and tumors present in the study had benign characters (15).

There were statistically significant differences between gender with the migration of impacted tooth/teeth in conjunction with the lesion and expansion of the lesion. This could be related with the type of lesion. The benign and aggressive lesions show different growth patterns. The radiographic findings of these lesions depend on morphologic findings and the relationship of adjacent structures. The size, growth stage, and localization of the lesions have effect on the radiographic features of the lesions, even one

having the same histopathological diagnosis. On the other hand, lesions with different histopathologic structures could share similar radiographic features (16,17).

Regarding histopathological diagnosis almost two third of the lesions were DC, followed by OKC, inflammation, ameloblastoma and glandular odontogenic cysts. In some studies, the relationship between the impacted tooth and the frequency of odontogenic cyst and tumors has been discussed. Curran et al. (18) showed that the frequency of the lesions associated with impacted teeth was DC, OKC, odontoma, ameloblastoma, calcifying epithelial odontogenic tumor, carcinoma, and myxoma, respectively. Shoaee et al. (19) reported that the three most common lesions associated with impacted teeth were DC, OKC, and ameloblastoma. In a previous study, the most common type of lesion associated with impacted third molar teeth was defined as DC followed by OKC, ameloblastoma, paradental cyst, and glandular odontogenic cyst (20). Our results show similarity with recent research indicating DC is the most common lesion associated with impacted teeth (10,15,21).

According to our results, majority of the patients with DC were between 31-50 years old, while other lesions were more common among patients <30 years old. The migration and root resorption in the adjacent tooth and expansion were seen in the evaluated cases in our study. DC was more common among males compared to females. The studies have reported that the DC is usually seen more common in adolescents and young adults between 20-40 years. In addition, it has been shown that DC is typically enveloping the crown of the impacted, leads to migration and is resorbed of adjacent tooth/teeth, and makes expansion buccally or medially, especially in large lesions radiographically (22,23). Also, DC is reported to be seen more common among males in the literature (24). Our findings show similarity with previous studies.

We found that other lesions associated with impacted teeth were OKC, inflammation, ameloblastoma and glandular odontogenic cyst. Many studies have shown that these lesions are associated with impacted teeth (18-20). It has reported that OKC usually appears between second and fourth decades. This lesion is frequently located at posterior body of the mandible and ramus and represented as unilocular

Table 5. The distribution and statistical analysis of the variables according to age						
Variables	Items	<30 n (%)	31-50 n (%)	51> n (%)	Total n (%)	p value
Location of the lesion	Anterior region of the jaws	13 (40.6)	17 (41.5)	8 (28.6)	38 (37.6)	0.507
	Posterior region of the jaws	19 (59.4)	24 (58.5)	20 (71.4)	63 (62.4)	
Internal structure of the lesion	Unilocular	31 (96.9)	36 (87.8)	25 (89.3)	92 (91.1)	a
	Multilocular	1 (3.1)	5 (12.2)	3 (10.7)	9 (8.9)	
Periphery of the lesion	Well-defined	30 (93.8)	35 (85.4)	26 (92.9)	91 (90.1)	a
	Scalloped shape	2 (6.2)	6 (14.6)	2 (7.1)	10 (9.9)	
Impacted tooth/teeth in conjunction with the lesion	38	7 (21.9)	12 (29.3)	9 (32.1)	28 (27.7)	0.652
	48	9 (28.1)	12 (29.3)	11 (39.3)	32 (31.7)	
	13, 23 or 43	7 (21.9)	10 (24.4)	5 (17.9)	22 (21.8)	
	Other teeth	9 (28.1)	7 (17.0)	3 (10.7)	19 (18.8)	
The relationship between impacted tooth/teeth and lesion	The lesion related with crown of the impacted tooth/teeth	12 (37.5)	20 (48.8)	9 (32.1)	41 (40.6)	a
	The lesion related with root of the impacted tooth/teeth	4 (12.5)	4 (9.8)	7 (25.0)	15 (14.9)	
	The lesion related with both crown and root of the impacted tooth/teeth	16 (50.0)	17 (41.5)	12 (42.9)	45 (44.6)	
Migration of impacted tooth/teeth in conjunction with the lesion	No migration	12 (37.5)	26 (63.4)	15 (53.6)	53 (52.5)	0.011*
	Migration towards buccal direction	14 (43.8)	10 (24.4)	3 (10.7)	27 (26.7)	
	Migration towards lingual/palatal direction	6 (18.8)	5 (12.2)	10 (35.7)	21 (20.8)	
Direction of expansion	Buccal expansion	7 (21.9)	14 (34.1)	7 (25.0)	28 (27.7)	a
	Bucco-lingual or buccal-palatal expansion	22 (68.8)	23 (56.1)	18 (64.3)	63 (62.4)	
	Lingual or palatal expansion	3 (9.4)	4 (9.8)	3 (10.7)	10 (9.9)	
Effects on surrounding anatomical structures	No effect	6 (18.8)	9 (22.0)	6 (21.4)	21 (20.8)	0.934
	The lesion is adjacent to cortical boundary of anatomical structures	6 (18.8)	9 (22.0)	4 (14.3)	19 (18.8)	
	The lesion is related with surrounding anatomical structures	20 (62.5)	23 (56.1)	18 (64.3)	61 (60.4)	
Expansion of the lesion	Present	30 (93.8)	33 (80.5)	23 (82.1)	86 (85.1)	a
	Absent	2 (6.2)	8 (19.5)	5 (17.9)	15 (14.9)	
Cortical bone thinning related with the lesion	Present	30 (93.8)	36 (87.8)	23 (82.1)	89 (88.1)	a
	Absent	2 (6.2)	5 (12.2)	5 (17.9)	12 (11.9)	
Cortical bone perforation related with the lesion	Present	31 (96.9)	37 (90.2)	22 (78.6)	90 (89.1)	a
	Absent	1 (3.1)	4 (9.8)	6 (21.4)	11 (10.9)	
Root resorption in adjacent tooth/teeth	Present	13 (40.6)	21 (51.2)	15 (53.6)	49 (48.5)	0.548
	Absent	19 (59.4)	20 (48.8)	13 (46.4)	52 (51.5)	
Migration in adjacent tooth/teeth	Present	14 (43.8)	16 (39.0)	9 (32.1)	39 (38.6)	0.653
	Absent	18 (56.2)	25 (61.0)	19 (67.9)	62 (61.4)	
Histopathological diagnosis of the lesion	Dentigerous cyst	15 (46.9)	34 (82.9)	23 (82.1)	72 (71.3)	0.001**
	Other lesions	17 (53.1)	7 (17.1)	5 (17.9)	29 (28.7)	
Total		32 (31.7)	41 (40.6)	28 (27.7)	101 (100)	
aChi-square analysis is not appropriate. *p<0.05; **p<0.01						

or multilocular radiolucency having well-defined borders, with minimal mediolateral expansion. Also, it has been informed that OKC may include the crown of the impacted teeth resembling the DC in some cases (22). In contrast, ameloblastoma is a benign lesion, a locally invasive tumor. It has reported that ameloblastoma is usually seen in patients 20-50 years old and develops in the molar-ramus regions of the mandible. It has been presented that this lesion usually shows unilocular or multilocular radiolucency and performs root resorption and tooth migration on the radiograph (24). In a recent study, it was reported that OKC and ameloblastoma lead to mesial-distal displacement of the impacted teeth more frequently compared with DC. OKC and ameloblastoma showed more aggressive growth pattern with higher rate of bony discontinuity and cortical bone expansion compared with DC (22).

Conclusion

We can say that this study is one of the few multicenter studies when viewed from this angle. In this study, statistically significant differences were found between age groups and migration of impacted tooth/teeth in conjunction with the lesion and histopathological diagnosis of the lesion. The migration of impacted tooth/teeth towards buccal direction was more common in the patients ≤30 years old, while lingual/palatal migration was more common in the patients 51≥ years old.

The clinical and radiographic features of lesions located in jaws show some similarity according to their growth patterns. Majority of these lesions are benign but certain lesions are aggressive and show a destructive growth pattern. These affect the treatment choice, surgical techniques, and post-operative recurrence rates. CBCT findings could help the surgeons' initial diagnosis, pre-operative surgery planning and treatment choice. Knowing all clinical, radiological, and histopathological features of the lesions provide the surgeon to reach the correct diagnosis, thus the doctors achieve high success in treatment with the right treatment plan.

Ethics

Ethics Committee Approval: The present cohort study was designed as a survey and was approved by the Clinical Research Ethics Committee of Ankara University Faculty of Dentistry (approval no: 36290600/63, date: 30.11.2015).

Informed Consent: Retrospective study.
Peer-review: Internally peer-reviewed.
Authorship Contributions
Surgical and Medical Practices: Z.F.Z., Z.A., E.B., S.K., D.G., İ.P., Concept: Z.F.Z., Z.A., E.B., S.K., D.G., İ.P., Design: Z.F.Z., Z.A., Y.T.K., C.D., E.B., S.K., D.G., İ.P., Data Collection or Processing: Z.F.Z., Z.A., N.H., Y.T.K., C.D., E.B., İ.S., M.İ., D.G., Analysis or Interpretation: N.H., Y.T.K., C.D., İ.S., N.B., Literature Search: Z.F.Z., Z.A., N.H., Y.T.K., C.D., N.B., Writing: Z.F.Z.

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

Financial Disclosure: The authors declared that this study received no financial support.

References

1. Kaushal N. Is radiographic appearance a reliable indicator for the absence or presence of pathology in impacted third molars? Indian J Dent Res 2012; 23: 298.

2. Stathopoulos P, Mezitis M, Kappatos C, Titsinides S, Stylogianni E. Cysts and tumors associated with impacted third molars: is prophylactic removal justified? J Oral Maxillofac Surg 2011; 69: 405-8.

3. Wood NK, Goaz PW, Schwartz LJ. Pericoronal radiolucencies. In: Wood NK, Goaz PW, editors. Differential diagnosis of oral lesions. 3rd ed. St Louis: Mosby; 1985. P. 357-8.

4. Cabbar F, Güler N, Comunoğlu N, Sençift K, Cöloğlu S. Determination of potential cellular proliferation in the odontogenic epithelia of the dental follicle of the asymptomatic impacted third molars. J Oral Maxillofac Surg 2008; 66: 2004-11.

5. Regezzi JA, Sciubba JJ. Cysts of the oral region. In: Regezi JA, Sciubba JJ, editors. Oral pathology; clinical pathologic correlations. 3rded. Philadelphia: WB Saunders Company; 1999. p. 288-322.

6. Deana NF, Alves N. Cone Beam CT in Diagnosis and Surgical Planning of Dentigerous Cyst. Case Rep Dent 2017; 2017: 7956041.

7. Scarfe WC, Farman AG, Sukovic P. Clinical applications of cone-beam computed tomography in dental practice. J Can Dent Assoc 2006; 72: 75-80.

8. Patil S, Halgatti V, Khandelwal S, Santosh BS, Maheshwari S. Prevalence of cysts and tumors around the retained and unerupted third molars in the Indian population. J Oral Biol Craniofac Res 2014; 4: 82-7.

9. Campbell JH. Pathology associated with the third molar. Oral Maxillofac Surg Clin North Am 2013; 25: 1-10, v.

10. Karabas HC, Ozcan I, Tekkesin MS, Tasyapan SA, Guray B, Atapek MM. Evaluation of Radiolucent Lesions Associated with Impacted Teeth: A Retrospective Study. Curr Med Imaging 2020; 16: 1332-9.

11. Kämmerer PW, Schneider D, Schiegnitz E, Schneider S, Walter C, Frerich B, et al. Clinical parameter of odontoma with special emphasis on treatment of impacted teeth-a retrospective multicentre study and literature review. Clin Oral Investig 2016; 20: 1827-35.

12. Kinard B, Hansen G, Newman M, Dennis P, Haeffs T, Perez S, et al. How well do we manage the odontogenic keratocyst? A multicenter study. Oral Surg Oral Med Oral Pathol Oral Radiol 2019; 127: 282-8.

13. El Khateeb SM, Abu-Hammad O, Fadel H, Dar-Odeh N. A Retrospective Analysis of Radiographic Jaw Findings in Young Women; Prevalence and Predictors. J Int Soc Prev Community Dent 2017; 7: 22-7.

14. Seyedmajidi M, Haghanifor S, Foroughi R, Hajiahmadi M, Mohammadpoor N. Histopathologic evaluation of normal radiolusent space around impacted third molar. J. Mashhad Dent 2011;35:99-106.

15. Gbotolorun OM, Olojede AC, Arotiba GT, Ladeinde AL, Akinwande JA, Bamgbose BO. Impacted mandibular third molars: presentation and postoperative complications at the Lagos University Teaching Hospital. Nig Q J Hosp Med 2007; 17: 26-9.

16. White SC, Pharoah MJ. Oral Radiology. Principles and Interpretation. 7th ed. Mosby: St Louis Missouri; 2004. p. 419.

17. Dunfee BL, Sakai O, Pistey R, Gohel A. Radiologic and pathologic characteristics of benign and malignant lesions of the mandible. Radiographics 2006; 26: 1751-68.

18. Curran AE, Damm DD, Drummond JF. Pathologically significant pericoronal lesions in adults: Histopathologic evaluation. J Oral Maxillofac Surg 2002; 60: 613-8.

19. Shoaee S, Khazaei P, Mashhadiabbas F, Varshosaz M, Sharifi F, HessarI H. Association between tooth impaction and odontogenic lesions: A matched case-control study. Med J Islam Repub Iran 2018; 32: 57.

20. Patil S, Halgatti V, Khandelwal S, Santosh BS, Maheshwari S. Prevalence of cysts and tumors around the retained and unerupted third molars in the Indian population. J Oral Biol Craniofac Res 2014; 4: 82-7.

21. Anand S, Kashyap B, Kumar GR, Shruthi BS, Supriya AN. Pericoronal radiolucencies with significant pathology: clinico-histopathologic evaluation. Biomed J 2015; 38: 148-52.

22. Lee JH, Kim SM, Kim HJ, Jeon KJ, Park KH, Huh JK. Characteristics of bony changes and tooth displacement in the mandibular cystic lesion involving the impacted third molar. J Korean Assoc Oral Maxillofac Surg 2014; 40: 225-32.

23. Henien M, Sproat C, Kwok J, Beneng K, Patel V. Coronectomy and dentigerous cysts: a review of 68 patients. Oral Surg Oral Med Oral Pathol Oral Radiol 2017; 123: 670-4.

24. Whaites E. Essentials of dental radiography and radiology. 3rd ed. Churchill Livingstone Elsevier: China; 2003. p. 296-7.

Original Article / Özgün Araştırma

Comparison of Long-term Clinical Outcomes of the Preferred Surgical Techniques in Secondary Hyperparathyroidism Cases

Sekonder Hiperparatiroidi Olgularında Tercih Edilen Cerrahi Tekniklerinin Uzun Dönem Klinik Sonuçlarının Karşılaştırılması

Emre Karakaya, Murathan Erkent

Başkent University Faculty of Medicine, Department of General Surgery, Ankara, Turkey

Address for Correspondence/Yazışma Adresi:
Emre Karakaya MD,
Başkent University Faculty of Medicine,
Department of General Surgery, Ankara, Turkey
Phone : +90 312 203 68 68
E-mail : dremrekarakaya@gmail.com
ORCID ID: orcid.org/0000-0002-4879-7974

Abstract

Objective: Secondary hyperparathyroidism (SHPT), that progresses with the deterioration of calcium-phosphorus metabolism detected in chronic kidney disease patients. Recurrence of SHPT may result in the recurrence of symptoms. Our aim was to compare the 5-year clinical results of autotransplantation after total parathyroidectomy (PTX-AT) with subtotal parathyroidectomy (SPTX).

Materials and Methods: We analyzed 140 patients retrospectively from January 2000 and October 2020 who were operated due to SHPT. Clinical and demographic characteristics of the patients, preoperative and postoperative (1st day, 1st month, 6th month, 1st year, 5th years) serum PTH, calcium (Ca), phosphorous (P) values and length of hospital stay (LOS) were compared in terms of operations performed. The Shapiro-Wilks test was used for analyzing normally distributed datas. Mann-Whitney U test used to evaluate of comparison of numerical data. Fisher's Exact or chi-square test was used for ratio comparisons or correlation. P<0.05 was considered statistical significance level.

Results: Of these 140 patients, 106 (75.7%) had SPTX. On the other hand, 34 patients (24.3%) underwent PTX-AT surgery. When the groups were compared in terms of the gender, age and comorbidities, the differences were statistically significant. Additionally, no statistically significant difference was found between the groups in terms of postoperative complications (p=0.206). The difference between the weights and sizes of the parathyroid glands removed between the operation groups was not statistically significant (p=0.751, p=0.176). The difference was not statistically significant between the groups in terms of PTH, Ca and P levels measured. LOS was statistically significantly longer in PTX-AT patients (p=0).

Conclusion: The surgical methods in the treatment of SHPT have no difference each other. Depending on the surgeon's preference, both surgical methods can be safely applied with high success rates.

Öz

Amaç: Sekonder hiperparatiroidi (SHPT) kronik böbrek yetmezliği hastalarında (CKD) görülen bozulmuş kalsiyum-fosfor metabolizması ile karakterizedir. SHPT'nin rekürrensi, semptomların tekrarlamasına neden olabilir. Bu çalışmanın amacı subtotal paratiroidektomi (SPTX) ile total paratiroidektomi ile birlikte ototransplantasyon (PTX-AT) yöntemlerinin 5 yıllık klinik sonuçlarını karşılaştırmaktır.

Gereç ve Yöntemler: SHPT nedeni ile Ocak 2000 ile Ekim 2020 tarihleri arasında ameliyat edilen 140 hastayı geriye dönük olarak taradık. Hastaların klinik ve

demografik özellikleri, ameliyat öncesi ve ameliyat sonrası (1. gün, 1. ay, 6. ay, 1. yıl, 5. yıl) serum PTH, kalsiyum (Ca), fosfor (P) değerleri ve hastane yatış süreleri yapılan ameliyatlar açısından karşılaştırıldı. Veri normal dağılımları Shapiro-Wilks testi ile değerlendirildi. Araştırma gruplarına göre sayısal ölçümlerin karşılaştırılması veri dağılımına uygun olarak Mann–Whitney U test ile değerlendirildi. Araştırma gruplarına göre oran karşılaştırmaları veya ilişki araştırmaları ki-kare veya Fisher Exact test ile araştırıldı. P<0,05 değeri istatistiksel olarak anlamlı kabul edildi.

Bulgular: Toplam 140 hastanın 106 (75,7%)'sına SPTX, 34 (24,3%)'üne ise PTX-AT ameliyatı yapılmıştır. Hastaların yaşları, cinsiyetleri ve komorbiditeleri açısından gruplar arası istatistiksel olarak anlamlılık saptanmamıştır. Gruplar ameliyat sonrası komplikasyonlar açısından karşılaştırıldığında fark istatistiksel olarak anlamlı değildi (p=0,206). Ameliyat grupları arasında çıkarılan paratiroid bezlerinin ağırlıkları ve boyutları arasındaki fark istatistiksel olarak anlamlı değildi (p=0,751, p=0,176). Gruplardaki PTH, Ca ve P değerleri arasındaki fark istatistiksel olarak anlamlı değildi. PTX-AT grubunda hastane yatış süresi istatistiksel olarak anlamlı şekilde daha uzun saptandı (p=0).

Sonuç: CKD hastalarında SHPT'nin cerrahi tedavisinde SPTX ile TPX-AT yöntemleri arasında herhangi bir fark yoktur. Cerrahın tercihine göre her iki cerrahi yöntem de yüksek başarı oranları ile güvenle uygulanabilir.

Introduction

Secondary hyperparathyroidism (SHPT) can be seen in approximately 85% of patients with chronic kidney disease (CKD). This disease needs attention during treatment period as it can have fatal complications (1). It is manifested by impaired calcium (Ca)-phosphorus (P) metabolism, calcifications in the vascular areas and disruption of bone turnover (2). Its pathophysiology involves an abnormal parathyroid hormone (PTH) response to hyperphosphatemia and hypercalcemia, coupled with 1,25-dihydroxyvitamin D deficiency, in end-stage renal disease (3). It may cause itching, bone pain, and cardiovascular complications that may negatively affect patient's social life. Treatment of SHPT composed of vitamin D preparations, calcimimetics and Ca-free phosphate binders (3). In cases resistant to medical treatment, parathyroidectomy (PTX) is an effective treatment to reduce the PTH level and equilibrate calcium levels and P levels. The most commonly used surgical methods in the treatment of SHPT are parathyroidectomy subtotally (SPTX) or auto-transplantation after total parathyroidectomy (PTX-AX) of a part of the normally appearing gland into the brachioradial muscle or sternocleidomastoid muscle from the neck. Disease recurrence, surgical morbidity, and the risk of hypoparathyroidism should be considered when selecting a surgical method. While the risk of hypocalcemia and permanent hypoparathyroidism is lower after SPTX, the risk of recurrence and thus the risk of the repeatiton of the surgical procedureis lower with PTX-AX (4). For these reasons, the debate on PTX techniques is still ongoing. Recurrence of SHPT may cause recurrence of symptoms and lead to increased morbidity and mortality secondary to cardiovascular problems as a result of increased PTH levels. In this study, our purpose was analyze the 5-year outcomes of PTX and to compare SPTX and PTX-AT techniques in CKD patients with SHPT.

Materials and Methods

In this study, we evaluated 348 patients who was performed surgical procedure for hyperparathyroidism between January 2000 and October 2020. We retrospectively reviewed the patients medical records. Among such patients, those with primary hyperparathyroidism or tertiary hyperparathyroidism, and those whose follow-up had not been performed at our center were excluded (Figure 1). So, the study included 140 patients. The demographic characteristics of the patients were analyzed. In addition, preoperative serum PTH, Ca, P levels were recorded. The corrected Ca levels were
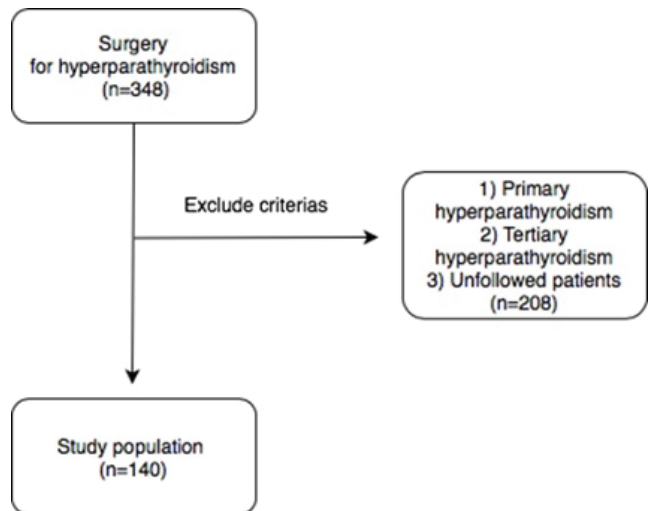
Figure 1. Flowchart of study design

determined on the basis of the serum albumin levels [corrected Ca level (mg/dL) = Total Ca level (mg/dL) + [0.8×(4.0-albumin (g/L)]. Serum PTH, Ca, P levels at first postoperative day, first month, the sixth month, first year, and fifth-year were recorded. The normal laboratory ranges were determined as Ca: 8,8-10 mg/dL, PTH: 12-88 pg/mL, P: 2.3-4.7 mg/dL. The largest gland was evaluated by preoperative imaging studies, and weight the excised parathyroid glands weights were measured and recorded. After surgery, the recurrent cases within the first six months were considered as persistence, and the recurrent cases after that period were considered as recurrence. The study groups were assessedin terms of clinical, demographic, biochemical, and pathological parameters. Also, comparasion was performed between the groups with respect to length of hospital stay (LOS). Additionally, the groups were compared in terms of postoperative complications, recurrence, and persistence rates.

Surgical Indications

Surgical indications were determined as failed medical therapy and/or dialysis; persistence of symptoms like bone pain and itching; a CxP product greater than 70; a high PTH level coupled with a Ca level above 11 mg/dL; parathyroid gland size greater than 1 cm or 500 mm^3 in imaging studies; and increased vascularity of the parathyroid gland in preoperative Doppler ultrasonography.

Surgical Technique

The patients were taken on dialysis the day before the surgery to eliminate electrolyte imbalances. The surgeon performed subtotal or total PTX operations according to his own decision. All surgical procedures were performed with the patient's head in extension, using the Kocher's incision under general anesthesia. Unilateral or bilateral thyroidectomy operation was carried out if a thyroid pathology was detected at the preoperative period or the parathyroid gland could not be visualized. Bilateral neck exploration was used in all surgical operations. In order to verify that the excised specimens were the true parathyroid glands, a frozen examination was performed. In addition, serum PTH level was intraoperatively measured 20 minutes after parathyroid gland excision to demonstrate an 80% drop in the PTH level compared to the preoperative level as an indication of procedural success. In patients who underwent SPTX, half of the

parathyroid gland with the most normal appearance was spared if it was smaller than 1 cm^3, or 1/3 to 1/4 of it if it was larger than 1 cm^3. In patients who underwent PTX-AT, after removing all parathyroid glands, the normal-appearing parathyroid tissue was cut into 1 mm pieces and auto-transplanted into the musculus sternocleidomastoideus or the musculus brachioradialis. We preferred the non-dominant forearm for auto-transplantation. After placing a minivac into the surgical site depending on the hemostatic status, the operation was ended. We removed the drains on the first day after surgery.

Statistical Analysis

The study was intended to have a retrospective design. For numbers and percentages we used categorical variables. Mean ± standard deviation or median (minimum-maximum) were used for numerical variables. The Mann-Whitney U test was used in the comparison of two groups that did not show normal distribution. Chi-square test and Fisher's Exact test were used to compare the proportions between the study groups. Statistical significance was set at $p<0.05$.

Results

Among 140 patients, 106 (75.7%) underwent SPTX, and 34 (24.3%) PTX-AT procedure. Seventy-eight (55.7%) patients gender were male, and 62 (44.3%) patients gender were female. The mean age of the study population was 44.9 (21-77) years. At the time of operation, 18 (12.9%) patients had Type 2 diabetes mellitus (DM); 45 (32.1%) had hypertension (HT); and 34 (24.3%) had coronary artery disease (CAD) as a comorbidity. The difference was not statistically between the groups with respect to age, gender distribution, and comorbidities (Table 1). A comparison of the study groups by postoperative complications showed no significant difference (p=0.206). Hoarseness was not observed in the SPTX group, while 2 (1.9%) patients in the same group developed no hematoma, and 16 (15.1%) patients developed no hypocalcemia. None of the patients who developed hematoma required re-operation. In the PTX-AT group, 1 (3%) patient suffered hoarseness and 3 (9.1%) patients suffered hypocalcemia while none of them developed a hematoma. When the groups were compared in terms of LOS, it was 2.09 (1-10) days in the SPTX group and 6 (1-12) days in the PTX-AT

group (p=0). Postoperative pathology examinations revealed no malignancy in any specimen. The mean parathyroid gland weight was 1895,08 (26-8900) mgr, and the mean parathyroid gland size was 14.06 (5-33) mm. There was a difference, but not significant, in terms of mean weight or mean size of the excised parathyroid glands (p=0.751, p=0.176, respectively) (Table 2).

Biochemical analysis revealed that the mean preoperative PTH level was 1621 (6.3-3573); the mean Ca level was 9.8 (6-12.9), and the mean P level was 5.45 (0.6-9.3). There was a difference between surgical techniques in terms of the levels of these parameters, but it was not significant. Similarly, the study groups were comparable in terms of PTH, Ca, P levels measured on the first day, first month, the sixth month, first year, and fifth-year postoperatively (Table 3).

Four (3.8%) patients in the SPTX group were found to have persistence, and 8 (7.5%) of them were found to have a recurrence. In the PTX-AT group, 2 (5.9%) patients had persistence, and 7 (20.6%) patients had a recurrence. No statistically difference was found in

terms of persistence and recurrence rates (p=0.678, p=0.064 respectively). While 14 (10%) of those who developed recurrence or persistence were re-operated, 126 (90%) patients continued to receive medical treatment.

Discussion

According to our study results, SPTX and PTX-AT are the similar surgical treatment method in terms of postoperative complications, pathological diagnosis, biochemical tests, or persistence rates during a long-term follow-up period.

Previous studies in the literature have reported that alterations in Ca and P values in patients with SHPT lead to increased mortality rates over the long term (5-7). Therefore, SHPT should be brought under control, either medically or surgically. We take the values defined by Kidney Disease: Impraving Global Outcomes as the reference for the treatment of SHPT at our center. However, PTH targets can be attained in only 22% of medically managed patients. Furthermore, it is well known that part of medically managed patients may show treatment incompliance (8,9). For

Table 1. Demographic and clinical parameters of the study population

	SPTX (n=106)	PTX + AT (n=34)	p-value
Age	70.58*	70.25*	0.967
Sex (female/male)	50/56	12/22	0.225
Comorbidity			
DM	12 (11.3%)	6 (17.6%)	0.338
HT	34 (32.1%)	11 (32.4%)	0.976
CAD	24 (22.6%)	10 (29.4%)	0.423

SPTX: Subtotal parathyroidectomy, PTX-AT: Total parathyroidectomy with auto-transplantation, DM: Diabetes Mellitus, HT: Hypertension, CAD: Coronary artery disease, *Mann-Whitney U test-mean rank level

Table 2. Comparison of specimens in terms of weight and size

	SPTX (n=106)	PTX + AT (n=34)	p-value
The weight of the parathyroid gland (mgr)	70.13*	71.66*	0.848
The size of the parathyroid gland (mm)	71*	68.94*	0.796

SPTX: Subtotal parathyroidectomy, PTX-AT: Total parathyroidectomy with auto-transplantation, *Mann-Whitney U test-mean rank level

Table 3. Comparison of the biochemical parameters between subtotal parathyroidectomy and auto-transplantation after total parathyroidectomy groups

		SPTX (n=106)	PTX-AT (n=34)	p-value
PTH level (pg/mL)	1 day	70.7*	69.87*	0.917
	1 months	70.3*	71.12*	0.919
	6 months	69.47*	73.71*	0.596
	1 year	70.44*	70.68*	0.977
	5 years	69.54*	73.49*	0.622
Calcium level (mg/dL)	1 day	72.37*	64.66*	0.334
	1 months	68.73*	76.03*	0.360
	6 months	74.02*	59.51*	0.069
	1 year	73.50*	61.15*	0.122
	5 years	73.69*	60.56*	0.100
Phosphorus level (mg/dL)	1 day	68.24*	77.54*	0.244
	1 months	71.24*	68.19*	0.703
	6 months	66.92*	81.66*	0.065
	1 year	68.79*	75.82*	0.379
	5 years	66.72*	82.28*	0.052

SPTX: Subtotal parathyroidectomy, PTX-AT: Total parathyroidectomy with auto-transplantation, PTH: Parathormone, *Mann-Whitney U test-mean rank level

these reasons, surgical treatment is recommended for SHPT cases in a majority of the current guidelines (4,10). So far, three surgical techniques have been defined for SHPT treatment: SPTX, TPX-AT, and TPX. Since TPX causes complications (severe hypocalcemia, adynamic bone diseases), it is avoided today as much as possible (11). Hence, we did not use the TPX technique in any surgically managed SHPT patient at our center.

After PTX operations patients may develop some complications including hypocalcemia, hoarseness due to recurrent laryngeal nerve injury, or hematoma formation. While hypocalcemia may occur at a higher rate due to the elimination of osteoclastic activity or the hungry bone syndrome at the postoperative period, other complications such as hoarseness and hematoma occur more rarely, at rates ranging between 1% and 5% (12,13). Similar with the other studies, we found that only 1-3% of the cases suffered complications like hoarseness and hematoma. Postoperative hypocalcemia is usually temporary and occurs due to ischemia of the remaining parathyroid glands. It is easily treated with intravenous Ca and vitamin D. In a study, Schneider et al. (13) investigated the relationship between the surgical procedure and postoperative hypocalcemia, but found no difference between surgical techniques. Our study similarly showed a greater rate of hypocalcemia (9-15%) than other complications although there was no significant difference. In our patients, all episodes of postoperative hypocalcemia were temporary and treated medically. Although adequate PTH lowering is usually achieved early after the surgery, PTH, Ca, and P levels may impair in the postoperative long term period. This is caused by hyperplasia of the remnant parathyroid gland secondary to long-term hemodialysis in patients undergoing SPTX; it may also occur in patients undergoing TPX-AT due to hyperplasia of the implanted parathyroid gland in the long term due to implantation of an excess amount of parathyroid tissue. In addition, regardless of the surgery, if there is an ectopic parathyroid gland, it may result in abnormalities of PTH, Ca, and P levels in later periods (14). In the other studies no significant difference was found between both methods in terms of PTH, Ca, and P levels in long term (15-18). Likewise,

our study did not show any significant difference during a 5-year follow-up period between groups.

In the literature, increased bone turnover, low preoperative serum Ca and postoperative complications are shown as the determinants of LOS (19,20). In these patients, after PTX, low serum Ca levels are detected and usually after surgery iv. Ca replacement is required. We did not find significant difference between the groups in terms of serum Ca and postoperative complications. However, we found significant difference in terms of LOS. LOS was significantly higher in the PTX-AT group. We think that this difference is due to the time required for the blood supply of parathyroid tissue, which autotransplanted during surgery, in the PTX-AT group.

In surgically treated SHPT cases persistence is defined as the lack of any postoperative fall in serum PTH level. Recurrence, on the other hand, is defined as an elevation of serum PTH level 6 months or later after surgery. Although a study by Melck et al. (21) showed somewhat higher recurrence and persistence rates in TPX-AT cases, that difference did not reach statistical significance. Higher rates in TPX-AT cases may result from a greater amount of implanted parathyroid gland (22). In accordance with the literature, our study found higher persistence and recurrence rates in the TPX-AT group despite failing to demonstrate any statistical significance.

As for the limitations of our study, the important limitations are that, number of patients is small and the unequal distribution of the groups was unequal. In addition, due to the retrospective design of our study, we could not reach demographic and follow-up datas of some patients. More detailed and effective study results may be obtained by a prospective multi-center study with a larger study population.

Conclusion

SHPT is an important, potentially fatal disorder that must be treated accordingly in patients with CKD. There is no difference between SPTX and TPX-AT techniques in treatment of SHPT. Depending on the surgeon's experience, both surgical techniques can be used safely with high success rates.

Ethics

Ethics Committee Approval: The present cohort study was designed as a survey and was approved by the Clinical Research Ethics Committee of Başkent University (no: KA20/485, date: 05.01.2021).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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

Financial Disclosure: The authors declared that this study received no financial support.

References

1. Steffen L, Moffa G, Müller PC, Oertli D. Secondary hyperparathyroidism: recurrence after total parathyroidectomy with autotransplantation. Swiss Med Wkly 2019; 149: w20160.

2. Zhang Y, Lu Y, Feng S, Zhan Z, Shen H. Evaluation of laboratory parameters and symptoms after parathyroidectomy in dialysis patients with secondary hyperparathyroidism. Ren Fail 2019; 41: 921-9.

3. Lau WL, Obi Y, Kalantar-Zadeh K. Parathyroidectomy in the Management of Secondary Hyperparathyroidism. Clin J Am Soc Nephrol 2018; 13: 952-61.

4. Kidney Disease: Improving Global Outcomes (KDIGO) CKD-MBD Work Group. KDIGO clinical practice guideline for the diagnosis, evaluation, prevention, and treatment of Chronic Kidney Disease-Mineral and Bone Disorder (CKD-MBD). Kidney Int Suppl 2009; 113: S1-130.

5. Mermel LA, Allon M, Bouza E, Craven DE, Flynn P, O'Grady NP, Raad II, Rijnders BJ, Sherertz RJ, Warren DK. Clinical practice guidelines for the diagnosis and management of intravascular catheter-related infection: 2009 Update by the Infectious Diseases Society of America. Clin Infect Dis 2009; 49: 1-45.

6. Hajjej Z, Nasri M, Sellami W, Gharsallah H, Labben I, Ferjani M. Incidence, risk factors and microbiology of central vascular catheter-related bloodstream infection in an intensive care unit. J Infect Chemother 2014; 20: 163-8.

7. Youn SH, Lee JC, Kim Y, Moon J, Choi Y, Jung K. Central Venous Catheter-Related Infection in Severe Trauma Patients. World J Surg 2015; 39: 2400-6.

8. Moe SM, Chertow GM, Coburn JW, Quarles LD, Goodman WG, Block GA, et al. Achieving NKF-K/DOQI bone metabolism and disease treatment goals with cinacalcet HCl. Kidney Int 2005; 67: 760-71.

9. Palmer SC, Nistor I, Craig JC, Pellegrini F, Messa P, Tonelli M, et al. Cinacalcet in patients with chronic kidney disease: a cumulative meta-analysis of randomized controlled trials. PLoS Med 2013; 10: e1001436.

10. Tominaga Y, Matsuoka S, Uno N. Surgical and medical treatment of secondary hyperparathyroidism in patients on continuous dialysis. World J Surg 2009; 33: 2335-42.

11. Chen J, Jia X, Kong X, Wang Z, Cui M, Xu D. Total parathyroidectomy with autotransplantation versus subtotal parathyroidectomy for renal hyperparathyroidism: A systematic review and meta-analysis. Nephrology (Carlton) 2017; 22: 388-96.

12. Koonsman M, Hughes K, Dickerman R, Brinker K, Dunn E. Parathyroidectomy in chronic renal failure. Am J Surg 1994; 168: 631-4; discussion 634-5.

13. Schneider R, Slater EP, Karakas E, Bartsch DK, Schlosser K. Initial parathyroid surgery in 606 patients with renal hyperparathyroidism. World J Surg 2012; 36: 318-26.

14. Tevfik A, Karakayalı FY, Yabanoğlu H, Moray G. Sekonder hiperparatiroidizm olgularında kriyoprezervasyonsuz total paratiroidektomi/önkol ototransplantasyon tekniğinin uzun dönem sonuçları. Cukurova Med J 2018; 43: 129-38.

15. Nicholson ML, Veitch PS, Feehally J. Parathyroidectomy in chronic renal failure: comparison of three operative strategies. J R Coll Surg Edinb 1996; 41: 382-7.

16. Sicard GA, Anderson CB, Hruska KA, Martin KJ, Etheredge EE. Parathormone levels after subtotal and total (autotransplantation) parathyroidectomy for secondary hyperparathyroidism. J Surg Res 1980; 29: 541-5.

17. Hargrove GM, Pasieka JL, Hanley DA, Murphy MB. Short- and long-term outcome of total parathyroidectomy with immediate autografting versus subtotal parathyroidectomy in patients with end-stage renal disease. Am J Nephrol 1999; 19: 559-64.

18. Gagné ER, Ureña P, Leite-Silva S, Zingraff J, Chevalier A, Sarfati E, et al. Short- and long-term efficacy of total parathyroidectomy with immediate autografting compared with subtotal parathyroidectomy in hemodialysis patients. J Am Soc Nephrol 1992; 3: 1008-17.

19. Stefanova D, Ullmann TM, Limberg J, Moore M, Beninato T, Zarnegar R, et al. Risk Factors for Prolonged Length of Stay and Readmission After Parathyroidectomy for Renal Secondary Hyperparathyroidism. World J Surg 2020; 44: 3751-60.

20. Alsafran S, Sherman SK, Dahdaleh FS, Ruhle B, Mercier F, Kaplan EL, et al. Preoperative calcitriol reduces postoperative intravenous calcium requirements and length of stay in parathyroidectomy for renal-origin hyperparathyroidism. Surgery 2019; 165: 151-7.

21. Melck AL, Carty SE, Seethala RR, Armstrong MJ, Stang MT, Ogilvie JB, et al. Recurrent hyperparathyroidism and forearm parathyromatosis after total parathyroidectomy. Surgery 2010; 148: 867-73; discussion 873-5.

22. Yumita S. Intervention for recurrent secondary hyperparathyroidism from a residual parathyroid gland. Nephrol Dial Transplant 2003; 18 Suppl 3: iii62-4.

Original Article / Özgün Araştırma

Potential Correlation Between Complicated Root Canal Morphologies of Mandibular Central Incisors and Radix Entomolaris in Mandibular Permanent First Molars in a Turkish Population: A Cone Beam Computed Tomography Analysis

Türk Popülasyonunda Mandibular Santral Kesici Dişlerdeki Komplike Kök Kanal Morfolojisi ile Mandibular Birinci Molar Dişlerdeki Radix Entomolaris Arasındaki Potansiyel Korelasyon: Konik Işınlı Bilgisayarlı Tomografi Analizi

● Duygu Göller Bulut[1], ● Zeliha Uğur Aydın[2]

[1]Bolu Abant İzzet Baysal University Faculty of Dentistry, Department of Oral and Maxillofacial Radiology, Bolu, Turkey
[2]Bolu Abant İzzet Baysal University Faculty of Dentistry, Department of Endodontics, Bolu, Turkey

Address for Correspondence/Yazışma Adresi:
Duygu Göller Bulut Assoc. Prof.,
Bolu Abant İzzet Baysal University Faculty of Dentistry, Department of Oral and Maxillofacial Radiology, Bolu, Turkey
Phone : +90 554 640 66 40
E-mail : duygugoller@hotmail.com
ORCID ID: orcid.org/0000-0003-4260-2520

Abstract

Objective: This study investigated the potential correlation between the presence of complicated canal configurations of mandibular central incisors (ManCIs) and the existence of radix entomolaris (RE) in the permanent mandibular first molars (ManFMs) in a Turkish population.

Materials and Methods: The prevalence of complicated ManCI root canal configurations and the prevalence of RE in ManFMs were investigated using cone beam computed tomography images of 534 patients. The correlation between complicated root canal configurations and the presence of RE was evaluated using multivariable logistic regression analysis.

Results: RE was observed in 18.6% (199/1068) of ManFMs, and 22.8% (244/1068) of ManCIs had complicated root canal configurations. There was a positive correlation between complicated ManCI root canal configurations and the presence of RE ($p<0.001$, $r=0.248$).

Conclusion: Within the limitations of the current study, the presence of RE was associated with the existence of complicated ManCI root canal configurations in this Turkish population.

Öz

Amaç: Bu çalışmada Türk popülasyonu üzerinde mandibular santral kesici dişlerde (ManSK) komplike kanal konfigürasyonunun varlığı ile daimi mandibular birinci azı dişlerinde (ManBM) radix entomolaris'in (RE) varlığı arasındaki potansiyel korelasyon araştırıldı.

Gereç ve Yöntemler: Toplamda 534 hastaya ait konik ışınlı bilgisayarlı tomografi görüntüsü üzerinde ManSK'lerde komplike kök kanal konfigürasyon prevalansı ve ManBM'lerde RE prevalansı araştırıldı. Karmaşık kök kanal konfigürasyonları ile RE varlığı arasındaki korelasyon çok değişkenli lojistik regresyon analizi kullanılarak değerlendirildi.

Bulgular: ManBM'lerin %18,6'sında (199/1068) RE gözlendi ve ManSK'lerin %22,8'inde (244/1068) karmaşık kök kanal konfigürasyonu saptandı. ManSK'lerde

karmaşık kök kanal konfigürasyonu varlığı ile ManBM'larda RE varlığı arasında pozitif bir korelasyon bulundu (p<0,001, r=0,248).
Sonuç: Mevcut çalışmanın limitasyonları dahilinde, Türk popülasyonunda ManBM'lerda RE varlığı ile ManSK'lerde karmaşık kök kanal konfigürasyonunun varlığı ilişkili bulundu.

Introduction

For successful endodontic treatment, the root canal system must be completely chemo-mechanically cleaned, prepared, and obturated (1). As is well known, these processes, which are essential for endodontic treatment, are affected by root canal anatomy (2). Therefore, knowledge of the main canals, additional canals or other anatomical variations for the elimination of incomplete instrumentation causing apical periodontitis may increase the success of endodontic treatment (1-3).

Mandibular permanent first molars (ManFMs) are frequently treated endodontically and usually have two roots, (distal and mesial) and three canals (two mesial and one distal). A common variation in ManFMs is the presence of a third root, which may be either buccal (radix paramolaris) or lingual [radix entomolaris (RE)] (1,4). This additional root may frequently cause procedural errors during endodontic treatment because it is smaller and separate from the other roots or fused with these roots. Radix paramolaris and RE are also curved. Previous studies showed that this anatomical variation needed to be considered in various dental procedures, such as surgical extractions, periodontal treatment, orthodontic treatment, and root canal treatment (1,4).

Mandibular central incisors (ManCIs) usually have a single root and a single canal. However, an additional canal or root is a common anatomical variation among ManCIs (2). To ensure the success of endodontic treatment and other dental procedures, extensive evaluations of this anatomical variation should be conducted prior to treatment (2,3).

Many studies have investigated the root canal anatomy in different populations using extracted teeth and periapical and panoramic radiographs (5,6). Although periapical and panoramic radiographs are important diagnostic tools for evaluating root canal configurations, they are not completely reliable due to limitations, such as distortion and superposition in the images obtained. Furthermore, studies of root canal configurations of extracted teeth are not always possible for legal and ethical reasons (6). Recently, cone-beam computed tomography (CBCT) has been widely used in dentistry because it allows clinicians to display an area in three different planes. The combination of CBCT images in the sagittal, coronal, and axial section eliminates the superposition of anatomical structures, giving the clinician a more complete view of the true three-dimensional morphology of root canal systems (7,8).

In the literature it was reported that the existence of RE in ManFMs may be associated with the presence of additional canals in ManCIs (9). Thus far, no study has investigated this association in a Turkish population. The aim of this study was to investigate the association between the existence of RE in ManFMs and the presence of complicated root canal configurations of ManCIs in a Turkish population.

Materials and Methods

Study Group

Due to the retrospective design of the study, the consent was not required from the patients. The study was approved by the Local Ethics Committee of Bolu Abant İzzet Baysal University (decision no: 2018/132 date: 06.09.2018). The study was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. The CBCT images included in this study were obtained from the Faculty of Dentistry of Bolu Abant İzzet Baysal University. In total, 1,250 CBCT images taken between 2014 and 2018 were obtained. Only CBCT images of good image quality in which the root canal configurations and canal orifices could be easily determined were included in the study. Only teeth without dental anomalies, periapical pathologies, root resorption, previous hemisection or root amputation treatment, large metallic restorations, coronal or post-core restorations, and root canal treatment were included. In all the included ManFMs and ManCIs, complete root formations were present bilaterally.

The final group included images obtained from 534 patients [319 females (59.7%) and 215 males (40.3%)] and 1,068 ManFMs and 1,068 ManCIs.

Imaging Procedures

The CBCT scans were obtained using an I-Cat imaging system (Imaging Sciences International, Hatfield, PA, USA) at 120 kVp and 15 mA, with a voxel size of 0.2 mm and an exposure time of 8.9 sec.

The presence or absence of RE and the existence of complicated ManCI canal configurations, including the type of configuration, were investigated using i-CAT vision Q 1.9 imaging software (Imaging Sciences International LLC. Hatfield, PA, USA). Tomography slices of 0.2 mm in the coronal, axial, and sagittal views were produced. To determine the number of canals and roots and the canal configurations in axial tomography slices, we investigated all sections throughout from the bottom of the pulp chamber to the root apex.

Morphological Analysis and Classification

Two observers evaluated all the CBCT images: an oral and maxillofacial radiologist with 6 years of experience and an endodontist with 6 years of experience. Observer calibration was achieved by having the two observers evaluate 20% of the CBCT scans at the beginning of the study. Intra-observer reliability was assessed by calculating the correlation coefficients (for observer one and observer two; 0.857 and 0.861, respectively). The observers were permitted to use image enhancement filters to change the intensity, contrast, or brightness of the images. A consensus was established in cases where there was inconsistency between the observers in the determination of morphological differences. Interobserver reliability was assessed when all the measurements were performed. The correlation coefficient was 0.908.

The presence of RE in the ManFMs was evaluated according to previous studies (9-11). The RE status of the patients was classified as follows (Figure 1 A-D):

No RE: RE was not found in either the left or right side;

Unilateral RE: RE was found in just one ManFM on either the left or right side, and no RE was detected in the other ManFM on the other side;

Bilateral RE: RE was found in both the right and left sides of ManFMs.

To determine the root canal configurations of ManCIs, a series of axial images and cross-sectioned images were examined from the cemento-
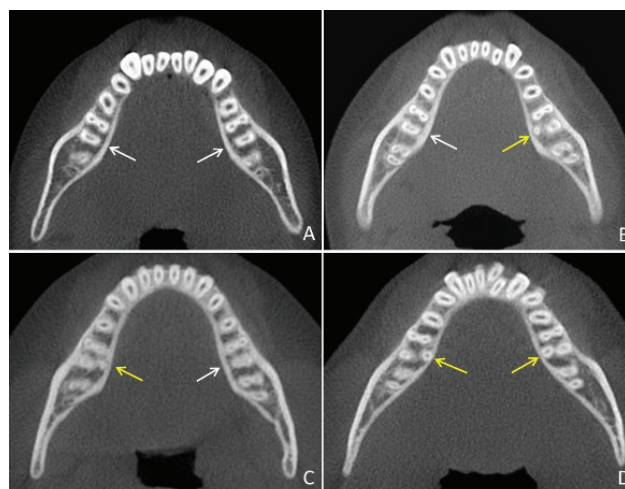
Figure 1. Axial CBCT images. (A) No RE, (B) unilateral RE on the left side, and (C) unilateral RE on the right side. (D) Bilateral RE (yellow arrows denote RE; white arrows denote no RE)

RE: Radix entomolaris, CBCT: Cone beam computed tomography

enamel junction to the root apex. The ManCI canal configurations were then classified in accordance with a system used in previous studies (9,12), as follows:

A single canal: the presence of one root and one canal.

A complicated canal: the occurrence of more than one root and canal.

The symmetric distribution arrangement of complicated ManCI canals was categorized as follows: bilateral single, unilateral complicated or bilateral complicated (Figure 2 A-C).

The root canal configuration of the teeth was classified according to Vertucci's classification (13).

Statistical Analysis

In each analysis, frequencies and percentages were calculated at tooth and patient levels. The chi-square test was used for examining differences in categorical variables, such as side (left/right) and sex (male/female), root canal configuration (single/complicated), and RE group (no RE, unilateral RE, or bilateral RE) (9). To assess the effect on the canal configurations of ManCIs, a multivariate logistic regression analysis was used and was simultaneously adjusted for other variables (side, age, and sex). The statistical analysis was performed using SPSS for Windows (Version 22.0; SPSS, Inc., Chicago, IL, USA). The statistically significant level was set at p<0.05.

Results

As shown by the results, 18.6% (199/1,068) of ManFMs had RE, and 22.8% (244/1,068) of ManCIs had a complicated root canal configuration. Only Vertucci's type I and type III canal configurations were
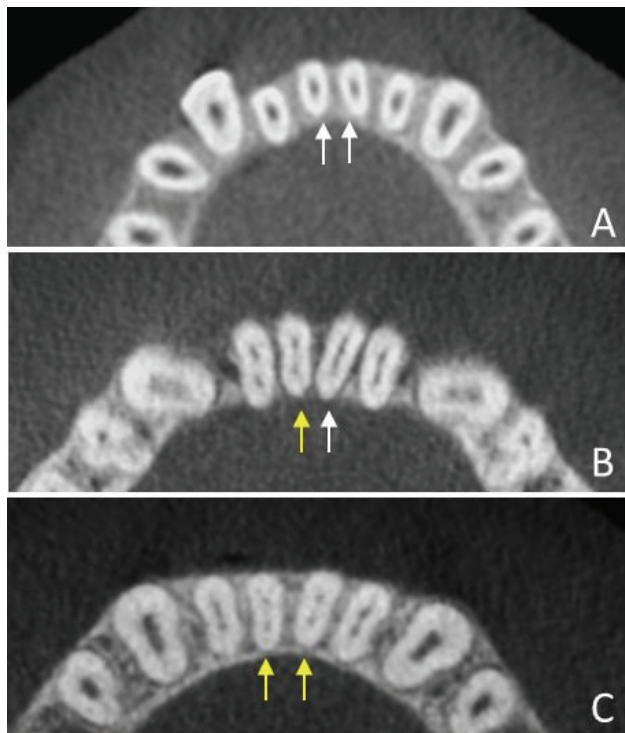
Figure 2. The root canal configuration of a ManCI in an axial CBCT image. (A) Bilateral single root canal configuration, (B) unilateral complicated root canal configuration, and (C) bilateral complicated root canal configuration (yellow arrows show RE; white arrows show no RE)

RE: Radix entomolaris, CBCT: Cone beam computed tomography, ManCIs: Mandibular central incisors

seen in ManCIs (Figure 3). In 74.2% of ManCIs, the canal configuration was type I, and it was type III in 25.8% of ManCIs.

Based on the CBCT images, 11.7% (63/534) of the patients had bilateral RE, and 12.1% (65/534) had unilateral RE (Figure 4A). Among the patients, 15.9% (86/534) had ManCIs with a bilateral complicated canal configuration, 73% (394/534) had ManCIs with a bilateral single canal, and 10% (54/534) had ManCIs with a unilateral complex canal configuration (Figure 4B).

Table 1 shows the prevalence of RE in ManFMs and complicated ManCI canal configurations according to side and sex. The prevalence of RE was higher in males (24.7%) than in females (13.8%) ($p=0.002$). The ratios of complicated ManCI canal configurations and RE in ManFMs were similar on the right and left sides ($p=0.215$ and $p=0.753$, respectively).
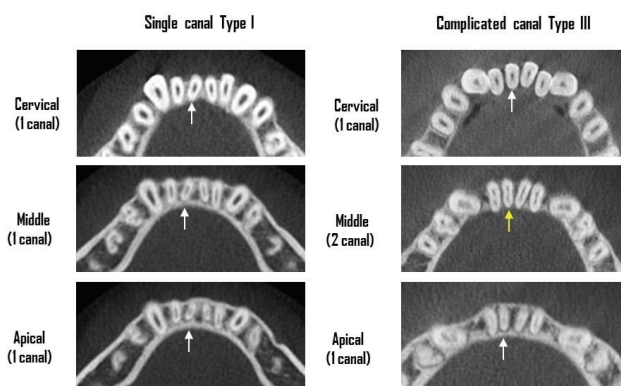
Figure 3. The root canal configuration of ManCIs according to Vertucci's classification type I and type III (white arrow: a single canal; yellow arrow: two canals)

ManCIs: Mandibular central incisors

Table 1. Distribution of ManCIs with complicated root canal configurations and ManFMs with RE by gender and side						
	ManCIs, n (%)		**p-value**	**ManFMs, n (%)**		**p-value**
	Single	**Complicated**		**Without RE**	**With RE**	
Total	824 (77.2)	244 (22.8)		869 (81.4)	199 (18.6)	
Gender						
Female	235 (73.7)	84 (26.4)	0.256	275 (86.2)	44 (13.8)	0.002*
Male	161 (74.9)	54 (25.1)		162 (75.3)	53 (24.7)	
Side						
Right	403 (75.5)	131 (24.5)	0.215	437 (81.8)	97 (18.2)	0.753
Left	421 (78.8)	113 (21.2)		402 (80.9)	102 (19.1)	
Single indicates the presence of one canal in ManCIs. Complicated indicates the occurrence of more than one canal in any CBCT section. *Statistical significance level was set at p<0.05, RE: Radix entomolaris, ManCIs: Mandibular central incisors, ManFMs: Mandibular first molars						
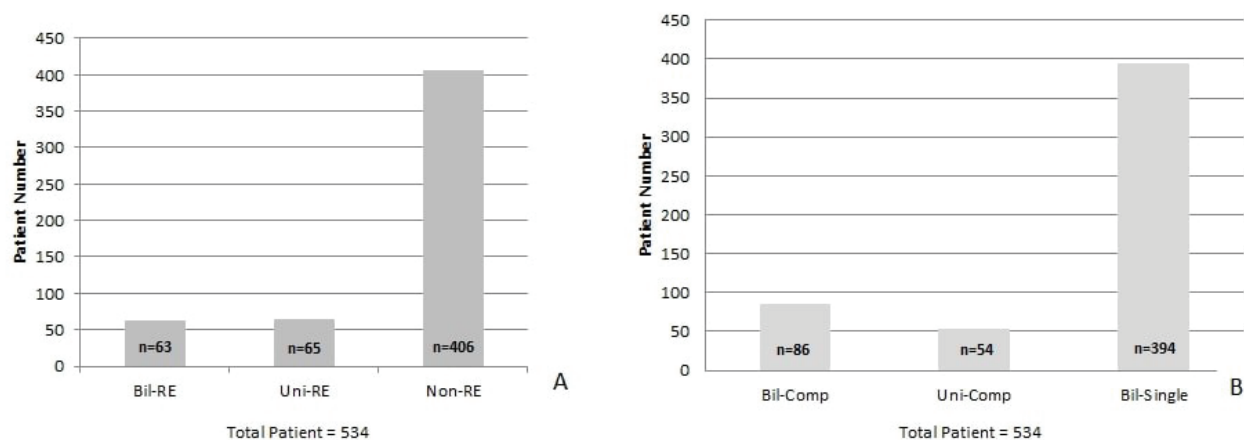
Figure 4. (A) Distribution patterns of unilateral RE and bilateral RE. (B) Distribution patterns of bilateral and unilateral complicated root canals
RE: Radix entomolaris
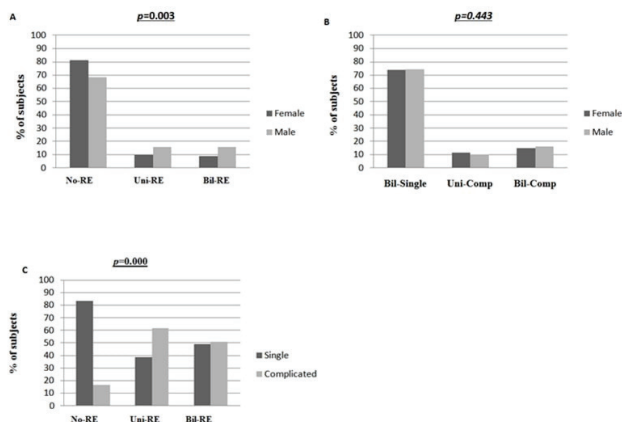
Figure 5. (A) Distribution patterns of ManFMs without/with RE (no RE, unilateral RE, and bilateral RE) based on sex. (B) Distribution patterns of ManCIs with bilateral single root canal configurations and complicated root canal configurations based on sex. (C) Distribution patterns of no RE, unilateral RE, and bilateral RE based on single and complicated root canal configurations
RE: Radix entomolaris, ManCIs: Mandibular central incisors, ManFMs: Mandibular first molars

There was a significant sex-related difference in the prevalence of RE in ManFMs (p=0.003) (Figure 5A), whereas there was no significant sex- or side-related related difference in the prevalence of complicated ManCI root canal configurations (p=0.443) (Figure 5B). There was a positive correlation between complicated ManCI root canal configurations and ManFMs with RE (p<0.001, r=0.248) (Figure 5C).

The multivariate analysis was adjusted for categorical variables, such as sex and sides. The adjusted odds ratios for the frequency of complicated ManCI root canal configurations in the bilateral RE group and unilateral RE group were 0.18 (95% confidence interval, 0.103-0.322; p<0.001) and 1.54 (95% confidence interval, 0.764-3.129; p=0.226), respectively (Table 2).

Discussion

Anatomical variations in root canal anatomy can cause difficulties in diagnosis and treatment. To ensure the success of dental treatments, the practitioner must have knowledge of the root canal anatomy. Although many studies have investigated the root canal anatomy of different tooth groups in different populations, only a few studies have investigated the relationship between variations in root canal anatomy (9,14).

In previous studies, the prevalence of RE in ManFMs differed, depending on the population (15,16). The prevalence of RE in ManFMs was reported to be higher in populations of Mongoloid origin (Chinese, Japanese, Korean, and Taiwanese) (5.8-43.6%) as compared with that in white populations (0.68-4.2%) (4). In this study, the prevalence of RE in ManFMs in a Turkish population was 18.6%. Although the etiology of RE is not known, previous research reported that external factors in the odontogenesis phase, penetrance of an atavistic gene, or a polygenetic system may influence the etiology (17). Studies also

Table 2. The Relationship between variables and the Frequency of complicated root canal configurations in ManCIs: Adjusted ORs and 95% CIs from multivariable logistic regression analysis

| | | ManCIs | | |
| | | 95% CI | | |
	Adjusted ORs	Lower	Upper	p-value
Gender				
Male	Referent			
Female	1.398	0.900	2.172	0.398
Side				
Left	Referent			
Right	1.211	0.910	1.613	0.190
RE group				
No-RE	Referent			
Bil-RE	0.18	0.103	0.322	<0.001*
Uni-RE	1.54	0.764	3.129	0.226

No-RE, no mandibular first molars with radix entomolaris bilaterally; Bil-DLR, radix entomolaris in both right and left mandibular first molars bilaterally; Uni-RE, one mandibular first molar with a radix entomolaris either at the left or right side and the other permanent mandibular first molar without a radix entomolaris. Analysis by multivariable logistic regression; the statistical significance level was set at p<0.05*. OR: Odds ratio, CI: Confidence interval, ManCIs: Mandibular central incisors

suggested that the presence of RE depended to a high degree on racial genetic factors (17,18). Therefore, we think that genetic differences between populations most likely explain the discord in the findings of the present study as compared with those in the literature.

A previous research reported root canal anatomy variations of ManCIs (19), with a number of studies reporting that ManCIs most commonly have type I or type III Vertucci root canal configurations (12,20). In line with these results, in this study, ManCIs with type I (74.2%) and type III (25.8%) root canal configurations were the most common.

Wu et al. (9) reported that there was a correlation between the presence of RE in ManFMs and complicated ManCI root canal configurations in a Taiwanese population. Similarly, we found a correlation between the presence of RE in ManFMs and complicated ManCI root canal configurations in this Turkish population. No previous studies have investigated this relationship in a Turkish population. Based on the findings of the present study, if RE should be present, and there should be a suspicion that complicated canal configuration in ManCIs.

Therefore, the results of this study can benefit clinical management.

A number of studies on symmetry in dental structures found that many anatomical variations were symmetrical and irrespective of the region (17,19,21). In patients with RE, Wu et al. (9) reported a 54.6% prevalence of the bilateral type in ManFMs. In this study, among those with RE, the prevalence of the bilateral type was 49.2%, and the prevalence of RE did not show a significant difference between the sides. In contrast, Schäfer et al. (15) detected no cases of bilateral RE in ManFMs in a German population. Similarly, other studies reported that anatomical variations differed significantly, depending on the side (16,18). According to some studies, the prevalence of RE was greater on the right side (16,22) whereas others found that it was greater on the left side (19). As stated previously in the literature (17), genetic variations between different populations most likely explain this difference.

Roots or canals in the same buccolingual plane in both ManFMs and ManCIs may cause superposition on two-dimensional radiographs. As a result, additional roots or canals may not be detected. To overcome this limitation and detect additional roots and canals, radiographs may be taken at different angles (23). However, the latter increases radiation exposure and is time consuming. In the identification of additional canals and roots, CBCT has been shown to provide more accurate results, without causing superposition and distortion as compared with two-dimensional radiographs (10). The voxel size is one of the most important factors that affects the image resolution in CBCT, with the smaller the voxel size, the better the quality and detail of the resulting image. However, a reduction in the voxel size in CBCT devices increases the exposure to radiation. In this study, CBCT images with a voxel size of 0.2 mm were obtained. This size is considered the maximum ideal size for evaluating root canal anatomy (23,24).

In a previous study, the presence of RE in ManFMs was reported to be related to a C-shaped canal configuration and radicular grooves in mandibular first premolar teeth (14). Zhang et al. (25) evaluated the morphological characteristics of RE in a Chinese population, and De Moor (26) classified RE according to the RE type. The relationship between the presence of RE and root canal configurations of teeth

other than ManFMs, in addition to the morphological characteristics of RE, should be determined in a Turkish population in future studies.

Conclusion

The results revealed a correlation between the presence of RE in ManFMs and the existence of complicated ManCI root canal configurations in this Turkish population. RE should be considered to be present in the presence of a complicated root canal configuration.

Ethics

Ethics Committee Approval: The study was approved by the Local Ethics Committee of Bolu Abant İzzet Baysal University (decision no: 2018/132 date: 06.09.2018).

Informed Consent: Retrospective study.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions:

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

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

Financial Disclosure: The authors declared that this study received no financial support.

References

1. Chen G, Yao H, Tong C. Investigation of the root canal configuration of mandibular first molars in a Taiwan Chinese population. Int Endod J 2009; 42: 1044-9.
2. Leoni GB, Versiani MA, Pécora JD, Damião de Sousa-Neto M. Micro-computed tomographic analysis of the root canal morphology of mandibular incisors. J Endod 2014; 40: 710-6.
3. Liu J, Luo J, Dou L, Yang D. CBCT study of root and canal morphology of permanent mandibular incisors in a Chinese population. Acta Odontol Scand 2014; 72: 26-30.
4. Song JS, Choi HJ, Jung IY, Jung HS, Kim SO. The prevalence and morphologic classification of distolingual roots in the mandibular molars in a Korean population. J Endod 2010; 36: 653-7.
5. Choi SJ, Lee JW, Song JH. Dental anomaly patterns associated with tooth agenesis. Acta Odontol Scand 2017; 75: 161-5.
6. Boschetti E, Silva-Sousa YTC, Mazzi-Chaves JF, Leoni GB, Versiani MA, Pécora JD, et al. Micro-CT Evaluation of Root and Canal Morphology of Mandibular First Premolars with Radicular Grooves. Braz Dent J 2017; 28: 597-603.
7. Fernandes NA, Herbst D, Postma TC, Bunn BK. The prevalence of second canals in the mesiobuccal root of maxillary molars: A cone beam computed tomography study. Aust Endod J 2019; 45: 46-50.
8. Alfawaz H, Alqedairi A, Alkhayyal AK, Almobarak AA, Alhusain MF, Martins JNR. Prevalence of C-shaped canal system in mandibular first and second molars in a Saudi population assessed via cone beam computed tomography: a retrospective study. Clin Oral Investig 2019; 23: 107-12.
9. Wu YC, Cheng WC, Weng PW, Chung MP, Su CC, Chiang HS, et al. The Presence of Distolingual Root in Mandibular First Molars Is Correlated with Complicated Root Canal Morphology of Mandibular Central Incisors: A Cone-beam Computed Tomographic Study in a Taiwanese Population. J Endod 2018; 44: 711-6.e1.
10. Huang RY, Cheng WC, Chen CJ, Lin CD, Lai TM, Shen EC, et al. Three-dimensional analysis of the root morphology of mandibular first molars with distolingual roots. Int Endod J 2010; 43: 478-84.
11. Wu YC, Su CC, Tsai YC, Cheng WC, Chung MP, Chiang HS, et al. Complicated Root Canal Configuration of Mandibular First Premolars Is Correlated with the Presence of the Distolingual Root in Mandibular First Molars: A Cone-beam Computed Tomographic Study in Taiwanese Individuals. J Endod 2017; 43: 1064-71.
12. Ahmed HM, Hashem AA. Accessory roots and root canals in human anterior teeth: a review and clinical considerations. Int Endod J 2016; 49: 724-36.
13. Vertucci FJ. Root canal anatomy of the human permanent teeth. Oral Surg Oral Med Oral Pathol 1984; 58: 589-99.
14. Wu YC, Cathy Tsai YW, Cheng WC, Weng PW, Su CC, Chiang HS, et al. Relationship of the Incidence of C-shaped Root Canal Configurations of Mandibular First Premolars with Distolingual Roots in Mandibular First Molars in a Taiwanese Population: A Cone-beam Computed Tomographic Study. J Endod 2018; 44: 1492-9.e1.
15. Schäfer E, Breuer D, Janzen S. The prevalence of three-rooted mandibular permanent first molars in a German population. J Endod 2009; 35: 202-5.
16. Tu MG, Tsai CC, Jou MJ, Chen WL, Chang YF, Chen SY, et al. Prevalence of three-rooted mandibular first molars among Taiwanese individuals. J Endod 2007; 33: 1163-6.
17. Chandra SS, Chandra S, Shankar P, Indira R. Prevalence of radix entomolaris in mandibular permanent first molars: a study in a South Indian population. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2011; 112: e77-82.
18. Curzon ME. Three-rooted mandibular permanent molars in English Caucasians. J Dent Res 1973; 52: 181.
19. Kayaoglu G, Peker I, Gumusok M, Sarikir C, Kayadugun A, Ucok O. Root and canal symmetry in the mandibular anterior teeth of patients attending a dental clinic: CBCT study. Braz Oral Res 2015; 29: S1806-83242015000100283.
20. Verma GR, Bhadage C, Bhoosreddy AR, Vedpathak PR, Mehrotra GP, Nerkar AC, et al. Cone Beam Computed Tomography Study

of Root Canal Morphology of Permanent Mandibular Incisors in Indian Subpopulation. Pol J Radiol 2017; 82: 371-5.

21. Johnsen GF, Dara S, Asjad S, Sunde PT, Haugen HJ. Anatomic Comparison of Contralateral Premolars. J Endod 2017; 43: 956-63.

22. Steelman R. Incidence of an accessory distal root on mandibular first permanent molars in Hispanic children. ASDC J Dent Child 1986; 53: 122-3.

23. Yew SC, Chan K. A retrospective study of endodontically treated mandibular first molars in a Chinese population. J Endod 1993; 19: 471-3.

24. Ji Y, Wen S, Liu S, Zhu M, Yao M, Wang T, et al. Could cone-beam computed tomography demonstrate the lateral accessory canals? BMC Oral Health 2017; 17: 142.

25. Zhang X, Xu N, Wang H, Yu Q. A Cone-beam Computed Tomographic Study of Apical Surgery-related Morphological Characteristics of the Distolingual Root in 3-rooted Mandibular First Molars in a Chinese Population. J Endod 2017; 43: 2020-4.

26. De Moor RJ, Deroose CA, Calberson FL. The radix entomolaris in mandibular first molars: an endodontic challenge. Int Endod J 2004; 37: 789-99.

Does Different Application Procedures Effect Hardness of Self Adherable Materials?

Farklı Uygulama Prosedürleri Kendinden Bağlanabilen Restoratif Materyallerin Sertliğini Etkiler mi?

● Başak Yazkan[1], ● Duygu Recen[2]

[1]Pamukkale University Faculty of Dentistry, Department of Restorative Dentistry, Denizli, Turkey
[2]İzmir Democracy University Faculty of Dentistry, Department of Restorative Dentistry, İzmir, Turkey

Address for Correspondence/Yazışma Adresi:
Duygu Recen Ph.D.,
İzmir Democracy University Faculty of Dentistry, Department of Restorative Dentistry, İzmir, Turkey
Phone : +90 232 260 10 01
E-mail : recenduygu@hotmail.com
ORCID ID: orcid.org/0000-0003-0613-386X

Abstract

Objective: To assess the effect of application procedures on the hardness of self-adherable materials after energy drink exposure.

Materials and Methods: Alkasite (self-cured), alkasite (dual-cured), (HGI), HGI + coating, HGI + heating, (GC), GC + coating, GC + heating, and nanohybrid composite (control) used. Samples from the main group were distributed into three subgroups (n=12): Red Bull, Burn, artificial saliva. The samples were dipped in solutions 2-min daily, up to 6 months. Surface hardness measurements were done after the specimen preparation and after they were kept in the solution for 1 week, 1 month and 6 months. Statistical analyses were done with Friedman tests, Kruskal-Wallis and Bonferroni post hoc tests (p<0.05).

Results: Dual-cured alkasite presented lower changes in Vickers hardness number after 6 months of immersion than self-cured alkasite (p<0.05). Coating application on HGI resulted in hardness advancement and coating application on GC significantly reduced hardness decrease in the Red Bull and Burn subgroups (p<0.05). Heating application, significantly decreased the hardness reduction in both HGI and GC (p<0.05).

Conclusion: Both coating and heating procedures on HGI may protect the hardness. Also, coating was more effective on HGI than on GC. Heating can be preferred than coating for GC. Dual-cured alkasite may present more resistance than self-cured alkasite

Öz

Amaç: Çalışmanın amacı farklı sertleştirme prosedürlerinin, enerji içeceklerine maruz kaldıktan sonra restoratif materyallerin sertliği üzerindeki etkisini değerlendirmektir.

Gereç ve Yöntemler: Alkasit (self-cured), alkasit (dual-cured), hibrit cam iyonomer (HGI), HGI + kaplama, HGI + ısıtma, cam karbomer (GC), GC + kaplama, GC + ısıtma ve nanohibrit kompozit (kontrol) kullanıldı. Her grup üç alt gruba ayrıldı (n=12): Red Bull, Burn, yapay tükürükte bekletme. Örnekler 6 ay boyunca günde 2 dakika solüsyonlara daldırıldı. Yüzey sertliği değerlendirmeleri başlangıçta, 1 hafta, 1 ay ve 6 ay sonra yapıldı. İstatistiksel analizler Friedman testi, Kruskal-Wallis ve Bonferroni post-hoc testleri kullanılarak yapıldı (p<0,05).

Bulgular: Dual sertleşmiş alkasit, 6 aylık bekletme sonrasında kendi kendine sertleşen alkasite göre Vickers sertlik sayısında (ΔVHN) daha düşük değişiklikler

gösterdi (p<0,05). Red Bull ve Burn alt gruplarında HGI üzerine kaplama uygulaması sertlik artışı ile sonuçlanmış ve GC üzerine kaplama uygulaması sertlik düşüşünü önemli ölçüde azaltmıştır (p<0,05). Isıtma uygulaması hem HGI hem de GC'de sertlik azalmasını önemli ölçüde azaltmıştır (p<0,05).

Sonuç: HGI üzerinde hem kaplama hem de ısıtma işlemi materyal sertliğini koruyabilir. Ayrıca kaplama uygulaması HGI üzerinde GC'ye göre daha etkili olmuştur. GC için kaplama yerine ısıtma uygulaması tercih edilebilir. Dual sertleşen alkasit, kendi kendine sertleşen alkasite göre daha fazla direnç gösterebilir.

Introduction

In an attempt to overcome drawbacks of conventional glass ionomers, such as prolonged setting time, dehydration, initial moisture sensitivity, enhanced high viscous glass hybrid materials have been developed (1,2). This glass technology has been modified with ultrafine, reactive glass particles and built up a much stronger matrix structure which allow extended indications of use even in stress-bearing areas (3). Also, nanotechnology offers a glass ionomer subgroup which is called glass carbomer. Material contains nano-sized powder particles and fluorapatite (4,5).

Since restorative materials are faced with many different erosive stimuli in oral environment, it has become important to strengthen mechanical properties with some additional applications. One of the additional enforcement is the use of heat. Heat application can be performed by using high-energy LED, halogen light source or ultrasonic excitation and it is expressed that heating significantly increases hardness of glass ionomers (6,7). An alternative application that has been shown as reinforcement is surface coating (8).

Another novel self adherable material, which stands out with its high compressive strength is alkasite. This product incorporates dimethacrylates in liquid and glass fillers, initiators, and pigments in powder (9-11). Setting reaction of this material can be done by two mechanisms: Self-cure and dual-cure (10).

Energy drinks, which are commonly preferred by students, long way drivers and athletes, have an erosive affect on restorative materials (11). It is reported that energy drinks may decrease the hardness of restorations due to their low pH and buffering capacity (11,12). However, there are few studies in literature about how heating or coating effect mechanical properties of glass ionomers.

Therefore, the aims of this study were to determine the effect of energy drinks on hardness regarding to additional heating or coating application on glass ionomers and different setting reaction mechanisms of alkasite (1,2). The following null hypotheses tested were: There would be no differences in the hardness of glass ionomers when coating or heating is applied; there would be no differences in the hardness of the alkasite whether hardened with self-cure or dual-cure (1,2).

Materials and Methods

In the power analysis (F=0.5), it was determined that 80% power could be obtained at 95% confidence level when at least 12 samples were taken per group in the study.

Three hundred and twenty four samples were prepared using a disc-shaped mold according to the manufacturer instructions (Table 1). Artificial saliva was prepared using 0.33 g of KH_2PO_4, 0.34 g of Na_2HPO_4, 1.27 g of KCl, 0.16 g of NaSCN, 0.58 g of NaCl, 0.17 g of $CaCl_2$, 0.16 g of NH_4Cl, 0.03 g of glucose, 0.2 g of urea, 0.002 g of ascorbic acid and 2.7 g of mucin in 1000 mL of distilled water. For the complete polymerization, specimens were stored in artificial saliva (37 °C, 24 hours) (13).

Dry polishing regimen was applied to the upper surfaces of all specimens with aluminum oxide impregnated discs by applying a light hand pressure, using a 10,000 rpm micromotor at low-speed. For the glass ionomer based materials, coatings were applied before and after polishing. Each group was randomly divided into three subgroups (n=12 per group) according to following immersion solutions: Red Bull (pH: 3.81, Red Bull GmbH, Austria); Burn (pH: 3.03, The Coca-Cola Company, Atlanta, GA, USA) and artificial saliva.

Before experiment, pH of energy drinks was measured with a pH meter (Waterproof pHep® 5 pH/Temperature Tester, Hanna Instruments Inc., Woonsocket, RI, USA). Samples were soaked in immersion solution for 2 minutes per day (23±1 °C). The samples were then washed with distilled water

and stored in fresh artificial saliva until the same application the next day. This cycle was repeated daily for six months over three immersion periods (14). All containers were closed to prevent immersion solutions from vaporizing. Energy drinks and artificial saliva were changed daily.

Microhardness measurements were done after specimen preparation and after they were kept in solution for 1-week, 1-month and 6-months. Using a microhardness tester (Duroline M, Metkon Instruments Inc., Bursa, Turkey) and a Vickers indenter, three tracks were made on the material surface at 100 mm intervals from each other by applying a static load of 200 g.

Statistical Analysis

Shapiro-Wilk test was used to evaluate whether the variables in the study were compatible with normal distribution. In comparing values obtained at different times for each groups, F statistic was applied for variables with normal distribution, and Friedman test for variables that were not normally distributed. Bonferroni post hoc test was preferred for binary comparison. Kruskal-Wallis test statistics were used to compare variables that did not show normal distribution. A value of <0.05 was considered as statistically significant.

Results

The mean hardness values of each group at baseline and after 1-week, 1-month, and 6-months of Redbull, Burn and artificial saliva immersion are presented in Table 2, Table 3 and Table 4, respectively. The column graphic showing changes of Vickers hardness number ΔVHN 1w (difference between baseline-1-week), ΔVHN 1m (difference between baseline-1-months) and ΔVHN 6m (difference between baseline-6-month) of each group is given in Figure 1.

Dual-cured alkasite presented the highest hardness values (p=0.0001). This group was followed by self-cured alkasite and nanohybrid composite resin, respectively (p=0.0001). The hardness values in hybrid glass ionomer (HGI) + coating were higher than HGI. On the other hand, there is no statistically significant difference between the hardness of glass carbomer + coating and the glass carbomer in all subgroups at baseline (p>0.05). Hardness of HGI + heating was higher than HGI (p=0.0001). Similarly, glass carbomer + heating presented higher values than glass carbomer (p=0.0001). The hardness values obtained after heating were found significantly higher than coating (p=0.0001).

Table 1. Materials used for each group and their application procedure		
Groups/Codes	**Material/Manufacturer/ Batch Number**	**Application**
Alkasite (self-cured)/ASC	Cention N/Ivoclar Vivadent AG, Bendererstrasse, Schaan, Liechtenstein/ W93722	Dispense powder and liquid, mix and add the remaining powder until a homogeneous consistency is achieved (45-60 s) (no light curing).
Alkasite (dual-cured)/ADC		Apply additional light for 20 s polimerized using a LED lamp at a distance of 1 mm (standard power curing mode of VALO™ Cordless, Ultradent, South Jordan, UT 84095, USA)
Hybrid glass ionomer/HGI	Equia Forte/ GC, Tokyo, Japan/1804061	Activate the capsule and mix in a high frequency mixer.
Hybrid glass ionomer + Coat/HGIC	Equia Forte Coat/ GC Europe, Leuven, Belgium	Apply Equia Forte Coat and apply light for 20 s (standard power curing mode of VALO™ Cordless).
Hybrid glass ionomer + Heat/HGIH		Additional light for 60 s with a LED lamp (standard power curing mode of VALO™ Cordless).
Glass Carbomer/GC	GCP Glass Fill/GCP Dental, Vianen, Netherlands/71712907	Activate the capsule and mix in a high frequency mixer for 15 s.
GlassCarbomer + Coat/GCC	GCP Gloss/GCP Dental, Vianen, Netherlands	Coat the surfaces with GCP Gloss and light cure for 60 s (GCP CarboLED, GCP Dental).
Glass Carbomer + Heat/ GCH		Additional light for 90 s (GCP CarboLED, GCP Dental).
Nanohybrid Composite/NC	Grandio So/VOCO GmbH, Cuxhaven, Germany/1806497	Apply the composite resin material and light cure for 40 s (standard power curing mode of VALO™ Cordless)
GCP: Good clinical practice		

Table 2. The mean surface hardness values and standard deviations of each group at baseline and after 1-week, 1-month, and 6-months of Redbull immersion

Group codes	Baseline	1-week	1-month	6-months
ASC	165.3±0.06[a,A]	161.5±0.08[a,B]	156.4±0.08[a,C]	147.5±0.08[a,D]
ADC	168.5±0.08[b,A]	164.4±0.07[b,B]	160.5±0.08[b,C]	156.5±0.08[b,D]
HGI	112.4±0.07[c,A]	98.5±0.08[c,B]	80.4±0.07[c,C]	69.4±0.07[c,D]
HGIC	118.4±0.09[d,A]	115.4±0.08[d,B]	98.4±0.09[d,C]	77.4±0.08[d,D]
HGIH	128.4±0.07[e,A]	123.5±0.09[e,B]	118.5±0.09[e,C]	110.5±0.07[e,D]
GC	70.5±0.08[f,A]	60.5±0.08[f,B]	49.5±0.08[f,C]	30.4±0.07[f,D]
GCC	68.3±0.07[g,A]	64.6±0.09[g,B]	52.5±0.07[g,C]	35.5±0.08[g,D]
GCH	83.5±0.08[h,A]	79.6±0.07[h,B]	71.4±0.06[h,C]	54.5±0.09[h,D]
NC	148.4±0.07[i,A]	144.5±0.07[i,B]	139.4±0.08[i,C]	134.5±0.078[i,D]

*Values indicated by different small letters on the same column and different big letters on the same line are statistically significantly different (p<0.0001)

Table 3. The mean surface hardness values and standard deviations of each group at baseline and after 1-week, 1-month, and 6-months of Burn immersion, intergroup comparisons in each evaluation point and intragroup comparisons between evaluation points

Group codes	Baseline	1-week	1-month	6-months
ASC	162.4±0.08[a,A]	158.4±0.09[a,B]	153.4±0,06[a,C]	144.4±0.08[a,D]
ADC	166.5±0.08[b,A]	162.4±0.07[b,B]	158.4±0.08[b,C]	155.4±0.08[b,D]
HGI	110.4±0.07[c,A]	95.4±0.06[c,B]	65.4±0.08[c,C]	57.5±0.07[c,D]
HGIC	115.4±0.07[d,A]	112.5±0.08[d,B]	94.4±0.06[d,C]	84.4±0.07[d,D]
HGIH	131.5±0.07[e,A]	127.5±0.09[e,B]	123.5±0.09[e,C]	118.5±0.08[e,D]
GC	65.4±0.06[f,A]	58.4±0.06[f,B]	47.5±0.09[f,C]	30.5±0.09[f,D]
GCC	66.5±0.08[f,A]	62.5±0.08[g,B]	51.4±0.07[g,C]	35.4±0.08[g,D]
GCH	81.5±0.07[g,A]	77.5±0.07[h,B]	71.4±0.07[h,C]	58.5±0.07[h,D]
NC	150.5±0.08[h,A]	146.4±0.08[i,B]	142.4±0.07[i,C]	137.5±0.08[i,D]

*Values indicated by different small letters on the same column and different big letters on the same line are statistically significantly different (p<0.0001)

Table 4. The mean surface hardness values and standard deviations of each group at baseline and after 1-week, 1-month, and 6-months of artificial saliva immersion, intergroup comparisons in each evaluation point and intragroup comparisons between evaluation points

Group codes	Baseline	1-week	1-month	6-months
ASC	168.4±0.07[a,A]	166.4±0.08[a,A]	164.4±0.06[a,B]	159.4±0.07[a,C]
ADC	171.4±0.07[b,A]	170.4±0.08[b,A]	169.5±0.08[b,A]	166.4±0.07[b,B]
HGI	115.4±0.08[c,A]	112.4±0.09[c,A]	98.5±0.08[c,B]	92.5±0.08[c,C]
HGIC	120.5±0.07[d,A]	119.4±0.07[d,A]	114.5±0.08[d,B]	108.4±0.07[d,C]
HGIH	131.5±0.08[e,A]	130.5±0.07[e,A]	126.4±0.08[e,B]	122.4±0.08[e,C]
GC	74.5±0.08[f,A]	70.5±0.08[f,B]	64.5±0.07[f,C]	53.4±0.07[f,D]
GCC	73.4±0.07[f,A]	71.5±0.08[f,A]	65.4±0.07[f,B]	53.3±0,05[f,C]
GCH	82.4±0.08[g,A]	81.3±0.04[g,A]	78.4±0.08[g,B]	72.5±0.07[g,C]
NC	153.4±0,08[h,A]	152.5±0.08[h,A]	151.4±0.06[h,B]	146.4±0.08[h,C]

*Values indicated by different small letters on the same column and different big letters on the same line are statistically significantly different (p<0.0001)
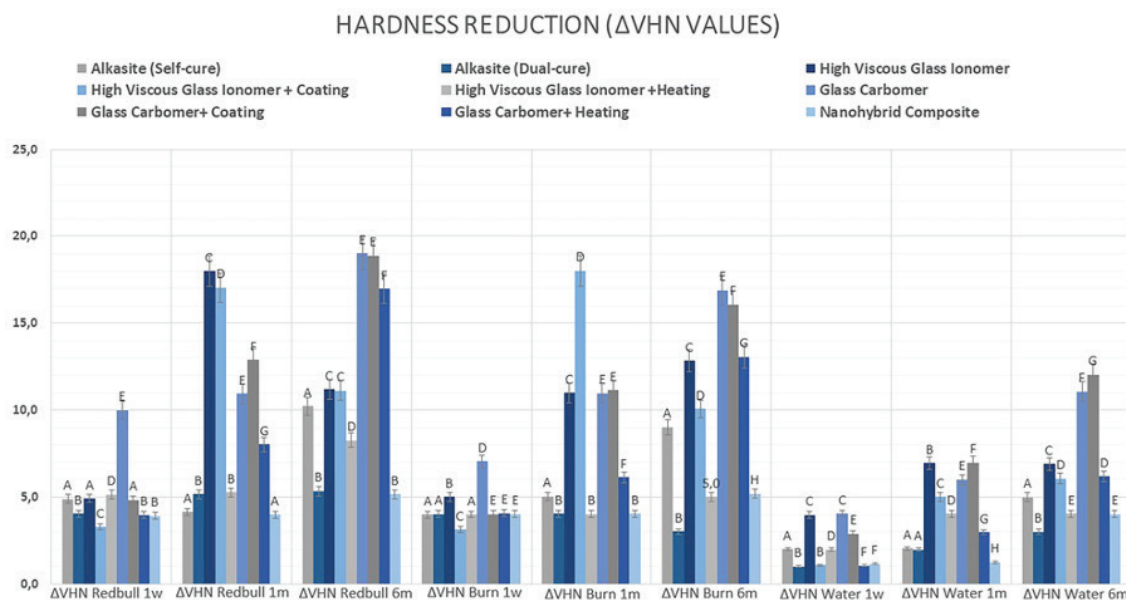
Figure 1. Column chart showing mean hardness change values of all groups after 1 week (ΔVHN 1w), 1 month (ΔVHN 1m) and 6 months (ΔVHN 6m)

After Red bull and Burn immersion, hardness was significantly decreased with the elapsed time in all groups (p=0.0001).

Dual-cured alkasite presented lower ΔVHN 6m than self-cured alkasite (p=0.0001). Coating on HGIs significantly resulted in hardness advancement and coating on glass carbomers significantly decreased hardness reduction in Red bull and Burn subgroups (p=0.0001). Heating, on the other hand, significantly decreased hardness reduction in both HGIs and glass carbomers (p=0.0001).

Discussion

Present study aimed to investigate whether different applications would effect surface hardness. In previous *in vitro* studies, materials were left in contact with acidic drinks for a long time (15,16). However, in oral environment, during consumption of drinks, restoration surfaces remain in contact with beverages for a very short time before being washed with saliva. Therefore, in this study, as descibed before by Erdemir et al. (14), the materials were dipped in energy drinks 2 minutes a day and then left in artificial saliva.

The present results reveal that coating and heating on HGI concluded in a decrease in the hardness reduction after energy drinks exposure. Therefore,

first null hypothese was rejected. This result is in agreement with Burdur and Sirin Karaarslan (17) who revealed varnish application increased the hardness of Equia Forte. Furthermore, in a different study it is reported that coated glass ionomer presented significantly higher hardness when compared no protection (18). Higher hardness obtained from coating can be interpreted as covering the surface against moisture is important for maintaining the hardening of the material. Jafarpour et al. (19) supported this interpretation with their study. It is reported that water sorption and solubility of restorative materials may decrease the mechanical properties and surface coating protects initial water contamination (17).

In this study, heating on glass carbomer increased hardness. However, coating on glass carbomers has not been found to be as successful as heating. Similarly, in a clinical study (20) researchers stated that good clinical practice (GCP) Gloss, had no effect on mechanical properties of restorative material, unlike GC Equia Coat. While the GCP Gloss varnish did not contain monomers, the varnishes used to protect the conventional glass ionomer cements consisted mainly of acrylic or methacrylic monomers. In many studies, light-curing, nano-filled, resin-based varnishes have been shown to be more successful than other surface coating materials (20).

In both HGI and glass carbomer, heating increased hardness more than coating. This finding is in line with the results of previous studies investigating the effect of heat treatment on the hardness of glass ionomers (21,22). Heat provided by LED light-curing units increases ion mobility during the initial stage of setting and causes acceleration in the hardening resulting in an improved setting reaction (23). This study revealed that heating using a high output light device is useful with regards to glass carbomer. Unlike the results of this study, some other studies indicated that heating had no effect on mechanical behaviour of the glass carbomer. This result was related to the structure of the glass carbomer material in these studies (6,24).

Alkasite presented the highest hardness, whether hardened as self-cure or dual-cure. This may be related to nanoparticle size of inorganic filling ingredient (25). However, when hardened with dual-cure, highest results were obtained. Therefore, second null hypothese was rejected. The higher hardness of dual-cured alkasite can be attributed to material's high polymer network density and high degree of conversion with a stable, efficient self-cure initiator (25). Unlike the results of present study, by Ilie (24) reported that additional light curing initially accelerates the polymerization kinetics and shortens the curing process, but does not change the final hardness. Different results of the present study may be attributed to additional light application can lead to higher values of degree of conversion and crosslinking, both straight related to the hardness. However, many factors which can affect result such as energy density, size and distribution of inorganic fillers should also be considered.

With all these results, there is a need for more *in vitro* and clinical studies to be carried out and only the hardness parameter was evaluated in the present study, and the amount of wear after long-term energy drinks exposure was not measured.

Conclusion

According to present study results:

- Heating can be preferred then coating in both HGIs and glass carbomers.

- Coating is more effective on HGIs than glass carbomers.

- Dual-cured alkasite may present more resistance than self-cured ones.

- Dual-cured alkasite may be a better alternative for patients on acidic diet when compared with glass ionomers.

Ethics

Ethics Committee Approval: Ethical approval is not required for this type of an *in vitro* material research article which does not involve humans, animals or exracted tooth.

Informed Consent: This study does not require patient consent.

Peer-review: Externally peerreviewed.

Authorship Contributions

Consept: D.R., B.Y., Design: D.R., B.Y., Data Collection or Processing: D.R., B.Y., Analysis or Interpretation: B.Y., Literature Search: D.R., B.Y., Writing: D.R., B.Y.

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

Financial Disclosure: The authors declared that this study received no financial support.

References

1. Savas S, Colgecen O, Yasa B, Kucukyilmaz E. Color stability, roughness, and water sorption/solubility of glass ionomer-Based restorative materials. Niger J Clin Pract 2019; 22: 824-32.

2. Ong J, Yap AU, Hong JY, Eweis AH, Yahya NA. Viscoelastic Properties of Contemporary Bulk-fill Restoratives: A Dynamic-mechanical Analysis. Oper Dent 2018; 43: 307-14.

3. Kutuk ZB, Ozturk C, Cakir FY, Gurgan S. Mechanical performance of a newly developed glass hybrid restorative in the restoration of large MO Class 2 cavities. Niger J Clin Pract 2019; 22: 833-41.

4. Moshaverinia A, Ansari S, Moshaverinia M, Roohpour N, Darr JA, Rehman I. Effects of incorporation of hydroxyapatite and fluoroapatite nanobioceramics into conventional glass ionomer cements (GIC). Acta Biomater 2008; 4: 432-40.

5. Tatlı EC, Ozer L. Evaluation of physical and mechanical properties of glass carbomer cement under in vitro conditions. Turk J Clin Lab 2018; 9: 281-6.

6. Menne-Happ U, Ilie N. Effect of heat application on the mechanical behaviour of glass ionomer cements. Clin Oral Investig 2014; 18: 643-50.

7. O'Brien T, Shoja-Assadi F, Lea SC, Burke FJ, Palin WM. Extrinsic energy sources affect hardness through depth during set of a glass-ionomer cement. J Dent 2010; 38: 490-5.

8. Kanik Ö, Turkun LS, Dasch W. In vitro abrasion of resin-coated highly viscous glass ionomer cements: a confocal laser scanning microscopy study. Clin Oral Investig 2017; 21: 821-9.

9. Mishra A, Singh G, Singh SK, Agarwal M, Qureshi R, Khurana N. Comparative Evaluation of Mechanical Properties of Cention N with Conventionally used Restorative Materials—An In Vitro Study. Int J Prosthodont Restor Dent 2018; 8: 120-4.

10. Cention N retrieved. Avaliable at URL: http://www.ivoclarvivadent.in/en-in/p/all/cention-n.

11. Yazkan B. Surface degradation evaluation of different self-adhesive restorative materials after prolonged energy drinks exposure. J Esthet Restor Dent 2020; 32: 707-14.

12. Cavalcanti AL, Costa Oliveira M, Florentino VG, dos Santos JA, Vieira FF, Cavalcanti CL. Short communication: In vitro assessment of erosive potential of energy drinks. Eur Arch Paediatr Dent 2010; 11: 253-5.

13. Ergücü Z, Türkün LS, Aladag A. Color stability of nanocomposites polished with one-step systems. Oper Dent 2008; 33: 413-20.

14. Erdemir U, Yildiz E, Eren MM, Ozel S. Surface hardness of different restorative materials after long-term immersion in sports and energy drinks. Dent Mater J 2012; 31: 729-36.

15. Ryge G, Foley DE, Fairhurst CW. Micro-indentation hardness. J Dent Res 1961; 40: 1116-26.

16. Yanıkoğlu N, Duymuş ZY, Yilmaz B. Effects of different solutions on the surface hardness of composite resin materials. Dent Mater J 2009; 28: 344-51.

17. Buldur M, Sirin Karaarslan E. Microhardness of glass carbomer and high-viscous glass Ionomer cement in different thickness and thermo-light curing durations after thermocycling aging. BMC Oral Health 2019; 19: 273.

18. Shintome LK, Nagayassu MP, Di Nicoló R, Myaki SI. Microhardness of glass ionomer cements indicated for the ART technique according to surface protection treatment and storage time. Braz Oral Res 2009; 23: 439-45.

19. Jafarpour D, Mese A, Ferooz M, Bagheri R. The effects of nanofilled resin-based coatings on the physical properties of glass ionomer cement restorative materials. J Dent 2019; 89: 103177.

20. Gurgan S, Kutuk ZB, Ergin E, Oztas SS, Cakir FY. Four-year randomized clinical trial to evaluate the clinical performance of a glass ionomer restorative system. Oper Dent 2015; 40: 134-43.

21. Kuter B, Eden E, Yildiz H. The effect of heat on the mechanical properties of glass ionomer cements. Eur J Paediatr Dent 2013; 14: 90-4.

22. Kleverlaan CJ, van Duinen RN, Feilzer AJ. Mechanical properties of glass ionomer cements affected by curing methods. Dent Mater 2004; 20: 45-50.

23. Gavic L, Gorseta K, Glavina D, Czarnecka B, Nicholson JW. Heat transfer properties and thermal cure of glass-ionomer dental cements. J Mater Sci Mater Med 2015; 26: 249.

24. Ilie N. Comparative Effect of Self- or Dual-Curing on Polymerization Kinetics and Mechanical Properties in a Novel, Dental-Resin-Based Composite with Alkaline Filler. Running Title: Resin-Composites with Alkaline Fillers. Materials (Basel) 2018; 11: 108.

25. Mazumdar P, Das A, Guha C. Comparative evaluation of hardness of different restorative materials (restorative GIC, Cention N, nanohybrid composite resin and silver amalgam) –an in vitro study. Int J Adv Res 2018; 6: 826-32.

Original Article / Özgün Araştırma

The Effect of Different Adhesive Resins, Composite Resins and Thermal Cycling on the Repair Bond Strength of Bulkfill Composite Resin

Bulkfill Kompozitlerin Tamir Bağlanma Dayanımı Üzerine Farklı Adeziv Rezinlerin, Kompozit Rezinlerin ve Termal Siklusun Etkisi

 Soley Arslan, Hacer Balkaya, Burhanettin Avcı, Ayşenur Doğan

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

Abstract

Objective: This study evaluated the influence of different adhesives, composites and thermal cycling on the repair bond strength of bulkfill composite.

Materials and Methods: A total of 54 bulkfill composite resin disks were obtained. The samples were stored in distilled water for 14 days and then the sample's surfaces were air-abraded with Al_2O_3 particles, etched with phosphoric acid, washed and dried. An adhesive was applied to the samples after the silane application. The samples were randomly divided into three main groups according to the type of adhesive resin used (a self-cured universal adhesive, a total-etch adhesive and a self-etch adhesive). After the adhesive application, four composite resin cylinders were placed on the sample surface using three different repair composites (bulkfill composite, microhybrid composite and nanohybrid composite). A total of 5000 thermal cycles were applied to half of the samples (n=12). A microshear bond strength test was performed using a universal tester. The data obtained were analyzed using Mann-Whitney U and Kruskal-Wallis tests.

Results: Of the repair composites, bulkfill composite tended to show the highest bond strength. There was no significant difference between the microhybrid and the nanohybrid composite groups (p>0.05). Generally, the adhesives did not significantly affect the repair bond strength.

Conclusion: The use of bulkfill composite to repair bulkfill composite provides more efficient repair bond strength. The tested adhesives can be used safely to repair bulkfill composite. The repair bond strength was not negatively affected after 5000 thermal cycles.

Öz

Amaç: Bu çalışmada bulkfill kompozit rezinlerin tamiri üzerine farklı adezivlerin, kompozitlerin ve termal siklusun etkisinin değerlendirilmesi amaçlanmıştır.

Gereç ve Yöntemler: Bu çalışmada 54 adet bulkfill kompozit rezin disk hazırlanmıştır. Örnekler 14 gün boyunca 37 °C'de distile suda tutulmuştur ve daha sonra örnek yüzeyleri Al_2O_3 partikülleri ile 10 sn kumlanmıştır, ardından fosforik asit ile dağlanmış, yıkanmış ve kurutulmuştur. Silan uygulamasından sonra numunelerin yüzeylerine adeziv rezin uygulanmıştır. Örnekler, kullanılacak adezive göre rastgele 3 ana gruba ayrılmıştır (bir self-cure üniversal adeziv, bir total-etch adeziv ve bir self-etch adeziv). Adeziv uygulaması sonrası, numune yüzeyine üç farklı tamir kompoziti (bulkfill kompozit, mikro hibrit kompozit ve nanohibrit kompozit) kullanılarak dörder adet kompozit silindir yerleştirilmiştir. Örneklerin yarısı 5000 termal döngüye tabi tutulmuştur (n=12). Mikromakaslama bağlanma dayanımı

testi, evrensel bir test makinesi kullanılarak gerçekleştirilmiştir. Veriler Mann–Whitney U ve Kruskal–Wallis testleri kullanılarak istatistiksel olarak analiz edilmiştir.

Bulgular: Tamir için kullanılan kompozit rezinler arasında bulkfill kompozit rezinler daha yüksek bağlanma dayanım değerlerine sahipti. Mikrohibrit ve nanohibrit kompozit grupları arasında anlamlı bir fark yoktu (p>0,05). Genel olarak, adeziv rezinler bağlanma dayanımını istatistiksel olarak anlamlı derecede etkilemedi.

Sonuç: Bulkfill kompozit rezinlerin tamirinde bulkfill kompozit rezin kullanımı daha verimli tamir bağlanma dayanımı sağlar. Test edilen adezivler, bulkfill kompozit rezinleri tamir etmek için güvenle kullanılabilir. Beş bin termal siklus tamir bağlanma dayanımını olumsuz etkilemedi.

Introduction

Composite resins (CR) are commonly used restorative materials in dental applications due to advantages such as aesthetic and adhesive characteristics of them, and they permit the use of a minimal invasive approach in restorative dentistry by preserving sound tooth structure (1). However, some problems such as microleakage, discoloration, wear, chipping or fracture of the restoration may occur depending on the degradation of the CR caused by various conditions such as pH and temperature changes in the oral environment, diet, and other factors (2,3). If the defects are minor, repairing of the restoration is a more appropriate approach because it is a protective, fast and simple approach.

The success of the repair of CR restorations depends on some factors, including the characteristics of the surface, the wettability of the adhesive resins, and the chemical compound of the CR (4). A durable adhesion between the aged restoration and the repair CR is essential for a successful repair procedure. In fact, adhesion to aged CR restorations can be quite challenging because of water absorption over time, and the reduction in the number of accessible C=C bonds that will react with the new CR (5).

Hybrid and especially microhybrid CRs can be used in both front and back teeth by successfully combining mechanical with aesthetic properties. Nanohybrid CRs offer advantages such as durability, low polymerization shrinkage, good polishability, ease of use, and superior aesthetic (6,7). In recent years, bulkfill CR that can be placed into a cavity with greater increments have been marketed. This class of CR helps to eliminate features such as marginal fractures associated with polymerization stress, and they have a high fluidity and can easily penetrate in hard to access cavities (8). The interaction between these different

CR should be well known in order to select the most appropriate repair technics.

The role of adhesive resin is to improve the wettability of the surfaces which have been mechanically treated and silanized (9). Universal adhesives (UA) were designed to be used in both direct and indirect restorative applications, including bonding to different substrates such as CRs, dental ceramics and alloys, and they could be used in all three application modes (etch-and-rinse, self-etch or selective-etch modes) (10). These adhesives would allow bonding to various surfaces without a priming agent such as silanes, or other surface primers which are dedicated and have to be separately placed. Recently, manufacturers have introduced different types of UA. The UA used in our study is a self-cured adhesive system in two-component form and it has been developed in order to be fully compatible with CRs in all three curing modes (light-cured, self-cured and dual-cured). Moreover, the manufacturer claims that it improves the adhesion of polymerizable resin materials to indirect restorative materials. However, to the best of our knowledge, there has been no study on the effect of self-cured adhesives on the repair bond strength (RBS) of bulkfill CRs.

Although there are many studies related to the repair of CRs in the literature, there is no consensus on which materials (adhesive resins and CR) are most appropriate for a successful repair process. Therefore, the present study aimed to investigate the early and late RBS of a bulkfill CR using different CR and different dental adhesive, using microshear bond strength test method. The hypotheses tested are as follows: 1) There is no difference in the RBSs of the tested CRs. 2) There is no difference in RBS with regard to the tested dental adhesives. 3) There is no difference between the RBS of the tested materials, with and without thermal cycling.

Materials and Methods

Preparation of the Samples

The materials used in this study, their compositions and batch numbers have been given in Table 1. In this study, fifty four composite discs (8 mm x 2 mm) were obtained using a bulkfill CR (Filtek Bulk Fill Posterior, 3M ESPE, MN, USA). The CR were packed into the teflon mold between two glass plates as bulk and polymerized for 20 s with an LED device (VALO Cordless; Ultradent Products, UT, USA, 1000 mW/cm²). Thereafter, the samples were embedded in autopolymerized acrylic resin by using PVC rings with their surface exposed. The surfaces of the samples were ground respectively with 400-grit and 600-grit sanding paper under water cooling and stored in distilled water at 37 °C for 14 days (6). The surfaces of the samples were air abraded for 10 s with aluminum oxide (Al_2O_3) particles (50-μm) 10 mm from the sample surface under a pressure of 4 bar and then they were etched with phosphoric acid for 30 s, washed for 60 s, and finally dried for 60 s.   A silane agent (Ultradent Silane, Ultradent, South Jordan, USA) was applied and 60 s was waited and the samples treated mechanically and chemically were then randomly divided into 3 main groups, each containing eighteen CR discs according to the dental adhesive applied (7):

Group 1: Tokuyama Universal Bond (Tokuyama Dental, Vicenza, Italy; TU; a self-cured universal adhesive resin), Group 2: Clearfil S3 Bond (Kuraray, Noritake, Japan; CS3; a self-etch adhesive resin), Group 3: Adper Single Bond 2 (3M ESPE, MN, USA; SB2; a total-etch adhesive resin).

The samples in each adhesive group were then deployed into three subgroups, each with six composite resin discs according to the repair composite used:

Group a: Filtek Bulkfill (FBF) Posterior (3M ESPE, MN, USA; FB; a bulkfill CR), Group b: Charisma Smart Composite (Heraeus Kulzer GmbH, Hanau, Germany; CSC; a microhybrid CR), Group c: Filtek Z550 (3M ESPE, MN, USA; FZ; a nanohybrid CR)

TU was prepared by mixing the double component in equal amounts and then applied to the sample surface. Mild air was then applied until the solvent evaporated and any curing was not applied. TU was used in self-cure mode. CS3 and SB2 were applied to the the sample surface for 10 s, and a uniform adhesive film was made using a mild air flow before being polymerized for 10 s with LED.

Table 1. Materials, compositions and batch numbers	
Materials	**Compositions**
Tokuyama Universal Bond Tokuyama Dental, Vicenza, Italy Batch 004	A) Phosphoric acid monomer (3D-SR), Bisphenol A di(2-hydroxy propoxy) dimethacrylate (Bis-GMA), Triethylene glycol dimethacrylate (TEGDMA), 2-Hydroxyethyl methacrylate (HEMA), MTU-6 (thiouracil monomer), Acetone B) γ-MPTES, Borate, Peroxide, Acetone, Isopropyl alcohol, Water
Clearfil S3 Bond Kuraray, Noritake, Japan Batch 6M0075	Bis-GMA, Sodium fluoride, 10 MDP, Colloidal silica, dl-Camphorquinone, Accelerators, Initiators, Hydrophilic aliphatic dimethacrylate, Hydrophobic aliphatic methacrylate, ethanol, and water
Adper Single Bond 2 3M ESPE, St Paul, MN, USA Batch N878242	Dimethacrylates, BisGMA, HEMA, a novel photoinitiator system, a methacrylate functional copolymer of polyacrylic and polyitaconic acids, ethanol, and water
Filtek Bulkfill Posterior 3M ESPE, St Paul, MN, USA Batch N853695	Urethane dimethacrylate (UDMA), Aromatic dimethacrylate (AUDMA), and 1,12-dodecane dimethacrylate, zirconia/silica and ytterbium trifluoride filler.
Charisma Smart Composite Heraeus Kulzer GmbH, Hanau, Germany Batch K010509	Barium Aluminum Fluoride glass, Bis-GMA, silicon dioxide
Filtek Z550 3M ESPE, St Paul, MN, USA Batch N887521	Bis-EMA, Bis-GMA, UDMA, TEGDMA, silica/zirconia (20 nm-3 μm, 81,8 vol%)
Ultradent Silane Ultradent, South Jordan, USA Batch BG3TD	MPS 5-15 wt %, pH 5,3 in Isopropanol

After the adhesive application, four CR cylinders were placed on the surface of each bulkfill CR disc using tygon tubes (1 mm x 1 mm) with repair composites. The CR used for repairing was polymerized with the same LED device for 20 s. The tygon tubes around the CR cylinders were carefully removed using a scalpel, and the samples were then kept in distilled water at 37 °C for 48 h. A total of 5000 thermal cycles (TC) (5-55 °C, a dwell time of 30 s) were performed to half of the samples in each group. The sample size was twelve in each group (n=12) (Figure 1). Figure 2 shows schematically how the samples were prepared.

Microshear Bond Strength Test

A microshear bond strength (µSBS) test was performed using a universal tester (Instron, Model 4444; Instron Corp., Canton, USA). A 0.25 mm thick
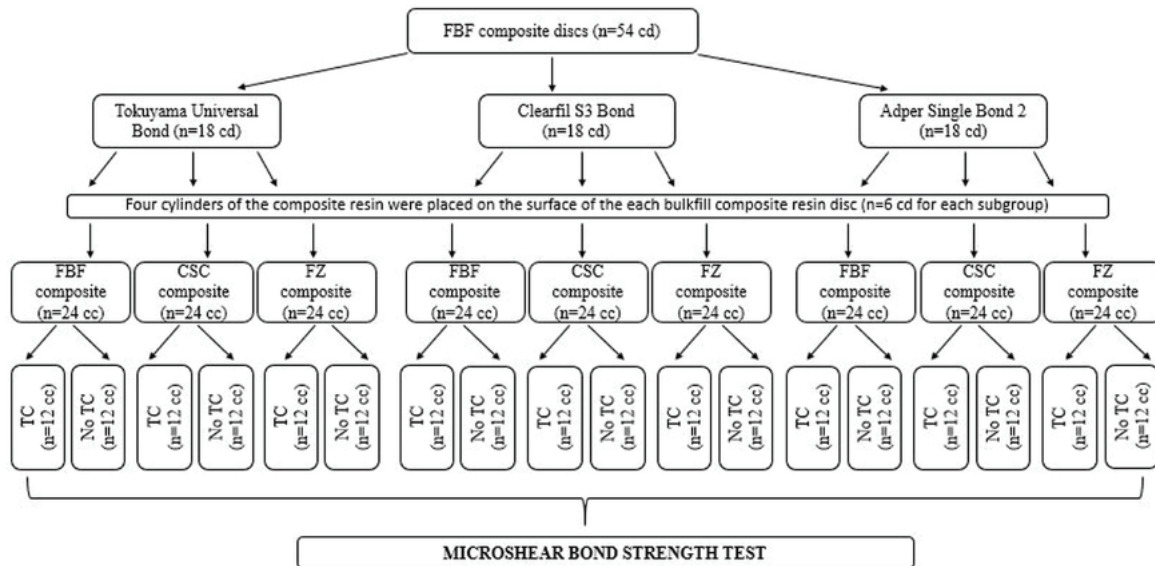
Figure 1. Study design and treatment groups
FBF: Filtek bulkfill, CSC: Charisma smart composite, FZ: Filtek Z55O, TC: Thermal cycling, cd: Composite discs, cc: Composite cylinders
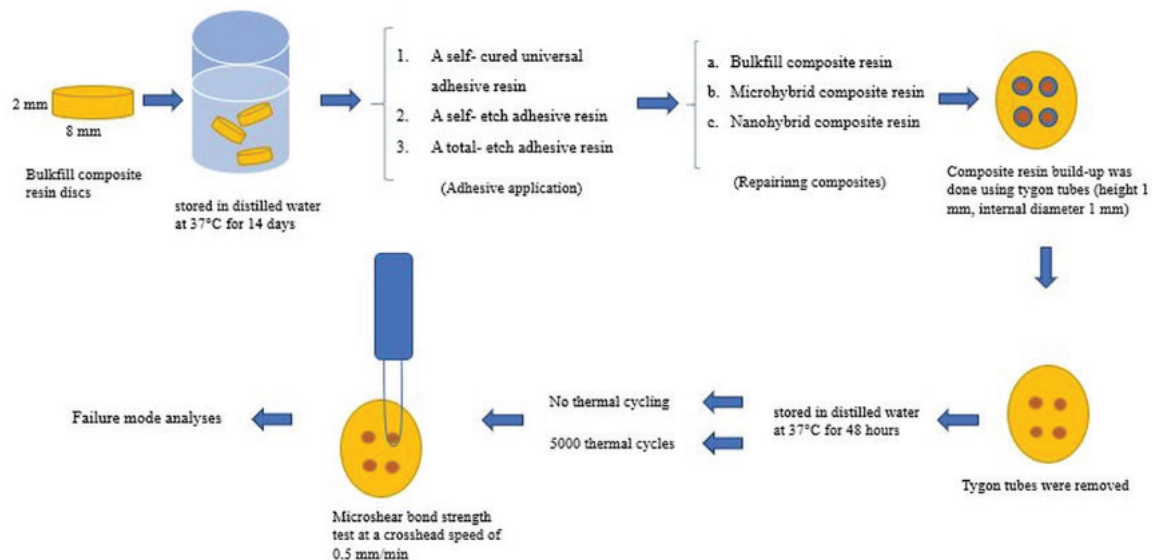
Figure 2. Shematic illustration of sample preparation and microshear bond strength test

wire loop which was placed around the CR cylinders contacting semicircularly were positioned at the bonding interface at a crosshead speed of 0.5 mm/min until failure occurred. µSBS test was applied to half of the samples without TC, while the other half of the samples were tested after the TC. The maximum force at failure was recorded in Newtons (N) and µSBS values were calculated as Megapascal (MPa) by dividing this force to the bonding area (mm²).

The failure areas were analyzed under an operation microscope (Stemi 1000, Zeiss, Germany) at 50× magnification to determine the type of failure, which

were classified as adhesive failure, cohesive failure or mixed failure (adhesive failure; fracture at the old CR-repair CR interface, cohesive failure; fracture in the old CR or repair CR, mixed failure; a combination of adhesive and cohesive failure).

Statistical Analysis

In this study, the SPSS 22 statistical package (IBM Inc, Chicago, IL, USA) were used for statistical tests. Descriptive statistics in the form of mean, standard deviation, median, frequency and ratio values were used with regard to the data. The distribution of variables was measured using the Kolmogorov

Table 2. The mean shear bond strength values (± SD), medians of the groups and statistically differences between the groups

Without TC	Tokuyama universal bond		Clearfil S3 bond		Single bond 2		p-value
	Mean ± SD	Median	Mean ± SD	Median	Mean ± SD	Median	
FBF	25.3±2.6	24.6	25.4±6.3	29.1^X	23±3.3*	22.7	0.21
CSC	20.3±5.2	19	19.3±6	19.6^Y	18.8±4.3	19	0.947
FZ	25.6±9.2	22.9	19.2±3.1	18.9^Y	20.7±6.4	19.8	0.249
p	0.064		0.035		0.073		
With TC	Tokuyama Universal Bond		Clearfil S3 bond		Single bond 2		p-value
	Mean ± SD	Median	Mean ± SD	Median	Mean ± SD	Median	
FBF	23.3±3.9	24.2^A	27.8±4.4	27.8^{B,X}	30.5±8.4*	31.1^{B,X}	0.013
CSC	22.1±7	21.6	24.8±8.3	25.8^{X,Y}	20.1±5.4	19.3^Y	0.353
FZ	25±7.2	26.8	19±3.2	18.6^Y	23.7±6.5	21.8^Y	0.06
p	0.345		0.003		0.01		

Different letters (X, Y) within columns indicate which groups have a statistically significant difference according to the type of composite resins (p<0.05). Different letters (A, B) within rows indicate which groups have a statistically significant difference according to the type of adhesive systems (p<0.05). Superscript "*" indicate the statistically difference between the groups with or without TC. SD: Standard deviation. TC: Thermal cycling, FBF: Filtek bulkfill, CSC: Charisma smart composite, FZ: Filtek Z55O

Table 3. Distribution of failure modes [n (%)]

Adhesive resins	Repair composite resins	Before TC			After TC		
		Adhesive	Cohesive	Mix	Adhesive	Cohesive	Mix
Tokuyama Universal bond	FBF	10 (83)	0 (0)	2 (17)	6 (50)	1(8)	5 (42)
	CSC	9 (75)	0 (0)	3 (25)	8 (67)	0 (0)	4 (33)
	FZ	9 (75)	0 (0)	3 (25)	8 (67)	0 (0)	4 (33)
Clearfil S3 bond	FBF	6 (50)	2 (17)	4 (33)	4 (33)	0 (0)	8 (67)
	CSC	5 (42)	2 (17)	5 (42)	6 (50)	0 (0)	6 (50)
	FZ	5 (42)	2 (17)	5 (42)	4 (33)	0 (0)	8 (67)
Single bond 2	FBF	12 (100)	0 (0)	0 (0)	10 (83)	0 (0)	2 (17)
	CSC	12 (100)	0 (0)	0 (0)	7 (58)	0 (0)	5 (42)
	FZ	12 (100)	0 (0)	0 (0)	8 (67)	0 (0)	4 (33)

FBF: Filtek bulkfill, CSC: Charisma smart composite, FZ: Filtek Z55O, TC: Thermal cycling

Simirnov test. Since the data distributions were not normal, non-parametric tests were used to determine the differences between the groups. Mann-Whitney U and Kruskal-Wallis tests were performed to detect any statistical differences between the variables, and to compare the groups.

Results

Table 2 gives the mean μSBS values of the samples. When the bond strengths of the groups without the TC were analyzed, no significant difference was found in terms of the adhesives used. Among the groups without TC, FBF showed higher μSBS when the repair composites were compared. Although FBF showed significantly higher μSBS than the other CR groups when used with CS3 (p=0.035), there was no statistically significant difference between the FZ repair composite group and the CSC repair composite group (p>0.05).

When the μSBSs of the groups with TC were examined, there was a statistically significant difference between the tested adhesives when FBF was used as the repair composite (p<0.05). However, there was no statistically significant difference between the adhesive resins which were used with other CR (FZ and CSC) (p>0.05). When TU was used as an adhesive, there was no significant difference between the CR (p>0.05). When CS3 was used as an adhesive, FBF showed the highest μSBS value. However, there was no statistically significant difference between the FBF and the CSC groups while there was a statistically significant difference between FBF and FZ groups (p>0.05). When SB2 was used as an adhesive, the FBF showed significantly higher μSBS than other CR groups (p<0.05). Furthermore, there was no significant difference between the FZ and the CSC groups (p>0.05).

When the RBS values of the groups before and after TC were compared, no statistically significant difference was observed except the SB2 + FBF group (p=0.018 for the SB2 + FBF group).

The fracture types of the samples are shown in Table 3. In our study, mostly the adhesive type failure was observed. This was followed by mixed and cohesive failures.

Discussion

Secondary caries, microleakage, discoloration, chipping and fracture are the most common reasons for failure of CR restorations. Recently, repairing of defective restorations has been recommended because repair has a limited risk of complications, and it reduces the loss of sound tooth structure when compared to total replacement. Furthermore, repair could decelerate the so-called restoration cycle, given that the replacement would lead to a greater scale of preparation (11).

Bulkfill CRs are very popular materials among clinicians as they offer ease of application, thereby eliminating time-consuming layering procedures. In literature, there is no study evaluating the effect of a silane containing self-cure adhesive in the repair of bulkfill composites. Therefore, in this study, the influences of thermal cycling, different CRs and adhesive resins on the RBS to a bulkfill CR were evaluated. The tested hypotheses, which suggest that there is no difference between the RBSs of different types of adhesives, and that thermal cycling does not influence the RBS of the bulkfill CR, were partially accepted. The null hypothesis which suggest that there is no difference between the RBSs of the tested CRs was rejected.

The use of convenient surface treatment methods, and the selection of the most suitable repair material and adhesive systems, are critical for successful repair. Different surface conditioning methods, including mechanical, chemical and physicochemical surface treatments, have been proposed in order to achieve a durable bonding between old and new CRs (12). Air abrasion with aluminum oxide is one of the most frequently-used mechanical roughening methods. In previous studies, it was found that air abrasion with Al_2O_3 (50-μm) significantly improved the RBS (9,13). In addition, the use of a silane agent during the repair of CR increases the wettability of the repair surface, and promotes chemical bonding by forming siloxane bonds between the silica-containing filler particles exposed on the surface to be repaired, and the resin matrix of the new resin layer (9,14). Due to the positive contributions mentioned above, the surfaces of the substrates were roughened with Al_2O_3 and a silane agent was applied in this study.

The adhesive system is another important factor in terms of improving the RBS between old and new CRs. The adhesive resins used in this study are in different generations, and contain different functional monomers and solvents. The SB2 is an etch-and-rinse adhesive which is water-ethanol disperse, contains both hydrophilic and hydrophobic resin monomers. When applied to a surface, monomer molecules orientate per the nature of the hydrophobic or hydrophilic surface, providing a good contact with the surface. The nature of SB2 creates a potential for adhesion on surfaces with different wetting properties (15). The CS3 is a self-etch adhesive which comprises 10-MDP (10-Methacryloyloxydecyl dihydrogen phosphate) and ethanol. 10-MDP, which is a functional monomer, can bond chemically to Ca^{+2} and make the bonding interface more resistant to degradation (16). The TU is a self-cured universal adhesive which contains an acidic three-dimensional self-reinforcing monomer (3D-SR), silane coupling agent (γ-MPTES), and acetone. The adhesive systems used in this study did not significantly affect the RBS of the tested groups. This may be due to the fact that they all contain different monomers all of which contribute to bond strength. Similarly, a previous study evaluated the influence of different adhesive systems (a self-etch adhesive, an etch-and-rinse adhesive and a universal adhesive) on the μSBS of repaired bulkfill CRs and it was found that the adhesive systems used did not significantly affect the RBS of the tested groups, and is therefore in consistent with the results of our study (17). Moreover, adhesive bonding to enamel is thought as the gold standard of bond strength between aged and fresh CRs (7). In literature, it was reported that a RBS between 15 and 30 MPa would be clinically acceptable, similar to CR to enamel bond strength (17). In the present study, the RBS values of all tested adhesive resins were within the acceptable range.

Bulkfill CRs have become increasingly popular among dental practitioners since they minimize some of the disadvantages of incremental layering, and decrease the application time (12). However, in the literature, there are a limited number of studies about the repair of aged bulkfill CRs with different types of CRs. In this *in vitro* study, this issue is also investigated, and the groups repaired with bulkfill CR showed higher μSBS than did the other CR groups. Our findings are in

agreement with previous studies, indicating that the use of the same CR is more appropriate when it comes repairing CR (12,17,18). Moreover, Cuevas-Suárez et al. (19) investigated the effect of different surface treatments on the bond strength of bulkfill CRs repaired with bulkfill or conventional nanoparticle composites. They reported that using the same bulkfill CR in the repair process could improve the effectiveness of the procedure (19). In contrast to findings of our study, in a study investigating the ability of posterior CR to repair aged bulkfill CR, the authors reported that the resin composite repair type did not affect the bond strength, and that the aged bulkfill CR could be effectively repaired with posterior CR (20). In addition, they emphasized the importance of the use of proper repair protocol, by specifying that the combined use of 10% hydrofluoric acid (HF) etching and adhesive resin would provide efficient RBS when the aged bulkfill CR is to be repaired using conventional CR. This discrepancy between the studies can be explained by the different surface treatment strategies, and the different materials used. Intraoral use of HF may be inconvenient because HF contamination to the skin or mucosa may cause necrosis in the deeper layers of the tissue (21). In addition, a calcium fluoride (CaF_2) precipitate is formed when HF is in direct contact with dentin and enamel. This precipitate prevents the adhesive resin infiltration into dentin tubules and the adhesion of CR is adversely affected (22). Moreover, the effectiveness of HF used for composite repair has been shown to be related to some properties of the inorganic filler such as the percentage, size, and type (23). Therefore, the use of HF as a routine procedure for composite repair is not recommended, especially if the composition of the old CR is not exactly known (24).

Aging has a key role in evaluating the RBS of CRs. Thermocycling is one of the commonly used aging methods in *in vitro* studies and simulates the stress created by changing the environmental temperature at the interface between materials (25). In the present study, 5000 TCs were performed between 5 and 55 °C with a dwell time of 30 s and it was found that the composite RBS was not negatively affected. Our findings are consistent with the results of a previous study which reported the RBS of CR was not affected by the aging conditions (5000 TCs) (25). On the other hand, in the literature, there are studies that 5000 TCs

significantly decrease the RBS of CR (3,4). Şişmanoğlu et al. (26) evaluated the influence of different universal adhesive resins and surface treatment methods on the RBS of bulkfill CRs, with and without TCs. They found that 5000 TCs significantly reduced the RBS with the exception of two adhesive group. They attributed this to the fact that these adhesives contained 10-MDP. In our study, 5000 TCs did not decrease the RBS in any groups, regardless of whether it contains 10-MDP. The difference between our results and those of these studies may be due to additional silane application, different ingredients and different chemical properties of the tested materials, and different testing conditions. However, it is estimated that 10000 TCs correspond to approximately one-year clinical functioning, when considering that thermocycling may occur 20-50 times a day (27). In this study, the samples were subjected to 5000 TCs which mimics a relatively short term of clinical function (approximately six-months). For this reason, thermal cycling may not adversely affect the RBS and it might be useful to perform additional studies with a larger number of cycles.

In the present study, a µSBS test method was preferred because it is considered an effective method for verifying the bond strength of materials, and it is a good representation of the forces clinically experienced by a restoration. Furthermore, the µSBS test has some advantages, including less rigorous sample preparation, and easier control of the bonding test area by means of tygon tubes (28).

This study was conducted in an *in vitro* environment without considering various factors such as saliva, dietary variables, and occlusal forces, and only CR samples were tested. In addition, the low number of TC is another limitation of our study. Therefore, further studies designed in *in vivo* and *in vitro* conditions are needed to confirm the results of this in vitro study.

Conclusion

Within the limitations of this study, it can be concluded that:

1. The use of bulkfill composite resin for repairing bulkfill composite resin would provide a more efficient RBS.

2. 5000 TCs (5 to 55 °C) did not negatively affect the composite repair bond strength.

3. All the tested adhesive systems can be used safely to repair bulkfill CRs.

However, additional studies with regard to bulkfill CR and self-cured adhesives should be performed.

Ethics

Ethics Committee Approval: In this study, ethics committee approval is not required because any material or tissue of human or animal is not used.

Informed Consent: This study does not require patient consent.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

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

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

Financial Disclosure: The authors declared that this study received no financial support.

References

1. Murdoch-Kinch CA, McLean ME. Minimally invasive dentistry. J Am Dent Assoc 2003; 134: 87-95.

2. Akova T, Ozkomur A, Uysal H. Effect of food-simulating liquids on the mechanical properties of provisional restorative materials. Dent Mater 2006; 22: 1130-4.

3. Rinastiti M, Özcan M, Siswomihardjo W, Busscher HJ. Effects of surface conditioning on repair bond strengths of non-aged and aged microhybrid, nanohybrid, and nanofilled composite resins. Clin Oral Investig 2011; 15: 625-33.

4. Ozcan M, Barbosa SH, Melo RM, Galhano GA, Bottino MA. Effect of surface conditioning methods on the microtensile bond strength of resin composite to composite after aging conditions. Dent Mater 2007; 23: 1276-82.

5. Eliasson ST, Tibballs J, Dahl JE. Effect of different surface treatments and adhesives on repair bond strength of resin composites after one and 12 months of storage using an improved microtensile test method. Oper Dent 2014; 39: E206-16.

6. Ergücü Z, Türkün LS, Aladag A. Color stability of nanocomposites polished with one-step systems. Oper Dent 2008; 33: 413-20.

7. Jung M, Sehr K, Klimek J. Surface texture of four nanofilled and one hybrid composite after finishing. Oper Dent 2007; 32: 45-52.

8. Burgess J, Cakir D. Comparative properties of low-shrinkage composite resins. Compend Contin Educ Dent 2010; 31: 10-5.

9. Fornazari IA, Wille I, Meda EM, Brum RT, Souza EM. Effect of Surface Treatment, Silane, and Universal Adhesive on Microshear Bond Strength of Nanofilled Composite Repairs. Oper Dent 2017; 42: 367-74.

10. Rosa WL, Piva E, Silva AF. Bond strength of universal adhesives: A systematic review and meta-analysis. J Dent 2015; 43: 765-76.

11. Loomans B, Özcan M. Intraoral Repair of Direct and Indirect Restorations: Procedures and Guidelines. Oper Dent 2016; 41: S68-78.

12. Koç-Vural U, Kerimova L, Baltacioglu İH, Kiremitçi A. Bond strength of dental nanocomposites repaired with a bulkfill composite. J Clin Exp Dent 2017; 9: e437-42.

13. Cho SD, Rajitrangson P, Matis BA, Platt JA. Effect of Er,Cr:YSGG laser, air abrasion, and silane application on repaired shear bond strength of composites. Oper Dent 2013; 38: E1-9.

14. Altinci P, Mutluay M, Tezvergil-Mutluay A. Repair bond strength of nanohybrid composite resins with a universal adhesive. Acta Biomater Odontol Scand 2017; 4: 10-9.

15. Kouros P, Koliniotou-Koumpia E, Spyrou M, Koulaouzidou E. Influence of material and surface treatment on composite repair shear bond strength. J Conserv Dent 2018; 21: 251-6.

16. Yoshida Y, Yoshihara K, Nagaoka N, Hayakawa S, Torii Y, Ogawa T, et al. Self-assembled Nano-layering at the Adhesive interface. J Dent Res 2012; 91: 376-81.

17. Oglakci B, Arhun N. The shear bond strength of repaired high-viscosity bulk-fill resin composites with different adhesive systems and resin composite types. J Adhes Sci Technol 2019; 33: 1584-97.

18. Gönülol N, Almasifar L, Cabadağ ÖG, Misilli T, Kalyoncuoğlu E. Shear Bond Strength of Aged Composite Restorations Repaired With a Universal Injectable Composite. Eur J Ther 2019; 25: 273-8.

19. Cuevas-Suárez CE, Nakanishi L, Isolan CP, Ribeiro JS, Moreira AG, Piva E. Repair bond strength of bulk-fill resin composite: Effect of different adhesive protocols. Dent Mater J 2020; 39: 236-41.

20. Ayar MK, Guven ME, Burduroglu HD, Erdemir F. Repair of aged bulk-fill composite with posterior composite: Effect of different surface treatments. J Esthet Restor Dent 2019; 31: 246-52.

21. Asvesti C, Guadagni F, Anastasiadis G, Zakopoulou N, Danopoulou I, Zographakis L. Hydrofluoric acid burns. Cutis 1997; 59: 306-8.

22. Loomans BA, Mine A, Roeters FJ, Opdam NJ, De Munck J, Huysmans MC, et al. Hydrofluoric acid on dentin should be avoided. Dent Mater 2010; 26: 643-9.

23. Lucena-Martín C, González-López S, Navajas-Rodríguez de Mondelo JM. The effect of various surface treatments and bonding agents on the repaired strength of heat-treated composites. J Prosthet Dent 2001; 86: 481-8.

24. Yesilyurt C, Kusgoz A, Bayram M, Ulker M. Initial repair bond strength of a nano-filled hybrid resin: effect of surface treatments and bonding agents. J Esthet Restor Dent 2009; 21: 251-60.

25. Magni E, Ferrari M, Papacchini F, Hickel R, Ilie N. Influence of ozone on the composite-to-composite bond. Clin Oral Investig 2011; 15: 249-56.

26. Şişmanoğlu S, Gürcan AT, Yıldırım-Bilmez Z, Gümüştaş B. Efficacy of different surface treatments and universal adhesives on the microtensile bond strength of bulk-fill composite repair. J Adhes Sci Technol 2020; 34: 1115-27.

27. Xie C, Han Y, Zhao XY, Wang ZY, He HM. Microtensile bond strength of one- and two-step self-etching adhesives on sclerotic dentin: the effects of thermocycling. Oper Dent 2010; 35: 547-55.

28. de Jesus Tavarez RR, Almeida Júnior LJDS, Guará TCG, Ribeiro IS, Maia Filho EM, et al. Shear bond strength of different surface treatments in bulk fill, microhybrid, and nanoparticle repair resins. Clin Cosmet Investig Dent 2017; 9: 61-6.

Plantar Sole Characteristics are Associated with Postural Stability

Plantar Taban Özellikleri Postüral Stabilite ile İlişkilidir

🄳 Ayfer Metin Tellioğlu[1], 🄳 Elif Aydın[2], 🄳 Eda Duygu İpek[1], 🄳 İlknur Can[3]

[1]Aydın Adnan Menderes University Faculty of Medicine, Department of Anatomy, Aydın, Turkey
[2]Aydın Adnan Menderes University Faculty of Medicine, Department of Physical Medicine and Rehabilitation, Aydın, Turkey
[3]Medipol University Esenler Hospital, Clinic of Physical Medicine and Rehabilitation, İstanbul, Turkey

Abstract

Objective: The foot is the endpoint of the lower extremity kinetic chain and the role of the anthropometric measurements of the plantar sole in postural steadiness has not been established well. This study aimed to assess the association between plantar sole dimensions and body sway.

Materials and Methods: One hundred ten young healthy volunteers were recruited for the study. The postural stability was measured using the modified Clinical Test of Sensory Interaction on Balance and the Tandem Stance test on a force platform. Anthropometric assessments were performed on a digital footprint image that was recorded by the same force platform. Maximum foot length, maximum foot width, heel width, medial longitudinal arch width, total contact area, foot index, Clarke's angle (CA) and Staheli Arch index (SAI) were recorded.

Results: Foot length and foot width had the reverse relationship with body sway when visual and somato-sensory inputs were limited. Postural stability was better with increasing foot contact area in cushioned and eyes closed conditions. Postural stability worsened with increasing values of SAI and CA when somato-sensory and visual inputs limited.

Conclusion: This study revealed that plantar sole dimensions and foot postural influence balance, especially when the somato-sensorial and visual systems are challenged together.

Address for Correspondence/Yazışma Adresi:
Eda Duygu İpek MD,
Aydın Adnan Menderes University Faculty of Medicine, Department of Anatomy, Aydın, Turkey
Phone : +90 256 218 20 00
E-mail : eda.cakir@adu.edu.tr

ORCID ID: orcid.org/0000-0002-9851-6157

Öz

Amaç: Alt ekstremite kinetik zincirinin son noktası ayaktır. Ayak tabanının antropometrik parametrelerinin postüral stabilite üzerindeki etkisi iyi belirlenmemiştir. Bu çalışmada ayak tabanının antropometrik değerleri ile vücut salınımı arasındaki ilişkiyi değerlendirmeyi amaçladık.

Gereç ve Yöntemler: Araştırmaya toplam 110 gönüllü katıldı. Postüral stabilite Tandem Stance testi ve Denge Üzerindeki Duyusal Etkileşim Klinik testi kullanılarak kuvvet platformunda ölçüldü. Antropometrik ölçümler platform tarafından kaydedilen dijital ayak izi görüntüsü üzerinde gerçekleştirildi. Maksimum ayak uzunluğu, topuk genişliği, maksimum ayak genişliği, ayak indeksi, toplam temas alanı, Clarke açısı (CA) ve Staheli Ark indeks (SAİ) değerleri kaydedildi. Verilerin istatiksel analizi %95 güven aralığında, SPSS versiyon 22.0 kullanılarak gerçekleştirildi, tanımlayıcı istatistikler ortalama ± standart sapma olarak ifade edildi, p<0,05 değeri istatiksel olarak anlamlı kabul edildi. Veriler arasındaki ilişkiler Pearson korelasyon analizi ile değerlendirildi.

Bulgular: Görsel ve somato-duyusal girdiler sınırlandırıldığında ayak genişliği ve ayak uzunluğu, vücut salınımı ile ters orantılı olarak bulundu. Postüral stabilitenin

gözler kapalı iken yumuşak zeminde ayak temas alanı arttıkça daha iyi olduğu tespit edildi. Görsel ve somato-duyusal girdiler sınırlı olduğunda CA ve SAİ'nin artan değerleri ile postüral stabilite kötüleşti.

Sonuç: Bu çalışma, ayak duruşu ve plantar taban boyutlarının özellikle görsel ve somato-duyusal sistemler sınırlandırıldığında dengeyi etkilediğini ortaya koymuştur.

Introduction

Postural stability represents the act of maintaining the body in a state of balance and requires constant integrations of vestibular, visual, musculoskeletal, and environmental factors. Postural steadiness and balance is fundamental to activities of daily living and sports. Aging, neurodegenerative diseases, musculoskeletal impairments such as contractures, muscle weakness, and limited range of motion interfere with postural control. Understanding factors that contribute to postural stability can help to improve motor strategies in sports and rehabilitation.

The foot is the endpoint of the lower extremity kinetic chain and contact surface of our body to the environment. The plantar sole bears and conducts all the forces of the body to the surface. Vice versa, the plantar sole carries tactile information from the supporting surface to the central nervous system in order to maintain balance. The foot has a relatively small base for maintaining postural control and small alterations of its structure may give cause for load changes during posture correction. Therefore, the maintenance of postural stability when performing functional tasks can be influenced by foot characteristics.

The association between foot and postural control has been investigated in many studies. Studies have shown foot posture, strength, range of motion, and foot angle are contributing factors on postural stability (1-5). However, there is little published data on the possible relation between plantar sole dimensions and postural stability. A previous study by Molikova et al. (6) established that postural stability was related to foot width and length. Another study conducted by Angın et al. (1) focused on the relation between standardized foot size (calculated as foot width divided by foot length) and postural sway on unilateral stance. The authors observed decreased sway velocity with increasing standardized foot size. However, the foot is a complex structure and every single part plays fundamental roles in functional activities. The size and settlement of every bone and joint can differ in all individuals and this is independent from body proportions, height, and weight. Accordingly, if we intend to understand the foot's contribution to balance, it is not sufficient to limit foot dimensions only to length and width.

Although some research has studied the influence of foot length and width on postural sway, there is still very little scientific understanding of the relation between postural stability and detailed anthropometric measurements of the foot sole. Therefore, the aim of this present study was to find and discuss any association between plantar sole dimensions and body sway.

Materials and Methods

Participants

In this study a cross-sectional design was used. One hundred ten young healthy volunteers from the student population of a medical faculty were recruited for the study. Exclusion criteria were a history of neurologic, visual or vestibular disorder and orthopedic disorders. The study was approved by the local ethics committee (protocol no: 2017/1192, date: 06.07.2017) and each participants signed informed consent form.

Postural Stability Assessment

Measurement of postural stability was performed using the modified Clinical Test of Sensory Interaction on Balance (mCTSIB) and the Tandem Stance test (TS) on a force platform (RScan International, Olen, Belgium) according to procedures described by Cohen et al. (7) This platform consisted of 4096 sensors with a data acquisition frequency of 300 Hz. The active sensor area was 325 mm x 488 mm, which was synchronized to a personal computer.

The mCTSIB consists of four conditions for balance assessment: eyes closed on a firm surface (EC), eyes open on a firm surface (EO), eyes closed on a foam-cushioned surface (CEC) and eyes open on a foam-cushioned surface (CEO). Each condition of the mCTSIB and TS were repeated 3 times for 10 seconds. Each mean value of three measurements

was taken as a final score and included into the statistical analysis.

Anthropometric Measurements

Anthropometric assessments were performed on a digital footprint image that was recorded by the force platform (Figure 1). All measurements were made using RSscan® software.
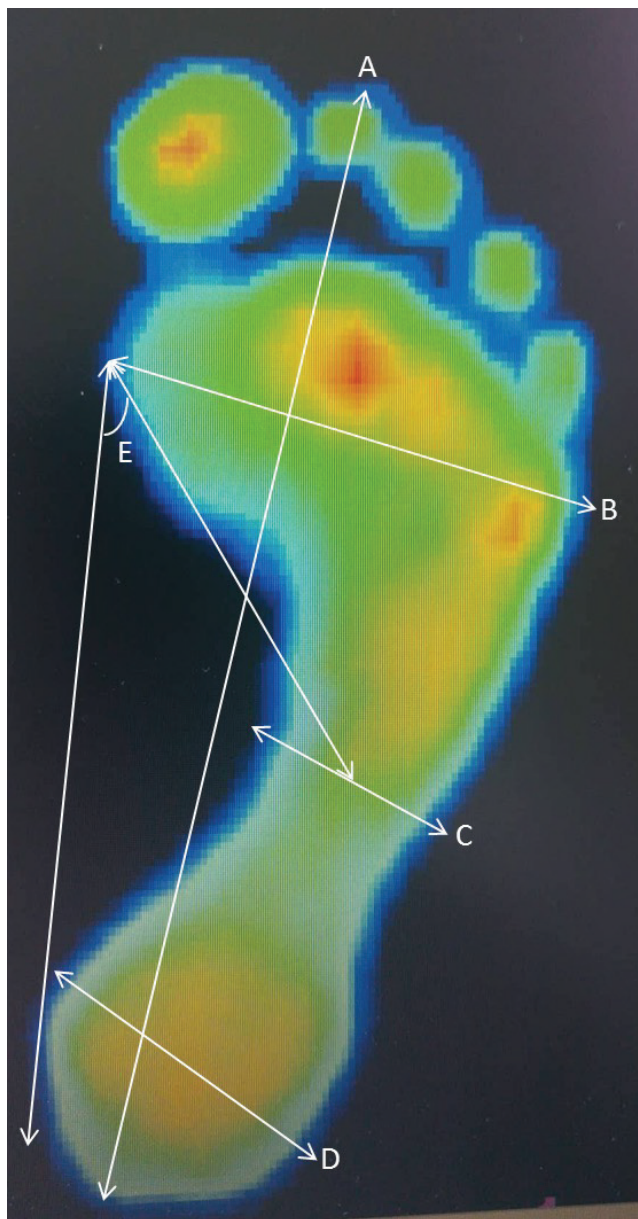
Figure 1. Representation of anthropometric measurements on digital footprint image. A line: maximum foot length; B line; maximum foot width; C line; medial longitudinal arch width; D line; heel width; Clarke's angle: E, Staheli Arch index: C/D

Maximum foot length and width were measured from the longest and widest points of the foot. Heel width and medial longitudinal arch width were recorded as well. Total plantar contact area was calculated using specific software. Also, foot index (FI) was calculated by dividing foot width (cm) by foot length (cm).

Clarke's angle (CA) is an objective, reliable and sensitive method for measuring the height of the internal longitudinal arch (8,9). It is defined as an angle between a line that joins the more internal point of the forefoot and the more internal point of the rear foot with another line that joins the more internal point of the forefoot with the deeper part of the footprint. While CA under 31 °C refers tendency to flat feet and/or pronation, above 41 °C refers to cavus foot.

The Staheli Arch index (SAI) is the midfoot width to hindfoot width ratio, which is calculated to illustrate foot arch development (10). A lower index value means a higher arch.

Statistical Analysis

Statistical analysis of the data was performed using SPSS version 22.0 at 95% confidence interval, descriptive statistics were expressed as mean ± standard deviation, $p < 0.05$ was considered significant. Relationships between variables were evaluated with Pearson's correlation test.

Results

In total, 110 subjects 44 females (height 165.4±7.2 cm, and weight 59.2±11.3 kg) and 66 males (height 178.1±6.6 cm, and weight 75.2±12.7 kg) participated in the study. The mean age of the subjects was 20.3±1.6 years.

Table 1 shows the results of the correlation analyses between mCTSIB and plantar sole anthropometric measurements. Foot length had reverse relationship with body sway when visual input was eliminated. This relation was more significant when both visual and somato-sensory input were limited. The same relationship was observed for foot width; postural sway decreased significantly with increasing foot width in cushioned EC measurements. CA was significantly related to body sway in the CEC position. The body became more stable with increasing CA. Postural stability was better with increasing foot contact area in cushioned EC conditions. The SAI was significantly

Table 1. Associations between the modified anthropometric measurements of the plantar sole and Clinical Test of Sensory Interaction scores

	AP-EO	ML-EO	TSW-EO	AP-EC	ML-EC	TSW-EC	AP-CEO	ML-CEO	TSW-CEO	AP-CEC	ML-CEC	TSW-CEC
Foot length	0.072	0.050	0.061	-0.169	-0.177	0.192*	-0.159	-0.156	-0.176	-0.215*	-0.206*	-0.229*
Foot width	-0.161	-0.023	-0.078	-0.066	-0.166	-0.133	-0.023	-0.025	0.033	-0.113	-0.098	-0.112
Heel width	0.100	0.132	0.049	0.124	0.092	0.005	-0.001	0.036	-0.052	0.045	-0.007	-0.008
MLA width	-0.042	-0.016	-0.056	-0.013	-0.043	-0.019	0.140	0.091	0.302**	0.075	0.025	-0.007
PCA	0.004	0.020	-0.095	-0.057	-0.133	-0.160	-0.081	-0.086	-0.107	-0.166	-0.146	-0.190*
FI	-0.211*	-0.069	-0.120	-0.072	-0.021	0.021	0.110	0.100	0.173	0.048	0.062	0.058
SAI	-0.053	-0.030	-0.053	-0.024	-0.050	-0.021	0.144	0.092	0.308**	0.073	0.027	0.025
CA	0.055	0.000	0.015	0.016	-0.033	-0.002	-0.019	0.019	-0.091	-0.204*	-0.061	0.027

AP: Antero-posterior, ML: Medio-lateral, EO: Eyes open, EC: Eyes closed, CEO: Cushioned eyes open, CEC: Cushioned eyes closed, TSW: Total sway, MLA: Medial longitudinal arch, FI: Foot index, PCA: Plantar contact area, SAI: Staheli Arch index, CA: Clarke's angle, *p<0.05, **p<0.01

related to total sway when somato-sensory input was limited. Postural stability worsened with increasing values of SAI. Wider heel width was significantly associated with greater antero-posterior body sway in TS when visual input was eliminated.

The associations between postural sway in a TS and anthropometric measurements of the plantar sole are represented in Table 2. There was no relationship between body sway in the TS position and plantar sole measurements.

The correlation analysis between foot width, FI, and body sway revealed no significant influence of these variables on postural stability.

Discussion

The present study investigated the impact of the structural properties of the foot to postural stability. The most significant body sway alterations observed were foot dimensions and foot posture when somato-sensory and visual inputs were limited. This result highlights the importance of foot structure on maintaining posture when other components of balance control are deprived.

In the present study, healthy individuals with smaller foot dimensions according to foot length, foot width and FI, demonstrated a significant increase in total body sway during bipedal stance on foam when their eyes were closed. This finding indicated that increasing foot dimensions are associated with better postural stability, especially on uneven

Table 2. Associations between anthropometric measurements of the plantar sole and postural sway in tandem stance

	AP-EO	ML-EO	TSW-EO
Foot length	-0.079	0.067	-018
Foot width	0.031	-0.036	-0.170
Heel width	0.021	0.134	-0.003
MLA width	0.059	0.133	0.095
PCA	-0.134	0.011	-0.091
FI	-0.042	-0.080	-0.088
SAI	0.059	0.124	0.103
CA	-0.158	-0.097	-0.060

AP: Antero-posterior, ML: Medio-lateral, EO: Eyes open, TSW: Total sway, FI: Foot index, PCA: Plantar contact area, SAI: Staheli Arch index, CA: Clarke's angle, *p<0.05, **p<0.01

surfaces and with limitation of visual input. This finding is reasonable because the foot neutralizes the forces produced by the body in order to provide postural correction. When somato-sensory and visual systems are challenged, people who have larger foot dimensions will have the advantage of a relatively wider base of support. In addition, people need more tactile stimulation from the surface when visual input is limited. A relatively larger contact surface area would provide more cutaneous sensation.

Parallel to our results, previous literature demonstrated diminished body sway in individuals with larger plantar contact area (1). Authors revealed that foot size was inversely related with postural

sway velocity. They explained this entity by the larger support surface. Different from these previous studies, we found that foot dimensions were not important until the surface became rough. This result is important for the performance of people doing outdoor recreational and sporting activities.

Footprint analysis is one of the clinical approaches for identifying flatfoot. The Staheli plantar arch index and CA were proposed as indicators of plantar arch development. Our findings revealed that participants exhibiting more pronated feet had poorer performance in postural control when their eyes were closed. We confirmed this result both with the SAI and CA.

Previous literature has presented contradictory findings about the effect of foot posture on postural stability. Angın et al.'s (1) findings revealed that people with prone foot posture had increased sway velocity of center of gravity in a unilateral stance when their eyes were closed. Similarly, in another survey conducted by Spink et al. (4) older volunteers with prone foot posture demonstrated worse performance in postural sway control on foam. In the same vein, Cobb et al. (11) demonstrated higher antero-posterior postural sway in younger people with forefoot varus. Contrary to these results, there are findings that support increased postural sway in individuals with less plantar contact surface. Lin et al. (12) found higher sway area with eyes closed on foam in children aged 4-5 years. On the other hand, their results were not significant in children aged between 8 and 9 years when the foot arch development had been occurred. Another study conducted by Hertel et al. (2) demonstrated higher postural sway area in young individuals with pes cavus. We found increased body sway scores among volunteers with more pronated feet. The findings of our study were confirmed with two common quantitative assessment systems that were designed for the analysis of flatfoot.

These results suggest that prone foot posture could be a contributing factor in impaired postural stability. There are a number of possible explanations for this: 1) with increasing arch index values, increased stress is placed on the osseous, muscular, and ligamentous supports of the foot arch, which may disturb proper weight bearing and provide higher postural sway; 2) it is reasonable to assume that increased pronation of the foot influences lower extremity kinematics, which could also influence postural control strategies.

Our results imply that the influence of the heel width to body sway is very subtle. A possible explanation for this is that forefoot joints are fundamental in giving elasticity to the foot during stance and they allow it to adapt better to unreliable surfaces. So, forefoot dimensions have an impact on balance. On the other hand, the rear foot comprises the ankle joint and the talocalcaneonavicular joint. The rear foot joints secure movement in at least one direction and the inclination angle of the ankle joint contributes to stability during stance. Hence, a foot with larger forefoot dimensions could be able to react and neutralize forces that contribute to body sway more sufficiently.

This study has several limitations. As our study did not include three-dimensional measurements of the foot structure, it is not clear whether our findings represent the real impact of foot structure on postural sway. In addition, postural control of the whole body requires the participation of the postural muscles of the trunk and lower extremities. Thus, the results of this study could be limited to the postural correction strategies of the foot but not whole-body postural control. Other anthropometric measurements of the body could influence balance but they were not the subject of this present study. These limitations could be the target of future studies.

Conclusion

There is very little scientific understanding of the relation between postural stability and detailed anthropometric measurements of the foot sole. Therefore, we were assess the association between plantar sole dimensions and body sway.

This study revealed that plantar sole dimensions and foot posture influence balance, especially when the somato-sensorial and visual systems are challenged together. It seems more reasonable to analyze foot characteristics that influence balance for guiding training facilities in sports and recreational activities in order to achieve a more stable balance and to prevent injuries.

Ethics

Ethics Committee Approval: The present cohort study was designed as a survey and was approved by the Clinical Research Ethics Committee of Adnan Menderes University (protocol no: 2017/1192, date: 06.07.2017)

Informed Consent: Written consents from participants had taken and kept in files separated for each one.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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

Financial Disclosure: The authors declared that this study received no financial support.

References

1. Angın S, Ilçin N, Yeşilyaprak SS, Simşek IE. Prediction of postural sway velocity by foot posture index, foot size and plantar pressure values in unilateral stance. Eklem Hastalik Cerrahisi 2013; 24: 144-8.

2. Hertel J, Gay MR, Denegar CR. Differences in Postural Control During Single-Leg Stance Among Healthy Individuals With Different Foot Types. J Athl Train 2002; 37: 129-32.

3. Cote KP, Brunet ME, Gansneder BM, Shultz SJ. Effects of Pronated and Supinated Foot Postures on Static and Dynamic Postural Stability. J Athl Train 2005; 40: 41-6.

4. Spink MJ, Fotoohabadi MR, Wee E, Hill KD, Lord SR, Menz HB. Foot and ankle strength, range of motion, posture, and deformity are associated with balance and functional ability in older adults. Arch Phys Med Rehabil 2011; 92: 68-75.

5. Bruyneel AV, Bertrand M, Mesure S. Influence of foot position and vision on dynamic postural strategies during the "grand plié" ballet movement (squatting) in young and adult ballet dancers. Neurosci Lett 2018; 678: 22-8.

6. Molikova R, Bezdickova M, Langova K, Holibka V, David O, Michalikova Z, et al. The relationship between morphological indicators of human body and posture. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 2006; 150: 261-5.

7. Cohen H, Blatchly CA, Gombash LL. A study of the clinical test of sensory interaction and balance. Phys Ther 1993; 73: 346-51; discussion 351-4.

8. Clarke HH. An objective method of measuring the height of the longitudinal arch in foot examinations. Res Q 1933; 4: 99-107.

9. Pauk J, Ihnatouski M, Najafi B. Assessing plantar pressure distribution in children with flatfoot arch: application of the Clarke angle. J Am Podiatr Med Assoc 2014; 104: 622-32.

10. Staheli LT, Chew DE, Corbett M. The longitudinal arch. A survey of eight hundred and eighty-two feet in normal children and adults. J Bone Joint Surg Am 1987; 69: 426-8.

11. Cobb SC, Tis LL, Johnson BF, Higbie EJ. The effect of forefoot varus on postural stability. J Orthop Sports Phys Ther 2004; 34: 79-85.

12. Lin CH, Lee HY, Chen JJ, Lee HM, Kuo MD. Development of a quantitative assessment system for correlation analysis of footprint parameters to postural control in children. Physiol Meas 2006; 27: 119-30.

Original Article / Özgün Araştırma

Comparison of the Nickel Titanium Alloy Archwires' Dimensions with the Mean Arch Dimensions of a Turkish Sample

Nikel Titanyum Alaşımı Ark Tellerinin Ark Boyutlarının Türk Örneklem Grubunun Ortalama Ark Boyutları ile Karşılaştırılması

 Berza Şen Yılmaz[1], Sevan Erkan[2], Ezgi Sunal Aktürk[1], Kübra Yaradanakul[3]

[1]Bezmialem Vakıf University Faculty of Dentistry, Department of Orthodontics, İstanbul, Turkey
[2]Bezmialem Vakıf University Faculty of Dentistry, İstanbul, Turkey
[3]Bezmialem Vakıf University Health Sciences Institute, Department of Orthodontics, İstanbul, Turkey

Address for Correspondence/Yazışma Adresi:
Ezgi Sunal Aktürk DDS, Asst. Prof.,
Bezmialem Vakıf University, Faculty of
Dentistry, Department of Orthodontics,
İstanbul, Turkey
Phone : +90 212 453 18 50
E-mail : sunalezgi@gmail.com

ORCID ID: orcid.org/0000-0001-8192-2784

Abstract

Objective: This study aimed to evaluate the harmony between the mandibular arch dimensions of a Turkish sample that has Class I occlusion without orthodontic treatment history and the arch dimensions of archwires produced by different manufacturers.

Materials and Methods: Mandibular dental casts were fabricated from 40 individuals with dentoskeletal Class I relationships, with minimal crowding. Dental casts were reduced to two dimensions using a photocopy machine, the intercanine distance, canine depth, intermolar distance and molar depth was recorded. The same parameters were measured in 8 rectangular archwires (named from A to H) marketed by 5 manufacturers and the measurement differences were compared.

Results: The archwire whose intercanine distance was the most suitable for the arch dimensions of the individuals in the study group was arch B, and the archwire with the most suitable intermolar distance was arch A. When the intercanine and intermolar distances were evaluated together, the archwire most compatible with the data of the individuals in the study group was arch A. The archwire that was the most incompatible with the arch dimensions of the individuals in the study group was arch D.

Conclusion: Differences were observed between the arch dimensions of all archwires evaluated in the study and the arch dimensions of the individuals in the study group. It may be advisable to take this finding into consideration in the maintenance of the orthodontic treatment results.

Öz

Amaç: Bu çalışmanın amacı ortodontik tedavi görmemiş, Sınıf I oklüzyon gösteren ve ortodontik tedavi geçmişi olmayan Türk bireylerden oluşan bir örneklem grubundan elde edilen mandibular ark boyutları ile farklı üretici firmalar tarafından üretilen ark tellerinin boyutları arasındaki uyumun değerlendirilmesidir.

Gereç ve Yöntemler: Bu araştırma kapsamında iskeletsel ve dental Sınıf I ilişkiye sahip, minimal çapraşıklığı bulunan toplam 40 bireyden mandibular alçı model elde edilmiştir. Ortodontik modeller fotokopi makinesi aracılığı ile iki boyuta indirgenmiş, interkanin mesafe, kanin derinliği, intermolar mesafe ve molar derinliği kaydedilmiştir. Aynı parametreler 5 üretici firma tarafından pazarlanan dikdörtgen kesitli 8 ark telinde (A'dan H'ye kadar isimlendirilmiş) de ölçülerek aradaki fark karşılaştırılmıştır.

Bulgular: Çalışmada incelenen ark tellerinden interkanin mesafenin çalışma grubundaki bireylerin ark boyutlarına en uygun olan ark telinin ark B, intermolar mesafesi en uygun olan ark telinin ise ark A olduğu gözlenmiştir. Hem interkanin hem de intermolar mesafeleri birlikte değerlendirdiğimizde ise çalışma grubundaki bireylerin verileri ile en uyumlu ark telinin ark A olduğu gözlenmiştir. Çalışma grubundaki bireylerin ark boyutları ile en uyumsuz olan ark telinin ise ark D olduğu belirlenmiştir.

Sonuç: Çalışmada değerlendirilen tüm ark tellerinin ark boyutları ile çalışma grubundaki bireylerin ark boyutları arasında farklılıklar gözlenmiştir. Ortodontik tedavi sonuçlarının idamesi konusunda bu bulguların dikkate alınması tavsiye edilebilir.

Introduction

The Straight Wire technique was developed by Lawrence Andrews and the brackets' design changes have made it possible to use preformed archwires, saving clinicians from time consuming bendings (1).

Many attempts have been made to find a universal arch form that will suit everyone (2,3). However, it is evident that a single arch form is not characteristic of a particular malocclusion and therefore customization of the archwires is accepted as always necessary (4). Archwires in various sizes and shapes are offered to clinicians in the market, and these characteristics closely affect orthodontic treatment results. Studies have shown that maintaining especially the intercanine width, the intermolar width, and the arch length contributes greatly to the stability of treatment results (5,6). A meta-analysis by Burke et al. (7) also emphasized that preservation of the original mandibular intercanine width is crucial for stability, as this distance tends to return to its pre-treatment value. Relapse is also known to occur almost ineluctably when the teeth are placed in an unstable position, outside the functional envelope. There is evidence that the most reliable way to maximize post-treatment stability is to maintain the original, pre-treatment arch form in which the teeth are presumed to be in a stable position (4).

In the ideal conditions, it is recommended to select the archwires individually by evaluating the arch sizes and arch shape. It is possible to bend stainless steel wires and customize both the arc properties. However, in most clinics, the archwires, especially Nickel Titanium (NiTi) archwires, are routinely used in different cross-sectional sizes but in one form. The treatment options in the lower jaw are limited compared to the upper, and the upper arc form is connected to its lower counterpart. Moreover, the lower intercanine distance is a crucial parameter known to be the key for stability of the orthodontic treatment outcomes. Considering all these main points, the use of NiTi archwires with standard shape and size can be problematic, especially in the lower jaw.

According to our literature research, no study has evaluated yet the harmony between the mean arch dimensions of the Turkish individuals and those of the archwires available in the market. The aim of this study is to evaluate the harmony between the mandibular arch dimensions of the best-selling archwires from different manufacturers and the mandibular arch dimensions obtained from Turkish individuals presenting Angle Class I occlusion with no orthodontic treatment history. We aimed to determine the wire that shows the best compatibility with the mean arc dimensions. As the null hypothesis, we suggested that there would be no difference between the selected archwires dimensions and the mean arch dimensions of the sample.

Materials and Methods

This clinical study was approved by Bezmialem Vakıf University Non-Invasive Ethics Committee (approval number: 54022451-050.05.04 date: 27.02.2018). The difference of 2.62 mm between the preformed and natural arch dimensions for the intermolar distance in the study by Oda et al. (8) was accepted as 3 on average. At least 35 cases were necessary to work with 95% confidence interval and to obtain 80% power for the present study.

Twenty female and 20 male volunteers who had not received any orthodontic treatment and who presented crowding less than 3 mm participated to the research and signed the informed consent form. They all presented Class I dentoskeletal relationship, and the ages ranged from 20 to 25. All the volunteers were in permanent dentition, had no missing, restored or impacted tooth, they all had ideal overjet-overbite, none of them had significant asymmetry and any craniofacial syndrome. The records of the individuals included in the study were collected in Bezmialem Vakıf University Faculty of Dentistry, Department of Orthodontics between April 2018 and May 2019.

Alginate impressions taken from the lower jaw were poured with hard plaster immediately after the procedure and special care was taken to leave no air bubbles. Two dimensional copies of the models were created using a printer with scanning feature (Develop Ineo4050).

In the study of Oda et al. (8), the buccolingual thickness of the central and canine brackets and those of molar tubes with 0.022-inch slot from 8 different manufacturers, were recorded and the average bracket and tube thicknesses were determined. We took reference from Oda et al.'s (8) average attachment thicknesses for central incisors, canines and first molars that are 1.34 mm, 0.75 mm and 0.73 mm respectively. The mean attachment thicknesses were added perpendicularly to the midpoints of the vestibular surfaces of the teeth in the mesiodistal direction on the 2-dimensional copies following the method described by Oda et al. (8). The bracket slot point (BSP), that is the surface where the archwire is likely to pass, was determined accordingly (Figure 1). The intercanine and intermolar distances were recorded at the BSP level (Figure 1). In addition, the canine and first molar depths were measured considering the BSP of the central tooth and finally, the mean arch dimensions were calculated.

While selecting the archwires, the local distributers of the manufacturers were interviewed and the best-selling products on the market were chosen. Eight

Figure 1. Bracket slot points at the central, canine and molar levels, intercanine and intermolar distances, canine (a) and molar (b) depths

different archwires from five manufacturers were selected for size analysis (Table 1).

Measurements on the arc wires included in the study were carried out on millimetric graph paper. The midline of the wires was overlapped with the midline determined on the graph paper, and the intercanine and intermolar distances were measured according to the mean canine and molar depths recorded (Figure 2).

Statistical Analysis

The comparative statistics were not applicable. The mean values, the standard deviations and the delta values were calculated with Microsoft Excel (Microsoft Windows, USA).

In order to determine measurement errors, half of the randomly selected study models were remeasured 2 weeks later by the same examiner (B.Y.). The Wilcoxon signed-rank test was performed to assess the difference between two measurements. Additionally, the dimensions of the preformed archwires at the canine and first molar levels were graphically compared with means of normal dental arch widths.

Results

Based on the measurements, the mean canine depth and the intercanine distance at BSP were recorded as 6.08±0.70 mm and 29.67±1.56 mm respectively. All the measurements were performed by an orthodontist (B.Y.) with 7-8 years of experience and no statistical difference was noted between the repeated measurements (p=0.65). The graphical comparison of the intercanine dimension of the

Table 1. Archwires whose dimensions were evaluated in the study	
Arch shape	**Manufacturer**
Europa II (arch A)	RMO (Denver, USA)
Bioform III (arch B)	RMO (Denver, USA)
Trueform I (arch C)	RMO (Denver, USA)
Damon (arch D)	Ormco (Glendora, Calif, USA)
Natural (arch E)	Highland Metal (Franklin, USA)
Form A (arch F)	GC Orthodontics (Breckerfeld, Germany)
Form III (arch G)	American Orthodontics (Sheboygan, USA)
VLP (arch H)	American Orthodontics (Sheboygan, USA)

cast measurements and the archwires is presented in Figure 3. Among the archwires that we evaluated in terms of intercanine distance, the most suitable archwire was found to be arch B (28.91 mm) (Figure 3). The second closest dimension was measured with arch E (30.44 mm). The archwire that presented the most incompatible dimension with the average intercanine distance of the study group was arch D (34.68 mm). It is noteworthy that the intercanine distances of the archwires evaluated in this study were larger than the mean intercanine distance of the Turkish individuals who participated in this study except one archwire (arch B) (Figure 3, Table 2).
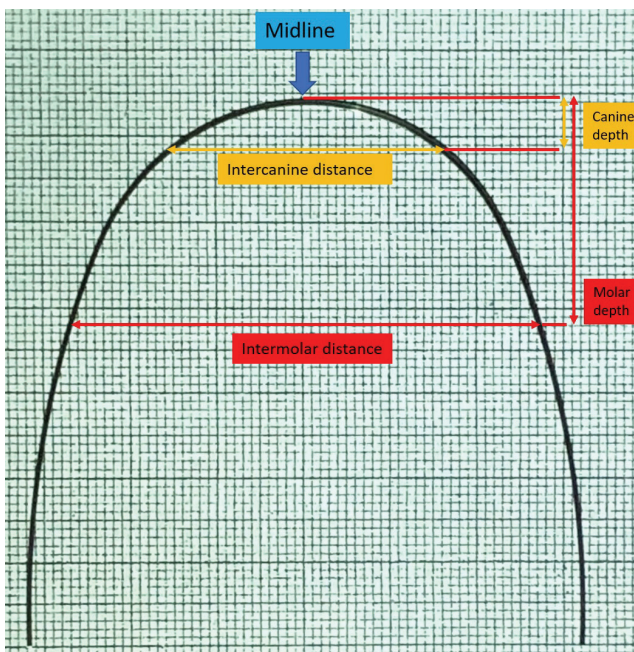
Figure 2. Measurement of the arch dimensions of a Damon (Ormco) archwire on graph paper
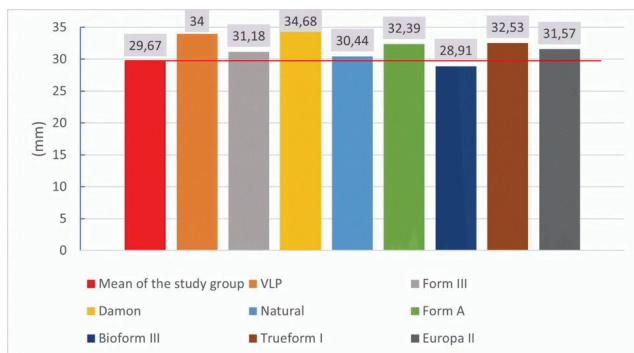
Figure 3. Intercanine distances (mm) of different archwires measured at mean canine depth

Measurements of individuals included in the study showed an average molar depth of 26.85±1.56 mm and a mean intermolar distance of 52.35±2.00 mm at BSP. The graphical comparison of the intermolar dimension of the cast measurements and the archwires is presented in Figure 4. Among the archwires we evaluated, the most suitable archwire in terms of intermolar distance was found to be arch A (52.26 mm) followed by be arch G (51.11 mm) (Figure 4). The most incompatible archwire for the intermolar distance was arch D (57.65 mm), like the intercanine distance. We also remarked that the intermolar distance of most of the archwires was narrower than the mean intermolar distance of the individuals (Figure 4, Table 2).

The delta (Δ) values of the intercanine and intermolar distances are presented at Table 2. Considering the archwires in terms of intercanine and intermolar distances at BSP, we found that the most compatible archwire with the mean values was arch A, followed by arch G (Table 2) (sum of the absolute difference values). Arch D was determined to be the most incompatible archwire in terms of intercanine and intermolar distances.

Discussion

NiTi archwires are preferred especially during the initial stage of the treatment since they have great superelastic and shape-memory properties. However, it is not possible to customize their form nor their dimensions. It has been reported that, the heavier NiTi archwires are more capable of changing the intercanine width during alignment and should be used with attention (9). Thus, by choosing the most appropriate NiTi archwire from the early stages of the treatment, the patient's chair time can be reduced by necessitating less customization when switching to stainless steel archwires. Moreover, clinicians try to choose the best fitting archwire to the original arch form and size of the patient to achieve a stable treatment outcome preserving the initial intercanine distance (10).

In the present study, we aimed to compare the arch dimensions of 8 different preformed archwires with the arch dimensions of a Turkish sample, to determine the most suitable archwire among the most popular ones on the market. The reason why the study was based on individuals presenting naturally the ideal

Table 2. The intercanine and the intermolar distances of the study group and the archwires

Arch shape	Intercanine distance	Intermolar distance	ΔIntercanine distance	ΔIntermolar distance
Europa II (arch A)	31.57	52.26	+1.90	-0.09
Bioform III (arch B)	28.91	48.10	-0.76	-4.25
Trueform I (arch C)	32.53	50.23	+2.86	-2.12
Damon (arch D)	34.68	57.65	+5.01	+5.3
Natural (arch E)	30.44	46.93	+0.77	-5.42
Form A (arch F)	32.39	51.05	+2.72	-1.30
Form III (arch G)	31.18	51.11	+1.51	-1.24
VLP (arch H)	34.00	55.93	+4.33	+3.58
Mean values recorded with the study group	29.67±1.56 mm	52.35±2.00 mm	-	-
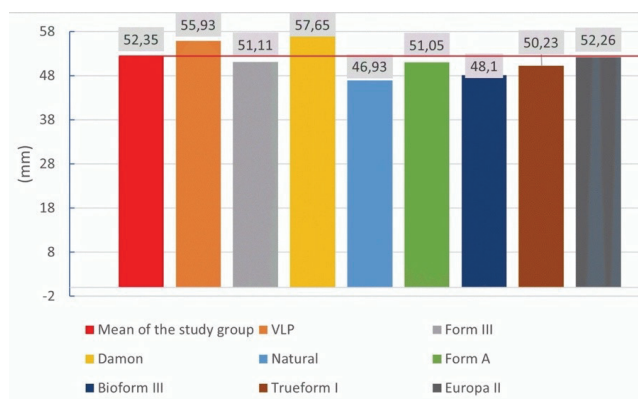
Figure 4. Intermolar distances (mm) of different archwires measured at the mean molar depth

occlusion is that the most common final goal of the orthodontic treatment is to achieve Class I occlusion.

Following the method of a similar previous study, we evaluated only the mandibular arch (8). The final occlusal relations are often planned based on the lower canine position since the therapeutic procedures in the mandible are more limited compared to those possible for the maxillary dentition. Thus, the maxillary arch form is strongly associated with the mandible and maintaining original mandibular canine width is an essential element in post-treatment stability (4,7,10).

In a study evaluating clinicians' choices when selecting archwires, NiTi archwires (99%) were preferred as the initial alignment wire (11). Although many clinicians stated that preservation of the intercanine width is particularly important, they stated that preservation of the pretreatment arch form was essential in the later but not in the early

stages of the treatment (11). However, the results of the study revealed that there is no conformity about how the arch form should be preserved. The authors concluded that even when clinicians desire to adapt their archwires with the intention of preserving the original arch form, the methods that they use is questionable.

The arch dimension and the arch form are considered as two essential factors in terms of teeth alignment, achieving esthetic results, and establishing occlusal stability (12). The effects of different variables on the arch size and form such as age, sex, malocclusion type and ethnicity have been previously evaluated (13-16). Bishara et al. (13) stated that the mandibular intercanine width increases until 13 years of age, but this dimension, on the average, was established by 8 years of age. On the other hand, the mandibular intercanine dimension is found to decrease slightly after 13 years of age. They reported that the decrease was significant only between 26 and 45 years of age and that the change in the intermolar width is not significant between 13 to 26 years of age. Therefore, we included individuals aged between 20 to 25 years in our study.

There are studies reporting contradictory results about the impact of the sex parameter on the arch size (13,15,17). We included equal numbers of male and female individuals to minimize the bias related to gender differences.

Nowadays, most of the manufacturers produce their archwires based on American or European arch forms. In a study comparing the arch forms of Turkish and North American individuals, it was reported that North Americans have greater molar depth and lower

molar width/depth ratio in comparison to the Turkish individuals, however, no significant difference was found for the transverse widths at the canine and molar levels between groups. The authors also stated the most frequent arch form seen was the ovoid arch form among the Turkish individuals and the tapered form was more common in the White American group (16). In another study by Nojima et al. (14), significantly larger width measurements at the canine and molar levels were recorded with the Japanese individuals compared to a group consisted of North American Whites, and the anteroposterior length to canine and molar widths ratios were also found to be greater for the Japanese individuals. In harmony with those findings, Kook et al. (18) reported larger and wider dental arches in a Korean population in comparison to a group consisted of White Americans and they stated that most of the preformed archwires are too narrow for many Asian patients. Similarly, it was reported that the commercially available preformed archwires had narrower widths at both canine and molar levels compared to the mean values recorded with a Japanese population (8). The preformed archwires that closely matched the mean arch dimensions were Orthos and Vari-Simplex (Ormco, Glendora, Calif, USA) large types. We referred to the mean average bracket and tube thickness calculated by Oda et al. (8) but we did not evaluate the same archwire brands since we selected the archwires based on the commercial data provided by the local providers. Unlike Oda et al.'s (8) results, all examined archwires were wider at the canine level compared to the mean value obtained from individuals in our study group. In contradiction, most of the evaluated archwires were narrower at the first molar level compared to the mean intermolar measurements value. This difference may be explained with ethnic differences. The intercanine distance being narrower, and the intermolar width being larger than the archwires dimensions may be related to the ovoid arch form that is common among Turks as previously reported (16).

The selection of the archwires was based on the reports of the local providers. A further study may evaluate a larger number of archwires from different manufacturers with various shapes. We suggested the fabrication of the archwires is a standard process and we measured one sample of each brand. This is the reason why the lack of a statistical analysis may be considered as a limitation of the study design, but instead of a statistical comparison, a graphical comparison was provided.

The sample of this study consisted of individuals presenting Class I occlusion without orthodontic treatment history, but since there are heterogeneous ethnic backgrounds in the Turkish population, it may be needed to perform further investigations in a larger sample size of people to be able to make a more accurate clinical assumption. In addition, it will be beneficial to carry out further studies to determine the most suitable arch form and size for different genders and different malocclusion types for the Turkish population. Moreover, we believe that the use of three-dimensional technologies could be useful to determine a more precise BSP since they provide a more accurate visualization and control of the tooth surface.

Conclusion

The important conclusions drawn from the present study are as follows:

1. The all examined archwires had larger dimensions in comparison with the mean of the study group at the canine level, whereas most of the evaluated archwires were narrower at the first-molar level.

2. Europa II (RMO, Denver, USA) was the archwire presenting the closest dimensions to the mean widths both at the canine and the molar levels among the evaluated archwires. One the other hand, the most incompatible archwire with both canine and molar width means was Damon (Ormco, Glendora, Calif, USA).

The null hypothesis was rejected. Clinicians are advised to consider these findings in the selection of NiTi archwire whose shape cannot be customized.

Authorship Contributions

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

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

Financial Disclosure: The authors declared that this study received no financial support.

References

1. Andrews LF. The straight-wire appliance. Br J Orthod 1979; 6: 125-43.

2. Hawley CA. Determination of the normal arch, and its application to orthodontia. Dent Cosmos 1905; 47: 541-52.

3. Currier JH. A computerized geometric analysis of human dental arch form. Am J Orthod 1969; 56: 164-79.

4. Felton JM, Sinclair PM, Jones DL, Alexander RG. A computerized analysis of the shape and stability of mandibular arch form. Am J Orthod Dentofacial Orthop 1987; 92: 478-83.

5. Glenn G, Sinclair PM, Alexander RG. Nonextraction orthodontic therapy: posttreatment dental and skeletal stability. Am J Orthod Dentofacial Orthop 1987; 92: 321-8.

6. Little RM. Stability and relapse of dental arch alignment. Br J Orthod 1990; 17: 235-41.

7. Burke SP, Silveira AM, Goldsmith LJ, Yancey JM, Van Stewart A, Scarfe WC. A meta-analysis of mandibular intercanine width in treatment and postretention. Angle Orthod 1998; 68: 53-60.

8. Oda S, Arai K, Nakahara R. Commercially available archwire forms compared with normal dental arch forms in a Japanese population. Am J Orthod Dentofacial Orthop 2010; 137: 520-7.

9. Ahmed M, Shaikh A, Fida M. Evaluation of conformity of preformed orthodontic archwires and dental arch form. Dental Press J Orthod 2019; 24: 44-52.

10. Braun S, Hnat WP, Leschinsky R, Legan HL. An evaluation of the shape of some popular nickel titanium alloy preformed arch wires. Am J Orthod Dentofacial Orthop 1999; 116: 1-12.

11. McNamara C, Drage KJ, Sandy JR, Ireland AJ. An evaluation of clinicians' choices when selecting archwires. Eur J Orthod 2010; 32: 54-9.

12. Boley JC, Mark JA, Sachdeva RC, Buschang PH. Long-term stability of Class I premolar extraction treatment. Am J Orthod Dentofacial Orthop 2003; 124: 277-87.

13. Bishara SE, Jakobsen JR, Treder J, Nowak A. Arch width changes from 6 weeks to 45 years of age. Am J Orthod Dentofacial Orthop 1997; 111: 401-9.

14. Nojima K, McLaughlin RP, Isshiki Y, Sinclair PM. A comparative study of Caucasian and Japanese mandibular clinical arch forms. Angle Orthod 2001; 71: 195-200.

15. Olmez S, Dogan S. Comparison of the arch forms and dimensions in various malocclusions of the Turkish population. Open J Stomatol 2011; 1: 158-64.

16. Celebi AA, Keklik H, Tan E, Ucar FI. Comparison of arch forms between Turkish and North American. Dental Press J Orthod 2016; 21: 51-8.

17. Ward DE, Workman J, Brown R, Richmond S. Changes in arch width. A 20-year longitudinal study of orthodontic treatment. Angle Orthod 2006; 76: 6-13.

18. Kook YA, Nojima K, Moon HB, McLaughlin RP, Sinclair PM. Comparison of arch forms between Korean and North American white populations. Am J Orthod Dentofacial Orthop 2004; 126: 680-6.

Effect of Pediatric Toothpastes Based on 500 to 1450 ppm Sodium Fluoride and Amine Fluoride with Different Detergents on Oxidative Stress and Cell Viability

500-1450 ppm Sodyum Florür ve Amin Florür Bazlı, Farklı Deterjan İçerikli Pediatrik Diş Macunlarının Oksidatif Stres ve Hücre Canlılığı Üzerine Etkisi

● Firdevs Kahvecioğlu[1], ● Hayriye Esra Ülker[2], ● Gül Tosun[1], ● Mutlu Özcan[3]

[1]Selçuk University Faculty of Dentistry, Department of Pediatric Dentistry, Konya, Turkey
[2]Selçuk University Faculty of Dentistry, Department of Restorative Dentistry, Konya, Turkey
[3]University of Zürich, Division of Dental Biomaterials, Center for Dental and Oral Medicine, Clinic for Reconstructive Dentistry, Zürich, Switzerland

Abstract

Objective: This study evaluated the effect of sodium fluoride and amine fluoride pediatric toothpastes with different detergents on oxidative stress and cell viability.
Materials and Methods: Pediatric toothpastes containing sodium fluoride (Sensodyne Pronamel-SP; Ipana Kids-IK; Signal Kids-SK; Oral B-OB) and amine fluoride (Elmex-EL; Elmex Junior-EJ) were obtained. While SP, IK, EL contained cocamidopropyl betaine detergent, SK, OB sodium lauryl sulfate and EJ had olaflur detergents. Toothpaste samples were diluted with medium at different predetermined concentrations. L929 fibroblast cells were exposed to toothpaste extracts at 37 °C for 2 min. Cell viability was tested using methyl tetrazolium test, while the formation of reactive oxygen species (ROS) was detected using flow cytometry.
Results: The decreasing concentration ratio from 1:1 to 1:32 decreased the cytotoxicity (p<0.05) except EL. The cytotoxicity of original pediatric toothpaste extracts (1:1) were significantly different, compared to the negative control group (p<0.05), except IK and EL (p>0.05). All toothpastes tested increased the number of ROS in L929 cells (p<0.05).
Conclusion: Pediatric toothpastes containing sodium fluoride with sodium lauryl sulfate presented more cytotoxic effect.

Öz

Amaç: Bu çalışmada, sodyum florür ve amin florür içeren pediatrik diş macunlarının farklı deterjan içeriklerinin oksidatif stres ve hücre canlılığı üzerine etkisi değerlendirilmiştir.
Gereç ve Yöntemler: Sodyum florür (Sensodyne Pronamel-SP; Ipana Kids-IK; Signal Kids-SK; Oral B-OB) ve amin florür (Elmex-EL; Elmex Junior-EJ) içeren pediatrik diş macunları kullanıldı. SP, IK, EL kokamidopropil betain deterjan içerirken, SK, OB sodyum lauril sülfat ve EJ olaflur deterjan içerikliydi. Diş macunu numuneleri önceden belirlenmiş farklı konsantrasyonlarda medyum ile seyreltildi ve L929 fibroblast hücreleri, diş macunu ekstraktlarına 37 °C'de 2 dakika maruz bırakıldı.

Hücre canlılığı metil tetrazolyum testi kullanılarak test edilirken, reaktif oksijen türlerinin oluşumu (ROS) flow sitometrisi kullanılarak tespit edildi.

Bulgular: 1:1'den 1:32'ye azalan konsantrasyon oranı, EL haricinde sitotoksisiteyi azalttı (p<0,05). Orijinal pediatrik diş macunu ekstraktlarının (1:1) sitotoksisitesi, negatif kontrol grubuna (p<0,05) kıyasla, IK ve EL haricinde (p>0,05) önemli ölçüde farklıydı. Test edilen tüm diş macunlarını, L929 hücrelerindeki ROS miktarını artırdı (p<0,05).

Sonuç: Sodyum florür içeren ve sodyum lauril sülfat içeren pediatrik diş macunları daha fazla sitotoksik etki göstermiştir.

Introduction

Tooth brushing using a toothpaste is an important oral hygiene practice that is beneficial for dental and gingival health, aids dental plaque removal, and prevents dental caries, especially in children (1). Toothpastes generally comprise abrasive, surface-active, moisturizing, gelling and/or binding, flavoring, preservative, and staining agents; sweeteners; and fluorides (2). The ideal toothpaste for children should comprise ingredients compatible with fluoride delivery to ensure adequate fluoride availability, minimal abrasivity, and consequently, a pleasant brushing experience (1,2).

The efficacy of fluoride-containing toothpastes depends on their fluoride concentration, frequency of use, volume of toothpaste used, and rinsing habits after brushing (3). Children between the ages of two-four and five-seven years swallow 34% and 13% toothpastes, respectively, while older children and adults ingest only 6% (4). Therefore, dentists should be aware of the potential adverse effects of toothpastes and fluorides and counsel patients and/or parents accordingly. Since many decades, prescription of fluoride toothpastes in carefully selected patients has been effective in preventing dental caries (5). However, some toothpaste components such as detergents may be harmful because of their foaming ability (6). Sodium lauryl sulfate (SLS), which is the most commonly used detergent in mouthwashes and toothpastes, is capable of denaturing proteins (7). Short-term use of toothpastes with ≤2% SLS content is considered harmless (5). Cocamidopropyl betaine (CAPB) is another commonly used detergent in toothpastes. CAPB-containing toothpastes are believed to be less irritating and can alleviate symptoms of dry mouth (8). Amine fluoride (AF) in toothpastes can also act as a detergent and affects L929 fibroblast cells (9).

Limited information is available in the literature on commercially available pediatric toothpastes for children ≤12 years old regarding the cytotoxic effects on gingival fibroblasts and L929 mouse fibroblasts and potential for causing oral squamous cell carcinoma (9,10).

Reactive oxygen species (ROS) are generated during mitochondrial oxidative metabolism. Oxidative stress refers to the instability caused by excess ROS or oxidant production. Various chemicals can disrupt the stable cellular redox balance, resulting in increased levels of ROS and subsequent cell death through apoptosis (11).

Therefore, in this study, we evaluated the effects of sodium fluoride- and AF-based pediatric toothpastes containing different detergents on oxidative stress and cell viability. The null hypothesis was that the type of detergent in sodium fluoride- and AF-based toothpastes in different dilutions would not have a significant effect on oxidative stress and cell viability of L929 murine fibroblast cells.

Materials and Methods

This study was approved by the Selçuk University Faculty of Dentistry Ethics Committee (decision no: 2015/01, date: 08.10.2015). Commercial pediatric toothpastes containing sodium fluoride (Sensodyne Pronamel-(SP), 1450 ppm; Ipana Kids-IK, 500 ppm; Signal Kids-(SK), 500 ppm; Oral B-OB, 500 ppm) and AF (Elmex-EL, 500 ppm; Elmex Junior-EJ, 1400 ppm) were obtained. SP, IK, and EL comprised CAPB; SK and OB contained SLS, and EJ comprised olaflur (Table 1).

The toothpastes were diluted in a medium (50 w/v%), homogenized using a vortex (WisemixVM-10; Daihan Scientific Co., Ltd., Seoul, South Korea), centrifuged (Hettich 320R Centrifuge, maximum speed: 15000 rpm, Germany), filtered, and immediately used in experiments. The original extracts (1:1) were diluted at 1:2, 1:4, 1:8, 1:16, and 1:32 in the medium.

Cytotoxicity Testing

L929 cells were seeded on each well of a 96-well plate at a density of 2×10^4 cells/well and incubated at 37 °C for 24h cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine

Table 1. Chemical composition of toothpastes used in the present study			
Group	Detergent content	Fluoride content	Composition
SP	Cocamidopropyl betaine	Sodium fluoride 1450 ppm	Aqua, sorbitol, hydrated silica, glycerin, PEG-6, cocamidopropyl betaine, xanthan gum, aroma, sodium fluoride, sodium saccharin, sucralose, titanium dioxide, sodium hydroxide, limonene
IK	Cocamidopropyl betaine	Sodium fluoride 500 ppm	Sorbitol, aqua, hydrated silica, aroma, cocamidopropyl betaine, benzyl alcohol, carbomer, mica, sodium chloride, sodium fluoride, sodium phosphate, sodium saccharin, trisodium phosphate, xanthan gum, Cl16255, Cl77891
SK	Sodium lauryl sulfate	Sodium fluoride 500 ppm	Sorbitol, aqua, hydrated silica, PEG-32, sodium lauryl sulfate, cellulose gum, sodium saccharin, sodium fluoride, mica, calcium gluconate, tocopheryl acetate, glycerin, limonene, phenylcarbinol, Cl12490, Cl77891
OB	Sodium lauryl sulfate	Sodium fluoride 500 ppm	Aqua, sorbitol, hydrated silica, sodium lauryl sulfate, cellulose gum, aroma, sodium saccharin, carbomer, trisodium phosphate, sodium fluoride, limonene, Cl42090
EL	Cocamidopropyl betaine, olaflur	Aminfluorid 500 ppm	Aqua, sorbitol, hydrated silica, hydroxyethylcellulose, titaniumdioxide, cocamidopropyl betaine, olaflur, aroma, limonene, sodium saccharin, hydrochloric acid
EJ	Olaflur	Aminfluorid 1400 ppm	Aqua, hydrated silica, sorbitol, olaflur, hydroxyethylcellulose, aroma, limonene, PEG-40 hydrogenated, castoroil, titaniumdioxide, saccharin, hydrochloric acid
SP: Sensodyne pronamel, IK: Ipana kids, SK: Signal kids, OB: Oral B, EL: Elmex, EJ: Elmex junior			

serum, penicillin (150 IU/mL), and streptomycin (150 µg/mL). The cells were then exposed to 100 µL of toothpaste extracts, while the cell culture medium was used as a negative control. After 24h, cell survival was evaluated by assessing enzyme activity using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). The cells were exposed to 200 µL MTT solution (0.5 mg/mL) for 2h at 37 °C. The blue formazan precipitate was then dissolved by shaking for 30 min at room temperature in 200 µL dimethyl sulfoxide. Absorption was assessed using a spectrophotometer at 540 nm (BioTek Epoch, BioTek Instruments, Inc., Winooski, VT, USA). Three independent experiments were performed in four wells (n=12 per group). Optical density readings from cultures exposed to extracts were compared with untreated control cells (100%).

Statistical Analysis

The Shapiro-Wilk test was used to test the normal distribution of data. One-Way analysis of variance and Tukey's post-hoc test were used to compare cell survival (α=0.05).

Reactive Oxygen Species Measurements

ROS levels were monitored using the oxidation-sensitive fluorescent probe 2'7'-dichlorodihydrofluorescein diacetate (H2DCF-DA, Invitrogen Molecular Probes, Karlsruhe, Germany). Dichlorofluorescein, a non-fluorescent compound, is formed as a result of intracellular esterase activity. When oxidized, it converts to dichlorodihydrofluorescein diacetate (DCF-DA) and becomes fluorescent. L929 cells were seeded on each well of a 6-well plate at a density of 2×10^5 cells/well and incubated at 37 °C for 24h. Thereafter, the cells were exposed to toothpaste extracts for 2 min, with cells exposed to triethyleneglycol dimethacrylate (1 mmol/L TEGDMA, Sigma-Aldrich, Taufkirchen, Germany) as the positive control. Subsequently, L929 cells were incubated with 10 µM H2DCF-DA at 37 °C for 30 min. The cells were seperated using 0.05% trypsin/ethylenediamine tetraacetic acid and aggregated by centrifugation and washed with PBS. DCF fluorescence values were determined using flow cytometry (FACS Aria III, BD Biosciences, San Jose, CA, USA) at an emission wavelength of 519 nm and an excitation wavelength of 488 nm (FITC-1). The mean fluorescence values were obtained using histogram statistics (FACSDiva v6.1.3, BD Biosciences). Two wells were used in two independent experiments (n=4 per group), and the results were averaged. All further analyses were performed using the averaged values. Differences in ROS levels were statistically evaluated

Table 2. Effect of the original extracts and concentrations of pediatric toothpastes on the viability of L929 cells

Test groups	Toothpaste concentration (Mean ± SD)					
	1:1	1:2	1:4	1:8	1:16	1:32
SP	63.9±10.9^{aA}	81.1±7.9^{A}	82±5.6^{A}	103.4±51^{B}	107.6±4.3^{B}	111.9±10.2^{B}
IK	84.3±14.2	84.1±10.8	89.7±15.3	88.5±24.2	90.9±14.6	88.7±27.7
SK	7.6±1.1^{a}	8.0±0.6^{a}	8.3±1.1^{a}	8.4±1.5^{a}	10.8±1.9^{a}	26.2±5^{a}
OB	10.8±1.3^{aA}	10.1±0.8^{aA}	17.1±3.1^{aA}	59.4±11.7^{aB}	93.2±8.4^{B}	96.9±4.4^{B}
EL	102.7±4.0	102.8±11.3	96.9±10.0	104.2±5.5	85.0±7.4	87.1±4.7
EJ	18.0±1.6^{aA}	40.4±7.2^{a}	52.1±8.8^{aB}	56.2±9.9^{aB}	61.1±18^{aB}	85.4±9.4^{B}

SP: Sensodyne pronamel, IK: Ipana kids, SK: Signal kids, OB: Oral B, EL: Elmex, EJ: Elmex junior, SD: Standard deviation. Two min post-exposure was evaluated using the standard MTT assay. ^{A,B}: Different upper-case superscript letters indicate a statistically significant (p<0.01), ^{a,b}: Different lower-case superscript letters indicate a statistically significant (p<0.01)

Table 3. Generation of ROS in L929 cells after exposure to pediatric toothpastes

	Mean	Standard deviation
Negative control	1.00	0.00
Positive control	8.92	0.24
SP	5.29^{n,p,a}	0.49
IK	5.23^{n,p,a}	0.24
SK	2.90^{n,p,b}	0.31
OB	2.42^{n,p,b}	0.06
EL	1.95^{n,p,b}	0.03
EJ	8.57^{n,p,b}	0.01

SP: Sensodyne pronamel, IK: Ipana kids, SK: Signal kids, OB: Oral B, EL: Elmex, EJ: Elmex junior, p: Significant difference from positive control group, n: Significant difference from negative control group, ^{A,B}: Different upper-case superscript letters indicate a statistically significant (p<0.01)

using the Mann-Whitney U test (α=0.05). Multiple comparisons were performed using the Bonferroni post-hoc test (p<0.05).

Results

Cytotoxicity of Pediatric Toothpastes

Cell viability was significantly affected by the type and concentration (in ppm) of detergent (both p<0.05) in the toothpastes. The decrease in concentration from 1:1 to 1:32 decreased the cytotoxicity of toothpastes significantly (p<0.05), except that of EL (Table 2).

The effects of original extracts (1:1) and concentrations (1:2, 1:4, 1:8, 1:16, and 1:32) of pediatric toothpastes on the viability of L929 cells after 2 min of exposure were evaluated using the standard MTT assay. Varying degrees of cytotoxicity were determined from the experiments (Table 2).

The survival rates were significantly different between L929 cells exposed to 1:1 concentration of SP and cells in the negative control group (p<0.05). There were no significant differences in the survival rates between L929 cells exposed to IK and EL and cells in the negative control group (p>0.05). Significant differences were observed in the survival rates between the L929 cells exposed to all concentrations of SK and cells in the negative control group (p<0.05). However, significant differences were observed between cells in the negative control group and L929 cells exposed to 1:2, 1:4, and 1:8 concentrations of OB (p<0.05). Significant differences were observed in the survival rates between L929 cells exposed to all concentrations of EJ (except 1:32) and cells in the negative control group (p<0.05).

Production of ROS by Pediatric Toothpastes

ROS production was measured using the oxidation-sensitive fluorescent probe H2DCF-DA. The L929 cell cultures were exposed to the toothpastes in the cell culture medium for 2 min and cell cultures exposed to 1 mmol/L TEGDMA were used as positive control. The mean fluorescence intensities were compared with those of untreated control cultures (n=4). The amount of ROS was increased by approximately eight-fold with 1 mmol/L TEGDMA. All toothpastes showed significant increase in the amount of ROS in L929 cells (p<0.05) (Table 3). ROS values were significantly increased with SP, IK, SK, OB, EL, and EJ by 5.29, 5.23, 2.90, 2.42, 8.57, and 1.95-fold, respectively (p<0.05).

Discussion

Biocompatibility is a unique property exhibited by a substance that interacts with its environment. The biological response of substances varies with changes

in the host, method of substance application, or substance itself (12). When a specific biocompatible substance is applied to the host, the corresponding effect may be either cell damage or stimulation of the cellular synthesis of some proteins, leading to inflammation. The results of this study indicate the possibility of the contents of pediatric toothpastes to show toxic effects, which may increase in the presence of some ingredients, specifically SLS. Additionally, fluoride-rich toothpastes have toxic effects. In the current study, only SP and IK were not cytotoxic; other pediatric toothpastes showed cytotoxic effects on the L929 cells, partially proving our hypothesis.

Toothpastes and mouthwashes have been used for more than 3000 years and formulated to conceal malodor, remove tooth stains, and treat or prevent diseases of the teeth. The toothpastes generally contain detergents, foaming agents, preservatives, antimicrobial agents, moisturizers, and homogenizers. Each component has a specific function and provides different characteristics to the toothpaste (2,13,14). The complete removal of products designed to expectorate after use is not possible, even for adults. Generally, it is more difficult in young children. Therefore, the frequent/daily use of toothpastes increases the necessity to investigate the potential systemic toxicity of their components (5).

There are many studies in the literature on the cytotoxicity of toothpastes (9,15-17). Detergents, particularly SLS, have cytotoxic effects (9). The study of Cvikl et al. (9) was that SLS-and AF-containing toothpastes were more cytotoxic for L929 fibroblast cells than CAPB and Steareth-20-containing toothpastes. In our study, all concentrations of SK containing SLS were cytotoxic to L929 cells. However, the 1:16 and 1:32 dilutions of OB containing SLS had no cytotoxic effects. Both these groups showed similar levels of ROS production. This result shows that cytotoxicity is affected by concentration.

Moore et al. (13) observed that detergents are associated with cell membrane disruption *in vitro*, a finding consistent with other *in vitro* studies; Cell incubation with SLS for 2 minutes reduces TERT-1 keratinocyte viability. Ghapanchi et al. (15) found that toothpastes containing SLS had various toxic effects on the primary epithelium and HeLa cells of the oral cavity. SLS showed the highest cytotoxicity at all time-points in a study by Tabatabaei et al. (18).

Elevated ROS levels affect the redox biological signals that maintain the physiological functions (19). SLS may accelerate cell death and rapidly eliminate deleterious microorganisms from the population (20). SLS initially interacts with cell membranes, causing an increase in intracellular Ca2+. Ca2+ stimulates the secretion of IL-1α due to calpain activation. IL-1α also stimulates ROS formation (21).

ROS production in cells is an important determinant of cell damage or redox signaling (19). ROS production was high in non-cytotoxic groups showing that cell damage occurs even when the cell does not lose its viability. In this study, CAPB containing SP, IK, and EL showed high ROS levels. All these toothpastes contain different concentrations and types of CAPB and fluoride. SP and IK contain 1450 ppm and 500 ppm sodium fluoride, respectively, and even 1:1 concentration of SP was cytotoxic. The difference between these two materials suggests that high doses of fluoride affect cell viability. Tabatabaei et al. (18) stated that the cytotoxicity of sodium fluoride was significantly correlated with time and increased over time. In other words, high concentrations for lesser durations and low concentrations for greater durations showed similar results.

The highest ROS level was found with EL containing 500 ppm AF, but EL containing 1400 ppm AF showed the lowest levels. This shows that low concentrations of AF leads to cell damage, even if the cell is alive. AF is also used as a detergent in toothpastes. Our results showed that all concentrations of EL and the 1:32 concentration of EJ were nontoxic. The determination of the most appropriate fluoride concentration for pediatric toothpastes requires comprehensive personalized evaluation after meticulous risk assessment. Toothpastes with lower fluoride concentrations can be recommended for children at risk of high fluoride toothpaste sensitivity (22). In general, children should brush their teeth twice a day using a fluoride and age-appropriate amount of toothpaste (23).

CAPB is also used as a detergent in toothpastes. No toxic effects were observed with any concentration of IK. Corroborating the results of our study, Cvikl et al. (10) proposed that children should only use CAPB-containing dentifrices that are specially formulated for them. Despite its foaming ability, CAPB is less harmful than SLS (24).

Recent studies on cell viability, cytotoxicity, and genotoxicity have revealed the potential adverse effects of toothpaste ingredients (9,14,16). However, the conducive environment of the oral cavity differs from *in vivo* conditions, and many factors, such as saliva, mucus layer, creatinine levels, blood flow, and normal flora, can protect the oral environment from harmful effects (15,16). Children older than six years have mixed dentition, and usually use adult dentifrices with higher fluoride concentrations and higher foaming properties, attributed to higher surfactant concentrations (1). This might explain the differing results for cell viability at varying ages after stimulation with toothpastes from the same manufacturer (10).

Further studies are needed to investigate the suitability of the oral cavity as a target tissue. Nonetheless, cell culture is an excellent method for assessing the mechanisms of incompatibility reactions. Alternatively, data from cytotoxicity tests, implantation studies, or cell culture models should be used to evaluate the biocompatibility of toothpastes.

Conclusion

Pediatric toothpastes containing sodium fluoride with SLS are more cytotoxic. AF-containing toothpastes with 1400 ppm fluoride are more cytotoxic than with 500 ppm fluoride, but ROS levels are greater at 500 ppm fluoride. Toothpastes containing CAPB are less toxic.

Ethics

Ethics Committee Approval: This study was approved by the Selçuk University Faculty of Dentistry Ethics Committee (decision no: 2015/01, date: 08.10.2015).

Informed Consent: This study does not require patient consent.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Design: F.K., H.E.Ü., Data Collection or Processing: F.K., Analysis or Interpretation: H.E.Ü., G.T., M.Ö., Literature Search: F.K., Writing: F.K., H.E.Ü., G.T., M.Ö.

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

Financial Disclosure: The study was supported by scientific research coordinator of Selçuk University (project no: 16401027).

References

1. Stovell AG, Newton BM, Lynch RJ. Important considerations in the development of toothpaste formulations for children. Int Dent J 2013; 63 Suppl 2: 57-63.

2. Davies R, Scully C, Preston AJ. Dentifrices--an update. Med Oral Patol Oral Cir Bucal 2010; 15: e976-82.

3. Kanduti D, Sterbenk P, Artnik B. Fluoride: A Review of Use and Effects on Health. Mater Sociomed 2016; 28: 133-7.

4. Barnhart WE, Hiller LK, Leonard GJ, Michaels SE. Dentifrice usage and ingestion among four age groups. J Dent Res 1974; 53: 1317-22.

5. Schmalz G, Arenholt-Bindslev D, editors. Biocompatibility of dental materials (Vol 1). Berlin: Springer; 2009.

6. Souza-Rodrigues RD, Ferreira Sda S, D'Almeida-Couto RS, Lachowski KM, Sobral MÂ, Marques MM. Choice of toothpaste for the elderly: an in vitro study. Braz Oral Res 2015; 29: 1-7.

7. Kitchin PC, Graham WC. Sodium alkyl sulfate as a detergent in tooth paste. J Am Dent Assoc and Dent Cosmos 1937; 24: 736-55.

8. Rantanen I, Tenovuo J, Pienihäkkinen K, Söderling E. Effects of a betaine-containing toothpaste on subjective symptoms of dry mouth: a randomized clinical trial. J Contemp Dent Pract 2003; 4: 11-23.

9. Cvikl B, Lussi A, Gruber R. The in vitro impact of toothpaste extracts on cell viability. Eur J Oral Sci 2015; 123: 179-85.

10. Cvikl B, Lussi A, Moritz A, Gruber R. Dentifrices for children differentially affect cell viability in vitro. Clin Oral Investig 2017; 21: 453-61.

11. Ray PD, Huang BW, Tsuji Y. Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling. Cell Signal 2012; 24: 981-90.

12. Sakaguchi RL, Powers JM, editors. Craig's restorative dental materials-e-book. Elsevier Health Sciences; 2012.

13. Moore C, Addy M, Moran J. Toothpaste detergents: a potential source of oral soft tissue damage? Int J Dent Hyg 2008; 6: 193-8.

14. Bruno M, Taddeo F, Medeiros IS, Boaro LC, Moreira MS, Marques MM, et al. Relationship between toothpastes properties and patient-reported discomfort: crossover study. Clin Oral Investig 2016; 20: 485-94.

15. Ghapanchi J, Kamali F, Moattari A, Poorshahidi S, Shahin E, Rezazadeh F, et al. In vitro comparison of cytotoxic and antibacterial effects of 16 commercial toothpastes. J Int Oral Health 2015; 7: 39-43.

16. Camargo SE, Jóias RP, Santana-Melo GF, Ferreira LT, El Achkar VN, Rode Sde M. Conventional and whitening toothpastes: cytotoxicity, genotoxicity and effect on the enamel surface. Am J Dent 2014; 27: 307-11.

17. Shahidi Z, Davani ST, Noori F, Tabatabaei MH, Sodeif F, Etemadi A, et al. Cytotoxicity and Antibacterial Activity of Toothpastes and Mouthwashes Available in the Iranian Market. Front Dent 2021; 18: 1-8.

18. Tabatabaei MH, Mahounak FS, Asgari N, Moradi Z. Cytotoxicity of the Ingredients of Commonly Used Toothpastes and

Mouthwashes on Human Gingival Fibroblasts. Front Dent 2019; 16: 450-7.

19. Schieber M, Chandel NS. ROS function in redox signaling and oxidative stress. Curr Biol 2014; 24: R453-62.

20. Goldshmidt H, Matas D, Kabi A, Carmi S, Hope R, Michaeli S. Persistent ER stress induces the spliced leader RNA silencing pathway (SLS), leading to programmed cell death in Trypanosoma brucei. PLoS Pathog 2010; 6: e1000731.

21. Mizutani T, Mori R, Hirayama M, Sagawa Y, Shimizu K, Okano Y, et al. Sodium Lauryl Sulfate Stimulates the Generation of Reactive Oxygen Species through Interactions with Cell Membranes. J Oleo Sci 2016; 65: 993-1001.

22. Kidd EA, Fejerskov O, editors. Dental caries: The disease and its clinical management. Blackwell Munksgaard; 2003.

23. Early Childhood Caries: IAPD Bangkok Declaration. Int J Paediatr Dent 2019; 29: 384-6.

24. Almohefer SA, Levon JA, Gregory RL, Eckert GJ, Lippert F. Caries lesion remineralization with fluoride toothpastes and chlorhexidine - effects of application timing and toothpaste surfactant. J Appl Oral Sci 2018; 26: e20170499.

Original Article / Özgün Araştırma

The Effect of Niacin and Melatonin Supplementation on the Antioxidant System and Lipid Peroxidation in Exercised Rats

Egzersiz Yaptırılan Ratlarda Niasin ile Melatonin Takviyesinin Antioksidan Sistem ve Lipid Peroksidasyonu Üzerine Etkisi

Adem Keskin[1], Aslıhan Büyüköztürk Karul[2]

[1]Aydın Gynecology and Pediatrics Hospital, Clinic of Biochemistry, Aydın, Turkey
[2]Aydın Adnan Menderes University Faculty of Medicine, Department of Biochemistry, Aydın, Turkey

Address for Correspondence/Yazışma Adresi:
Adem Keskin phD,
Aydın Gynecology and Pediatrics Hospital,
Clinic of Biochemistry, Aydın, Turkey
Phone : +90 535 372 11 43
E-mail : adem.keskin2@saglik.gov.tr
ORCID ID: orcid.org/0000-0003-1921-2583

Abstract

Objective: The aim of this study was to examine the effect of niacin and melatonin supplementation on the antioxidant system and lipid peroxidation in exercised rats.
Materials and Methods: The rats were divided into four groups. Running exercise was done for ten days. Niacin and melatonin were given before exercise.
Results: The malondialdehyde levels of the melatonin group were lower than those of the other groups. Malondialdehyde levels of the niacin + melatonin group were higher than the control groups super oxide dismutase (SOD) levels of the melatonin group were higher than those of the other groups. SOD levels of the niacin + melatonin group were lower than those of the other groups. Catalase levels of the melatonin group were higher than those of the niacin groups. Glutathione peroxidase levels of the control group were lower than those of the other groups. Total oxidant levels of the control group were lower than those of the niacin groups. Total antioxidant level (TAS) levels of the control group were lower than those of the other groups. TAS levels of the niacin + melatonin group were lower than those of the niacin or melatonin groups. Melatonin levels of the melatonin groups were higher than those of the other groups.
Conclusion: Melatonin supplementation decreased malondialdehyde levels and increased antioxidant parameters. It can be said that it has a higher antioxidant effect compared to niacin. Co-administration of niacin and melatonin increased oxidation parameters and decreased SOD and catalase levels. These enzymes indicated that they were used in response to increased oxidant status.

Öz

Amaç: Bu çalışmanın amacı, egzersiz yaptırılan ratlara verilen niasin ve melatonin desteğinin antioksidan sistem ve lipid peroksidasyonuna olan etkisini incelemektir.
Gereç ve Yöntemler: Ratlardan 4 grup oluşturuldu. On gün koşu egzersizi yaptırıldı. Egzersiz öncesi niasin ve melatonin verildi.
Bulgular: Melatonin grubunun malondialdehit düzeyleri, diğer gruplara göre daha düşüktü. Niasin + melatonin grubunun malondialdehit düzeyleri, kontrol grubuna göre daha yüksekti. Melatonin grubunun Süper oksit dismutaz (SOD) düzeyleri, diğer gruplara göre daha yüksekti. Niasin + melatonin grubunun SOD düzeyleri, diğer gruplara göre daha düşüktü. Melatonin grubunun katalaz düzeyleri, niasin gruplarına göre daha yüksekti. Kontrol grubunun glutatyon peroksidaz düzeyleri, diğer gruplara göre daha düşüktü. Kontrol grubunun total oksidan seviye düzeyleri, niasin gruplarına göre daha düşüktü. Kontrol grubunun total antioksidan seviye

(TAS) düzeyleri, diğer gruplara göre daha düşüktü. Niasin + melatonin grubunun TAS düzeyleri, niasin veya melatonin gruplarına göre daha düşüktü. Melatonin gruplarının melatonin düzeyleri diğer gruplara göre daha yüksekti.

Sonuç: Melatonin takviyesi, malondialdehit düzeylerini azalttı ve antioksidan parametreleri artırdı. Niasine göre daha yüksek bir antioksidan etkiye sahip olduğu söylenebilir. Niasin ve melatoninin birlikte uygulanması oksidasyon parametrelerini arttırdı ve SOD ve katalaz seviyelerini azalttı. Bu enzimler, artan oksidan durumuna yanıt olarak kullanıldıklarını gösterdi.

Introduction

In recent years, studies on exercise have reported that in addition to its beneficial effects on health, exercise also has oxidant effects depending on the type and intensity of the exercise (1,2). It is known that regular exercise has beneficial effects on health; however, its molecular mechanisms on peripheral tissues have not been examined adequately yet (1). Especially intense exercise and hypoxia are reported to have cumulative effect on stress (2). It is known that contracting skeletal muscles form free radicals and cellular structures are exposed to oxidative damage during long lasting intense exercise (3,4).

It is reported that melatonin detoxifies free radicals such as OH^- and H_2O_2 and therefore can prevent the harmful effects of these on biomolecules (5). Melatonin also protects the membrane against lipid peroxidation by attaching to the outer surface of the phospholipid layer of the cell membrane and contacting the radicals before the membrane (3).

There are contradictory studies about the application of melatonin before exercise; it has been shown that giving 1 mg/kg of melatonin to rats undergoing acute exercise reduces oxidative damage (4). On the other hand, in another study investigating the effects of melatonin in rats exposed to intense exercise, it has been shown that melatonin has no effect on total antioxidant status (TAS) and total oxidant status (TOS) (6).

The first substance in the synthesis of melatonin and niacin is tryptophan. Niacin is the precursor of NAD+/NADH and NADP+/NADPH that undertake important metabolic events in living cells. It has an active role in preventing many pathological processes with the effect of this metabolic cofactor (7). In studies conducted with niacin, it is reported to affect a large number of antioxidant enzymes; however, there are contradictory studies on the issue (8).

When studies conducted on oxidant state and antioxidant capacity with both melatonin and niacin were examined, no studies were found in which the effects of melatonin and niacin, which have their origins from a common, essential amino acid, were evaluated together. There are also some contradictory studies such as the insufficiency of their antioxidant capacity. In line with this information, the aim of this study was to investigate the effects of administering melatonin and niacin, which originate from the same amino acid, separately or together on lipid peroxidation and antioxidant capacity in the face of oxidative stress caused by exercise.

Materials and Methods

In this study, Procedures on animals were performed in Aydın Adnan Menderes University (ADU) Faculty of Medicine Experimental Animal Center. All analyzes were performed in ADU Central Laboratory. The ethics committee decision required for the study was obtained from ADU Animal Experiments Local Ethics Committee Center (Decision no: 64583101/2018/039, date: 23.03.2018). This study is the study of the doctoral thesis completed at ADU, Institute of Health Sciences, Department of (Medicine) Biochemistry. This study was supported by ADU Scientific Research Unit.

Procedures Applied on Rats

In the study, 40 rats weighing 380-465 grams were used. Rats were divided into four groups (n=10). Group 1= control group (only exercise was done), group 2= niacin group (exercise + niacin), group 3= melatonin group (exercise + melatonin), group 4= niacin + melatonin combo group (exercise + niacin + melatonin).

Rats exercise treadmill jogging at 15 min/day and 20 m/min speed without inclination for 10 days. Melatonin (Sigma-Aldrich) was given at a dose of 5 mg/kg intraperitoneally 30-45 minutes before exercise. Niacin (Sigma-Aldrich) was given orally at a dose of 360 mg/kg 30-45 minutes before exercise.

Approximately 7 mL of blood samples were collected by intracardiac, one day after the last

exercise. Blood specimens taken were transferred to ethylenediaminetetra-acetic acid tubes for catalase study and to biochemistry tubes for other studies. Serum samples were used for Malondialdehyde (MDA), TOS, TAS, melatonin, super oxide dismutase (SOD) and glutathione peroxidase (GPX)measurment.

Biochemical Methods

Catalase, MDA, SOD, GPX, TOS, TAS and melatonin levels were determined by spectrophotometric methods. Shimadzu Uv-1700 (Japan) device was used for catalase. TOS and TAS analysis were performed with the Rel Assay (Turkey) kit. Melatonin measurment was performed with the Elabscience kit. Biotek Epoch (Canada) device was used for SOD, MDA, GPX, TOS, TAS and melatonin analysis.

Statistical Analysis

SPSS 22.0 Windows program was used to analyze the data. Results were given as mean ± standard deviation. The groups were compared One-Way ANOVA. As a post hoc test, Tukey test was used for analyzed parameters MDA, Catalase, TOS, TAS and melatonin, and Dunnett T3 test was used for SOD and GPX.

Results

The rats were weighed every day of the exercise. Along with exercise, weight loss was observed in groups.

The group melatonin (2.09±0.67 μmol/L) gave lower MDA concentrations in comparison to control (3.01±0.53 μmol/L), niacin (3.23±0.50 μmol/L) and niacin + melatonin (3.77±0.54 μmol/L) groups (p<0.01) (Figure 1). However, higher MDA levels were found in the group received niacin + melatonin (3.77±0.54 μmol/L) in comparison to control (3.01±0.53 μmol/L) and melatonin (2.09±0.67 μmol/L) groups (p<0.001).

SOD levels were higher in group melatonin (12.52±2.57 U/mL) in comparison to control (8.74±1.08 U/mL), niacin (8.37±1.01 U/mL) and niacin + melatonin (6.01±0.61 U/mL) groups, while low SOD levels were observed in the group niacin + melatonin (6.01±0.61 U/mL) in comparison to control (8.74±1.08 U/mL), niacin (8.37±1.01 U/mL) and melatonin (12.52±2.57 U/mL) groups (p<0.01) (Figure 2).

Catalase levels were higher in the group melatonin [11.47±2.19 k/gr hemoglobin (Hb)] in comparison to niacin (8.19±2.48 k/gr Hb) (p<0.05) and niacin
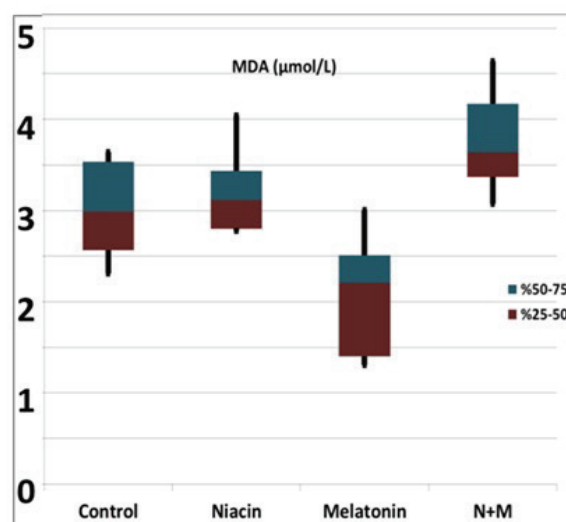
Figure 1. MDA levels of the groups
MDA: Malondialdehyde
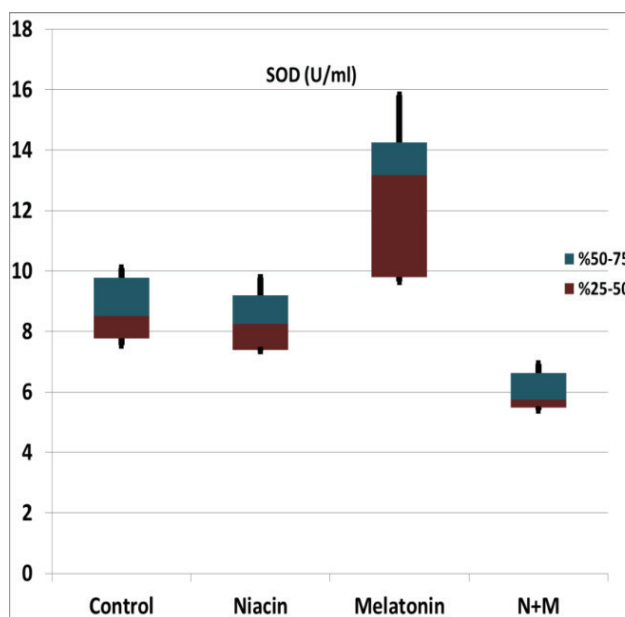
Figure 2. SOD levels of the groups
SOD: Super oxide dismutase

+ melatonin (6.95±1.95 k/gr Hb) (p<0.001) groups (Figure 3).

GPX levels were low in the control group (526.00±99.54) in comparison to melatonin (1029.92±249.34), niacin (1033.44±198.05) and niacin + melatonin (1152.33±313.19) combo groups (p<0.001) (Figure 4).

TOS levels were low in the control group (11.46±2.01 μmol H2O2 Equiv./L) in comparison to

niacin (15.43±2.69 µmol H2O2 Equiv./L) and niacin + melatonin (14.89±2.99 µmol H2O2 Equiv./L) groups (p<0.05) (Figure 5).

Lower levels of TAS was found in the control group (797.61±44.2 µmol Trolox Equiv./L) in comparison to melatonin (1205.32±38.84 µmol Trolox Equiv./L), niacin (1188.83±54.27 µmol Trolox Equiv./L) and niacin + melatonin (1113.56±72.11 µmol Trolox Equiv./L) combo groups (p<0.001). Additionally,

niacin+melatonin group (1113.56±72.11 µmol Trolox Equiv./L) showed lower levels in comparison to melatonin (1205.32±38.84 µmol Trolox Equiv./L), niacin (1188.83±54.27 µmol Trolox Equiv./L) groups (p<0.05) (Figure 6).

Melatonin levels were higher in the groups received melatonin (189.09±39.85 pg/mL) and niacin
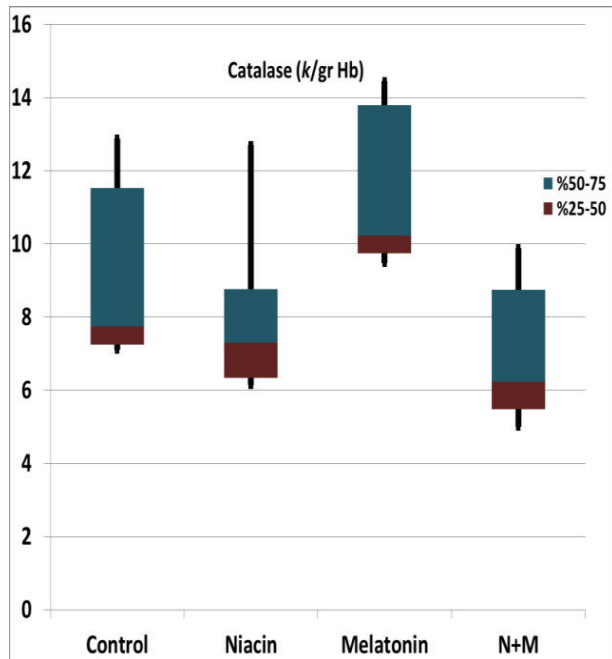
Figure 3. Catalase levels of the groups

Figure 5. TOS levels of the groups
TOS: Total oxidant status

Figure 4. GPX levels of the groups
GPX: Glutathione peroxidase
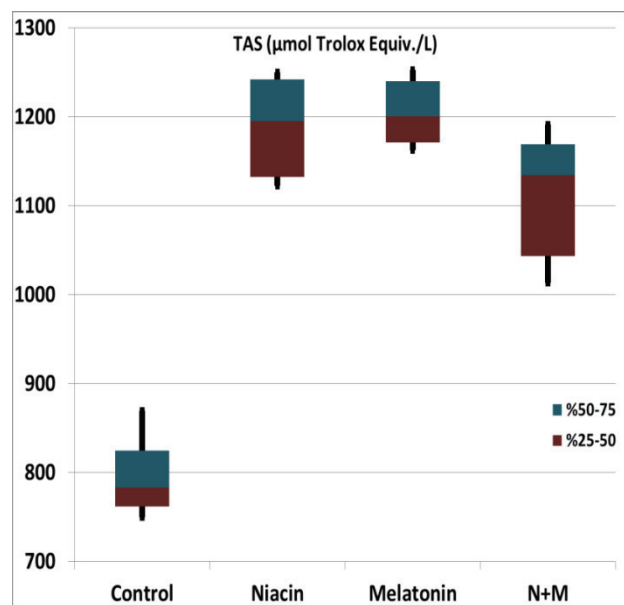
Figure 6. TAS levels of the groups
TAS: Total antioxidant level

+ melatonin (189.71±31.48 pg/mL) in comparison to control (135.72±26.81 pg/mL), niacin (142.80±24.80 pg/mL) groups (p<0.05) (Figure 7).
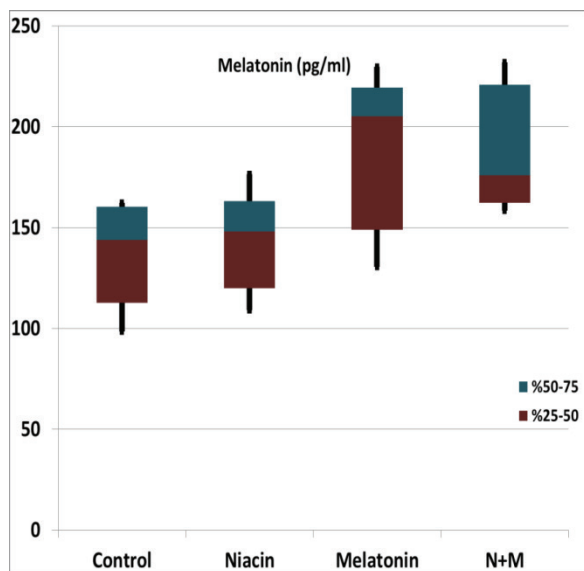
Figure 7. Melatonin levels of the groups

Discussion

It is known that moderate and intense exercise increases oxidant activity, is harmful for the organism and can threaten health. In our study, our aim was to examine the antioxidant effects of melatonin and niacin, which are reported to have antioxidant effect but the effects of which can change depending on the type of physical activity, separately and in combination on exercised rats.

Oxidative stress is known to play a role in a large number of physiological situations such as aging or exercise and in various diseases. Although regular mild exercise seems to be useful for health and oxidative stress, acute and tiring workouts of anaerobic and aerobic exercise may trigger a review of systems (ROS) over production (9). Though exercise is a source of stress that creates oxidative stress by increasing the production of free oxygen radicals, It is also known to affect antioxidant enzyme activity to develop resistance against oxidative stress (10).

In our study, rats were exposed to treadmill exercise with no elevation for 10 days. The control group was just exposed to exercise and the other three groups that exercised were given niacin, melatonin, niacin + melatonin, respectively.

The group that experienced the most significant weight loss during exercise was the group niacin+melatonin together. Malondialdehyde levels were higher in the group with niacin and melatonin together than in the other groups. MDA levels of the melatonin group were found to be lower than the other groups. In exercised rats, melatonin alone was found to decrease lipid peroxidation. MDA levels of the group that was given only niacin were found to be significantly higher than those of the group that was given only melatonin. It can be said that niacin is not as effective as melatonin in preventing lipid peroxidation.

It has been shown that long term exercise in adult men increases MDA levels and that melatonin significantly decreases MDA increase (11). In our study, similarly, MDA was lower in the group melatonin when compared with the other groups.

In another study which compared obese children and healthy subjects, oxidant-antioxidant state was examined in association with weight loss. Body mass index (BMI), fat mass and waist-hip ratio were found to decrease significantly, while MDA was found to increase significantly, when compared with the control group. MDA was correlated with whp, fat mass and BMI and antioxidant state was found to return to normal after six months of dietary restriction (12).

In a study in which 400 mg/kg niacin was given to rats and exposed to exercise, it was found that NAD content and energy consumption increased in skeletal muscle (13). In a study conducted on rats, it was found that melatonin application caused SOD levels to be higher (14). In this study, SOD levels of the group melatonin was found to be higher when compared with the other groups.

In a study in which the effect of aerobic exercise on lipid metabolism was investigated in rats with dietary hyperlipidemia, it was reported that malondialdehyde levels increased, while SOD levels decreased and SOD levels increased after MDA levels decreased (15). In our study, weight loss, decrease in SOD levels and increase in malondialdehyde levels were observed especially in the group niacin + melatonin.

Catalase is one of the antioxidant enzymes that catalyse the decomposition of H_2O_2 formed by SOD into molecular oxygen and water (16). In a study which examined the effect of melatonin against oxidative stress induced by exercise in healthy individuals, no

significant change was observed in catalase level (17). In our study, significant change was not found in the group melatonin when compared with the control group. In our study, weight loss and catalase were found to decrease in the group niacin+melatonin together when compared with the group melatonin.

In a study in which melatonin was made in athletes, pre and post-exercise GPX levels of the control group and the groups which received melatonin were examined. Post-exercise levels were found to be lower in both groups when compared with the pre-exercise. In the comparison between groups, the group that was given melatonin was found to be higher when compared with the control group (18).

Studies conducted on the antioxidant effect of niacin have been conducted on different living beings and cell cultures. Niacin has been found to decrease ROS production in hepatocyte cell cultures (19).

In our study, when compared with the control group, GPX levels of the other three groups were found to be higher. In all of the groups that received niacin, GPX level was found to be significantly high, unlike SOD, catalase and MDA levels.

When the analysed TOS levels were examined in our study, the levels of the two groups given niacin, which had more weight loss when compared with the other two groups, were found to be significantly high when compared with the control group. The kit we used in our study to determine TAS gives results about the total antioxidant state with TEAC method. TAS levels of the groups which were given melatonin and niacin were found to be significantly high when compared with the control group. These results show that niacin and melatonin show antioxidant separately and together.

In our study, when serum melatonin levels were examined, it was found that the two groups which received melatonin had significantly higher serum melatonin levels than the two groups which did not receive melatonin. This result showed that antioxidant and oxidant parameters were evaluated in suitable melatonin concentrations.

Melatonin increases the volume of muscle spindles and decreases ROS production (20). The indole ring of melatonin prevents free radical damage, it has also been shown to be very effective in increasing physical performance (21). The effect of giving melatonin on physical performance is still a disputable issue. In their meta analysis, Lopez-Flores et al. (22) reported that the effect of giving melatonin could differ depending on the type of physical activity. There are a large number of studies reporting that giving exogenous melatonin in experimental animal studies has antioxidant effects and repairs muscle damage in rats (23-25). In our study, similar to the results of the studies above, giving melatonin to rats increased antioxidant activity.

Conclusion

As a result, it can be said that the administration of exogenous melatonin to rats increases antioxidant activity and also shows higher antioxidant activity than niacin. It can be said that the administration of exogenous niacin to rats increases antioxidant activity (GPX, TAS). It can be said that the administration of exogenous niacin and melatonin combination to rats did not create a synergistic effect on antioxidant parameters.

Ethics

Ethics Committee Approval: The present cohort study was designed as a survey and was approved by the Local Ethical Committee for Animal Experiments of Aydın Adnan Menderes University (decision no: 64583101/2018/039, date: 23.03.2018).

Informed Consent: Experimental study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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

Financial Disclosure: The authors declared that this study received no financial support.

References

1. Górnicka M, Drywień M, Frąckiewicz J, Dębski B, Wawrzyniak A. Alpha-Tocopherol May Protect Hepatocytes Against Oxidative Damage Induced by Endurance Training in Growing Organisms. Adv Clin Exp Med 2016; 25: 673-9.
2. Pialoux V, Mounier R, Ponsot E, Rock E, Mazur A, Dufour S, et al. Effects of exercise and training in hypoxia on antioxidant/pro-oxidant balance. Eur J Clin Nutr 2006; 60: 1345-54.

3. Granger DN, Kvietys PR. Reperfusion injury and reactive oxygen species: The evolution of a concept. Redox Biol 2015; 6: 524-51.

4. Alonso M, Collado PS, González-Gallego J. Melatonin inhibits the expression of the inducible isoform of nitric oxide synthase and nuclear factor kappa B activation in rat skeletal muscle. J Pineal Res 2006; 41: 8-14.

5. Brzezinski A. Melatonin in humans. N Engl J Med 1997; 336: 186-95.

6. Ağırbaş Ö. Effects of Melatonin and Ascorbic Acid on DNA Damage and Oxidative Stress Emerging in Muscles in Rats on Which Intensive Exercise Stress Was Created. Erzurum. Atatürk University. 2013.

7. Burtis CA, Ashwood ER. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics 5th Ed. 2010.

8. Ilkhani F, Hosseini B, Saedisommeolia A. Niacin and Oxidative Stress: A Mini-Review. J Nutri Med Diet Care 2016; 2: 1-6.

9. Pingitore A, Lima GP, Mastorci F, Quinones A, Iervasi G, Vassalle C. Exercise and oxidative stress: potential effects of antioxidant dietary strategies in sports. Nutrition 2015; 31: 916-22.

10. Aguiló A, Tauler P, Pilar Guix M, Villa G, Córdova A, Tur JA, et al. Effect of exercise intensity and training on antioxidants and cholesterol profile in cyclists. J Nutr Biochem 2003; 14: 319-25.

11. Ziaadini F, Aminae M, MahsaRastegar MM, Abbasian S, Memari AH. Melatonin Supplementation Decreases Aerobic Exercise Training Induced-Lipit Peroxidation and Malondialdehyde in Sedentary Young Women. Pol J Food Nutr Sci 2017; 67: 225-32.

12. Mohn A, Catino M, Capanna R, Giannini C, Marcovecchio M, Chiarelli F. Increased oxidative stress in prepubertal severely obese children: effect of a dietary restriction-weight loss program. J Clin Endocrinol Metab 2005; 90: 2653-8.

13. Cantó C, Houtkooper RH, Pirinen E, Youn DY, Oosterveer MH, Cen Y, et al. The NAD(+) precursor nicotinamide riboside enhances oxidative metabolism and protects against high-fat diet-induced obesity. Cell Metab 2012; 15: 838-47.

14. Beck WR, Botezelli JD, Pauli JR, Ropelle ER, Gobatto CA. Melatonin Has An Ergogenic Effect But Does Not Prevent Inflammation and Damage In Exhaustive Exercise. Sci Rep 2015; 5: 18065.

15. Yang Y, Wu T, He K, Fu ZG. Effect of aerobic exercise and ginsenosides on lipid metabolism in diet-induced hyperlipidemia mice. Zhongguo Yao Li Xue Bao 1999; 20: 563-5.

16. Aydemir T, Kuru K. Purification and partial characterization of catalase from chicken erythrocytes and the effect of various inhibitors on enzyme activity. Turkish Journal of Chemistry 2003; 27: 85-97.

17. Kumar KV, Mur N. Effect Of Oral Melatonın On Exercıse-Induced Oxıdant Stress In Healthy Subjects. Indian Journal of Pharmacology 2002; 34: 256-9.

18. Ortiz-Franco M, Planells E, Quintero B, Acuña-Castroviejo D, Rusanova I, Escames G, et al. Effect of Melatonin Supplementation on Antioxidant Status and DNA Damage in High Intensity Trained Athletes. Int J Sports Med 2017; 38: 1117-25.

19. Ganji SH, Kashyap ML, Kamanna VS. Niacin inhibits fat accumulation, oxidative stress, and inflammatory cytokine IL-8 in cultured hepatocytes: Impact on non-alcoholic fatty liver disease. Metabolism 2015; 64: 982-90.

20. Stacchiotti A, Favero G, Rodella LF. Impact of Melatonin on Skeletal Muscle and Exercise. Cells 2020; 9: 288.

21. Escames G, Ozturk G, Baño-Otálora B, Pozo MJ, Madrid JA, Reiter RJ, et al. Exercise and melatonin in humans: reciprocal benefits. J Pineal Res 2012; 52: 1-11.

22. Lopez-Flores M, Luque-Nieto R, Moreira OC, Suarez-Iglasias D, Villa-Vicente JG. Effects of Melatonin on Sports Performance: A Systematic Review. Journal of Excise Physiology online. 2018; 21: 121-38.

23. Favero G, Stacchiotti A, Castrezzati S, Bonomini F, Albanese M, Rezzani R, et al. Melatonin reduces obesity and restores adipokine patterns and metabolism in obese (ob/ob) mice. Nutr Res 2015; 35: 891-900.

24. Salucci S, Battistelli M, Baldassarri V, Burini D, Falcieri E, Burattini S. Melatonin prevents mitochondrial dysfunctions and death in differentiated skeletal muscle cells. Microsc Res Tech 2017; 80: 1174-81.

25. Sayed RKA, Fernández-Ortiz M, Diaz-Casado ME, Aranda-Martínez P, Fernández-Martínez J, Guerra-Librero A, et al. Lack of NLRP3 Inflammasome Activation Reduces Age-Dependent Sarcopenia and Mitochondrial Dysfunction, Favoring the Prophylactic Effect of Melatonin. J Gerontol A Biol Sci Med Sci 2019;74:1699-708.

Several Shaping Characteristics of OneCurve Continuously Rotating System versus Three Different Kinematic Systems: ProTaper Universal, Twisted File Adaptive and WaveOne Gold

Üç Farklı Kinematik Sisteme Karşı OneCurve Sürekli Rotasyon Sisteminin Şekillendirme Özellikleri: ProTaper Universal, Twisted File Adaptive ve WaveOne Gold

Ali Türkyılmaz[1], Volkan Arıkan[2]

[1]Kırıkkale University Faculty of Dentistry, Department of Endodontics, Kırıkkale, Turkey
[2]Kırıkkale University Faculty of Dentistry, Department of Pedodontics, Kırıkkale, Turkey

Address for Correspondence/Yazışma Adresi:
Ali Türkyılmaz Ph.D.,
Kırıkkale University Faculty of Dentistry, Department of Endodontics, Kırıkkale, Turkey
Phone : +90 532 248 20 51
E-mail : turkyilmaz_a@hotmail.com
ORCID ID: orcid.org/0000-0003-0641-0062

Abstract

Objective: This study aimed to compare the curvature change, preparation time, resin removal amount and working length reduction properties of the OneCurve (0.25/0.06), ProTaper Universal F2 (0.25/0.06), Twisted File Adaptive SM2 (0.25/0.06) and WaveOne Gold Primary (0.25/0.07) using simulated root canal models.

Materials and Methods: A total of 67 plastic models were used. Three models were used to verify the initial curvature angle, the weight of the unprepared resin block and the initial root canal length. The remaining 64 models were divided into four groups of 16 samples. After preparation, the changes in parameters were measured again. Statistical analysis was done with SPSS 22.0 using one-way ANOVA and post hoc Tukey's tests and Kruskal–Wallis with Bonferroni corrections. Pearson and Spearmen correlation coefficients were also used. A p-value <0.05 was significant.

Results: Significant differences were observed between OneCurve, ProTaper Universal F2, Twisted File Adaptive SM2 and WaveOne Gold Primary file systems in terms of resin removal amount, root canal curvature change and preparation time (p<0.05). The correlations were statistically significant (p<0.05).

Conclusions: Twisted File Adaptive SM2 performed significantly less resin removal and curvature change. Furthermore, the Twisted File Adaptive and WaveOne Gold Primary instruments required less preparation time compared with OneCurve and ProTaper Universal F2.

Öz

Amaç: Bu çalışmanın amacı simüle edilmiş kök kanal modelleri kullanılarak OneCurve (0,25/0,06), ProTaper Universal F2 (0,25/0,06), Twisted File Adaptive SM2 (0,25/0,06) ve WaveOne Gold Primer (0,25/0,07) eğelerinin kurvatür değişimi, preparasyon süresi, rezin uzaklaştırma miktarı ve çalışma boyu kaybı özelliklerini karşılaştırmaktır.

Gereç ve Yöntemler: Toplam 67 plastik model kullanıldı. İlk eğrilik açısını, hazırlıksız reçine bloğunun ağırlığını ve ilk kök kanal uzunluğunu doğrulamak için üç model kullanıldı. Kalan 64 model, 16 örnekten oluşan dört gruba ayrıldı. Hazırlandıktan sonra parametrelerdeki değişiklikler tekrar ölçüldü. İstatistiksel analiz SPSS 22.0

ile tek yönlü ANOVA ve post hoc Tukey testleri ve Kruskal–Wallis ile Bonferroni düzeltmesi kullanılarak yapıldı. Ayrıca, Pearson ve Spearmen korelasyon katsayıları kullanıldı. P değeri <0,05 istatistiksel olarak anlamlı olarak kabul edildi.

Bulgular: Rezin uzaklaştırma miktarı, kök kanal eğiminde değişim ve preparasyon süresi açısından OneCurve, ProTaper Universal F2, Twisted File Adaptive SM2 ve WaveOne Gold Primary eğe sistemleri arasında önemli farklılıklar gözlendi (p<0,05). Korelasyonlar istatistiksel olarak anlamlıydı (p<0,05).

Sonuç: Twisted File Adaptive SM2 önemli ölçüde daha az miktarda rezin uzaklaştırdı ve eğim değişikliği gösterdi. Ayrıca, Twisted File Adaptive ve WaveOne Gold Primary enstrümanları, OneCurve ve ProTaper Universal F2 ile karşılaştırıldığında daha az preparasyon süresi gerektirdi.

Introduction

The ideal preparation preserves the original anatomy of the root canal with the smallest possible apical diameter and the largest possible root canal orifice diameter. In this manner, the mechanical preparation of curved canals needs more attention since several complications may arise during the preparation of these canals, such as thinning of the root canal walls or straightening of curved canal paths. Thus, an adequate entrance and optimal preparation of the root canal at the coronal third should be maintained to avoid over-instrumentation and outright perforation (1).

In recent years, advances in rotary instruments made of nickel-titanium (NiTi) have improved the effectiveness of root canal treatment notably, especially in curved canals. Their properties of superelasticity, resistance and shape memory are the main advantages of their use (2). Another benefit of NiTi files is in reducing the preparation time for root canal instrumentation, and facilitation of debris removal (3). On the other hand, the curvature of the canal is a potential problem for cyclic fatigue failure due to cyclic stresses on instruments by bending (4), and NiTi instruments have a risk of fracture due to the different curvature shapes of root canals in clinical application.

The OneCurve (OC; MicroMega, Besancon, France) is a recently produced NiTi endodontic file with a patent-protected heat treatment, known as C-Wire alloy technology (5). It is a continuously rotating rotary file that preparing of the entire root canal length with a single instrument; it has a pre-bendable property and a shape memory. The OC file has a similar tip size and taper (0.25/0.06) to its predecessors [One Shape (MicroMega) and Two Shape (MicroMega)] but has a new shape design (5). A triangular cross-sectional instrument ProTaper Universal F2 (PTU; Dentsply Maillefer, Ballaigues, Switzerland) has a gradual taper. The file system has registered metallurgy that enhance the resistance and flexibility to cyclic fatigue (6). The Twisted File Adaptive SM2 (TF Adaptive, Axis/SybronEndo Orange, CA, USA) is a NiTi instrumentation system that combines the continuous rotation motion and reciprocating motion. The instrument works in continuous rotation if there is little or no stress loading on the instrument. WaveOne Gold (WOG) Primary reciprocal files are the advanced version of WaveOne (WO) files (Dentsply Sirona, Ballaigues, Switzerland), with metallurgical properties modified from M-Wire to Gold-Wire to improve the fracture resistance (7).

Currently, the information on the shaping ability of the OC is limited, and studies comparing OC shaping characteristics with different kinematics in curved canals are insufficient. This study aimed to compare the OC with three different kinematic rotary systems; the continuous rotating PTU, the adaptive motion TF Adaptive and the reciprocating WOG on four different shaping characteristic properties of files curvature change, working length (WL) reduction, resin removal amount and preparation time and to provide an analysis of the correlations between interrelated shaping properties.

Materials and Methods

Ethical approval was not required since no human or animal subjects were involved. The study was planned to be conducted with four groups and the sample calculation was performed considering an alpha-type error of 0.05, a beta power of 0.80 and with 0.40 as the effect size using G* Power v.3.1 software (Heinrich Heine University, Dusseldorf, Germany). A minimum sample size of 16 for each group was determined.

Similar to previous studies (8-10), for standardization and simulation of the curved root canals of natural human teeth, plastic models (curved endo-training resin blocks; Dentsply Maillefer, Ballaigues, Switzerland) with an approximately

mean curvature of 33°, ISO 15 apical enlargement, and 16-mm, 0.02 taper canal space were used. Four groups were formed each containing 16 samples. An electronic balance (Mettler Toledo, Switzerland) with an accuracy of 0.0001 g was used to weight the unprepared plastic models before instrumentation, and weights were recorded. An experienced endodontist (A.T.) performed all procedures. An ISO size 15 stainless steel K-file (Dentsply Maillefer, Ballaigues, Switzerland) was set when the tip of the file was visible at the edge of the canal at 2.5x magnification under a dental microscope (OPMIpico version 8.0, Carl Zeiss Meditac AG, Germany), and initial WL was obtained by subtracting 0.5 mm from the length. All canals were prepared according to this initial WL up to size 25 enlargement.

Group 1: OneCurve (0.25/0.06). The models were prepared with the X-Smart Plus endodontic motor at 300 rpm and 2.5 N.cm torque with continuous rotation. The One Flare (0.25/0.09) (Micro Mega), ISO K-file (0.10/0.02), One G (0.14/0.03), ISO K-file (0.15/0.02) and OneCurve files were used. The Glide Path One G (Micro Mega, France) (250-400 rpm max 1.2 N.cm torque) was used with 300 rpm and 1.0 N.cm torque.

Group 2: ProTaper Universal F2 (0.25/0.06). The models were prepared with the X Smart Plus (Dentsply, Switzerland) endodontic motor in the ProTaper Universal program with continuous rotating motion at 300 rpm and at various torque values in accordance with the files used. The SX, ISO 015 K-File, S1, S2, F1 and F2 files were used.

Group 3: Twisted File Adaptive SM2 (0.25/0.06). The models were prepared with the Elements Motor (SybronEndo, Glendora, CA, USA) in the TF Adaptive program with adaptive motion. The ISO K-file (0.08/0.02 and 0.15/0.02), SM1 (0.20/0.04) and SM2 files were used.

Group 4: WaveOne Gold Primary (0.25/0.07). The models were prepared with the X-Smart Plus endodontic motor in the WOG program with reciprocating motion including own rpm and N.cm values. The One G and a primary file were used.

The models were instrumented with TF Adaptive using an 8:1 reduction handpiece for TF Adaptive and a 6:1 reduction handpiece for the other files. All instruments were inserted with light pressure, and an in-and-out pecking motion not exceeding 3 mm was applied to engage the resin canal surfaces. After three pecks, resin canal was flushed with 2 mL distilled water. The instrument was cleaned regularly using a moist sponge to remove resin particles from the flutes. Following the use of each file, a 15 K-sized file was placed up to the WL to unclog debris blockage. The preparation was continued until the file reached the WL established previously. Each instrument was discarded after preparation of two resin canals. The WL was established again after instrumentation to the nearest 0.25 mm.

Evaluation of Canal Curvature and WL Change

Pre- and post-instrumentation images were taken at 1.6x magnification (Carl Zeiss). As previously described by Schneider (11), a parallel line was drawn to the axis of the canal on the digital photo images. Another line was scribed from the apical foramen to the previous line at the point that the simulated root canal began to leave the long axis of the tooth. The angle created by two lines was accepted as a curvature parameter. The same procedure was done for the postoperative images. The change in curvature was measured by subtracting the postoperative angle from the pre-operative one.

The initial WL was verified with an ISO 15 K-file (Dentsply Maillefer, Ballaigues, Switzerland) until the file was just visible at the apical edge of the resin canal at 2.5x magnification under a dental microscope (Carl Zeiss Meditac AG, Germany), and 0.5 mm was subtracted to determine the WL. After instrumentation was completed, the WL was measured with a similar method and the difference between the initial and last value was recorded as the change in WL.

Evaluation of Resin Removal and Preparation Time

The removal amount of resin was determined by subtracting the weight of the prepared model from the weight of the unprepared model. The time elapsed during preparation including the operation period, instrument changes and irrigation application was recorded using a chronometer.

Statistical Analysis

All parameters were analyzed with SPSS 22.0 software (IBM, Armonk, NY). Normality was verified by using the Shapiro-Wilks test. The one-way analysis of variance and post hoc Tukey's tests were used for curvature change and WL change as there were normally distributed data. The resin amount

and preparation time data were not distributed normally, so the Kruskal-Wallis test with Bonferroni correction was used for these comparisons. P<0.05 was considered significant. In correlation analyses, Spearman's t-test (for non-parametric variables) was used for the association between resin removal amount and preparation time, and Pearson's t-test (for parametric variables) was used for the association between WL reduction and curvature change.

Results

Comparative investigation of the shaping ability properties of the files is presented in Table 1. The mean change of canal curvature presents a significant difference between the four groups (p=0.004). The PTU demonstrated a significantly curvature reduction when compared with the TF Adaptive and WOG. Preparation of the canals resulted in a mean loss in WL for all file systems, but statistically significant differences were not found in WL reduction between file systems (p=0.295).

A significant difference was found in the resin removal amount between the files (p=0.012). The TF Adaptive removed significantly less resin from the canals when compared to the PTU and WOG. There was a significant difference between groups in preparation time (p<0.001). The shortest mean preparation time was recorded with the WOG. The post-hoc test showed that the WOG file carried out preparation significantly faster than the OC or PTU.

A significant correlation between the shaping properties was found. The Pearson correlation coefficient (rs) between curvature change and WL change was equal to 0.381 (between 0.3 and 0.7) (p=0.003), showing a positive moderate correlation

between these two variables. The Spearman correlation coefficient (rs) between resin removal and preparation time was equal to 0.390 (between 0.3 and 0.7) (p=0.002), showing a positive moderate correlation between these two variables.

Discussion

The NiTi alloys were developed with thermal treatment technology, namely, M-wire (Dentsply Tulsa Dental Specialities, Tulsa, OK), controlled memory wire (CM wire, DS Dental, Johnson City, TN) or R-phase wire (SybronEndo, Orange, CA). Thermomechanical processing is an optimized technique to control the transformation and microstructure properties of NiTi alloys for enhanced mechanical properties (12). The OC is a single-file system that uses rotating motion, manufactured with C-wire thermo-mechanical technology. The file has a variable-cross-section design. The manufacturer claims that the instrument is developed to maintain effective preparation while protecting original root canal anatomy (13).

Meanwhile, the PTU is a much researched NiTi file system that uses M-wire thermo-mechanical properties. In TF Adaptive technology, R-phase heat treatment, forming of the mental core and designing of the special surface increase the strength, fatigue resistance and flexibility of the file (14). The WOG is a single-file system with similar kinematics to the WO, though the metallurgic property is changed to Gold-wire, which provides greater fracture resistance to the file and increases its flexibility when compared with NiTi and the M-wire alloy (15). The WOG size 25 with a 0.07 taper is suggested by the manufacturer to come at the ideal size for most root canals (16). The

Table 1. Differences in the degree of canal curvature change (°), WL reduction (mm), resin removal amount (mg) and preparation time (s) with OneCurve, Protaper Universal F2, Twisted File Adaptive SM2 and WaveOne Gold Primary

	Curvature change (°)	WL change (mm)	Resin removal amount (mg)		Preparation time (s)	
File type	Mean ± SD	Mean ± SD	Min-max	Mean rank	Min-max	Mean rank
OneCurve	6.75±2.75[a,b]	0.28±0.19[a]	2.40-4.60	32.97[a,b]	191-345	35.13[a,b]
ProTaper Universal	8.06±3.11[a]	0.35±0.16[a]	1.20-9.80	34.87[b]	248-567	51.60[a]
TF Adaptive	4.83±201[b]	0.23±0.15[a]	0.90-4.10	17.90[a]	122-233	19.60[b,c]
WaveOne Gold	5.11±2.44[b]	0.25±0.21[a]	2.70-9.70	37.07[b]	106-231	15.67[c]
p-value	p=0.004	p=0.295	-	p=0.012	-	p<0.001

*In each column the values followed by the same superscript letter do not differ (p<0.05), TF: Twisted file, Min-max: Minimum-maximum, WL: Working length

cross-section of the file is designed with two cutting edges on an off-centered parallelogram.

The continuous rotating motion was the first kinematic movement used in endodontic motors. This technique aimed to decrease the preparation time and cost and increase the shaping quality and safety (3). Another rotation technique is adaptive motion, which works with its own motor (Elements Motor, Sybron Endo) including a special mode that combines of continuous and reciprocating rotation.

The present study investigated the shaping ability of four NiTi instruments working with three different kinematic motions in simulated moderate curvature canals. The four main findings obtained from four shaping ability features were that: (i) less flexible instruments caused more canal straightening even if the instruments compared have similar tapers; (ii) similar to previous reports (9,17,18), WL reduction occurred with all instruments regardless of the taper, flexibility or alloy technology; (iii) although the files used in the study have similar taper angles, the equilateral triangular cross-sectioned (two sharp cutting edges) TF Adaptive removed less resin than the One Curve (S-shaped near the shaft and triangular-shaped at the tip), ProTaper (convex triangular cross-section) or WOG (parallelogram-shaped cross-section); (iv) using a single-file system with reciprocating motion resulted in faster preparation than the others.

Reduction in WL in curved canals is one of the main concerns for the preparation of the curved root canals has been emphasized by numerous studies (19,20). This unpredictable change may lead to overpreparation and overfilling, which may result in foreign-body reaction, delayed periapical healing (9) or postoperative pain (21). In the present study, root canal curvature and root canal length were correlated with WL reduction. The straightening of a severe curvature influenced WL modification. On the contrary, it has been reported that straight canals do not cause a significant WL decrease after instrumentation (22). Essentially, minimal reduction in WL for canals with curvatures is predictable since NiTi file systems prepare root canals with the crown-down method, and in curved canals, the preparation of the coronal section of the canal facilitates the placement of the file in the apical section by straightening the curvature at the coronal part. A study described a mean WL reduction of 0.22 mm after canal preparation with NiTi files (23). Similar to this result, the present study found the average reduction in WL to be 0.28±0.18 mm in curved canals regardless of the file type. Also, the positive correlation between WL change and canal curvature corroborates the results of previous studies (19,20). In line with the present study, it was previously reported that different kinematic and metallurgic properties could not protect the original anatomy of root canals and caused a modification in WL (9,18,20). Although the WOG has a more tapered structure (0.07) than the other files, it caused a similar curvature change when compared to the TF Adaptive and OC instruments. Its flexibility may have contributed to the control of curvature change.

The preparation time is calculated by measuring the total preparation time, which includes active instrumentation, instrument changes and irrigation application (24), or the active instrumentation time only (18). In this study, to obtain more realistic results that could reflect clinical conditions, using a chronometer, the total duration of preparation was recorded.

The findings revealed that the use of WOG instruments showed a significantly faster preparation than the use of PTU or OC instruments, but that difference was not statistically significant against the TF Adaptive. The shortest mean preparation time was recorded for the WOG against the other files used. The WOG is a single-file system that works in a reciprocating motion. The files that run with a continuous rotating motion use a series of files, while reciprocating system have only one main file used to enlarge the root canal generally. This presents an advantage in terms of preparation time for practitioners. When acrylic blocks were used, the weight of the removed acrylic resin gives a measurement with which to understand the enlargement capacity of the file. In the present study, TF Adaptive caused less significant resin removal when compared to the PTU or WOG. Different parameters mentioned above could be effective in this complex interrelationship, warranting further investigation. The positive correlation between resin removal and preparation time reveals that the systems with shorter preparation times cause greater dentin removal from the canals. This is presumably the result of more aggressive preparation. While a shorter preparation time is an important feature of

rotary systems, a balance between a quick and safe preparation would be more desirable for clinicians.

In the present study, the taper angles of the files in different groups were not standardized, which can be considered as one of the limitations of the study, as this may have prevented higher correlation values from being obtained. Another limitation is the resin blocks used. These blocks provide a standard condition that cannot perfectly mimic the anatomic irregularities and variabilities in root canal systems, thus; the results may not definitively reflect *in vivo* conditions (3).

Conclusion

Considering the experimental conditions of the present study, OC did not show a superior shaping performance versus the other files used. To obtain more specific information about the shaping ability of the OC, further examinations comparing with different-generation instruments on simulated canals and/or extracted teeth are needed.

Ethics

Ethics Committee Approval: Ethical approval was not required since no human or animal subjects were involved.

Informed Consent: Informed consent was not required since no human subjects were involved.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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

Financial Disclosure: The authors declared that this study received no financial support.

References

1. Ruddle CJ. Nickel-titanium rotary instruments: current concepts for preparing the root canal system. Aust Endod J 2003; 29: 87-98.
2. Arens FC, Hoen MM, Steiman HR, Dietz GC Jr. Evaluation of single-use rotary nickel-titanium instruments. J Endod 2003; 29: 664-6.
3. Ahn SY, Kim HC, Kim E. Kinematic Effects of Nickel-Titanium Instruments with Reciprocating or Continuous Rotation Motion: A Systematic Review of In Vitro Studies. J Endod 2016; 42: 1009-17.
4. Peters OA. Current challenges and concepts in the preparation of root canal systems: a review. J Endod 2004; 30: 559-67.
5. Serafin M, De Biasi M, Franco V, Angerame D. In vitro comparison of cyclic fatigue resistance of two rotary single-file endodontic systems: OneCurve versus OneShape. Odontology 2019; 107: 196-201.
6. Arias A, Singh R, Peters OA. Torque and force induced by ProTaper universal and ProTaper next during shaping of large and small root canals in extracted teeth. J Endod 2014; 40: 973-6.
7. Özyürek T. Cyclic Fatigue Resistance of Reciproc, WaveOne, and WaveOne Gold Nickel-Titanium Instruments. J Endod 2016; 42: 1536-9.
8. Liu Y, Qiu N, Xue M, Wang C, Yu X. Comparison of Shaping Ability of Five Nickel-Titanium Rotary Instruments in Simulated Curved Canals. Journal of Dental and Oral Health 2017; 3: 1-5.
9. Tien M, Tjoa H, Zhou M, Abbott PV. Comparative Study of Four Endodontic File Systems to Assess Changes in Working Length during Root Canal Instrumentation and the Effect of Canal Curvature on Working Length Change. J Endod 2020; 46: 110-5.
10. Donnermeyer D, Viedenz A, Schäfer E, Bürklein S. Impact of new cross-sectional designs on the shaping ability of rotary NiTi instruments in S-shaped canals. Odontology 2020; 108: 174-9.
11. Schneider SW. A comparison of canal preparations in straight and curved root canals. Oral Surg Oral Med Oral Pathol 1971; 32: 271-5.
12. Hieawy A, Haapasalo M, Zhou H, Wang ZJ, Shen Y. Phase Transformation Behavior and Resistance to Bending and Cyclic Fatigue of ProTaper Gold and ProTaper Universal Instruments. J Endod 2015; 41: 1134-8.
13. Micro-Mega. The One Curve Brochure. Available at: https://micro-mega.com/wp-content/uploads/2020/11/60301900-A_Flyer-One-Curve-Portfolio-EN_web.pdf. Accessed March 10, 2021.
14. Gambarini G, Gergi R, Naaman A, Osta N, Al Sudani D. Cyclic fatigue analysis of twisted file rotary NiTi instruments used in reciprocating motion. Int Endod J 2012; 45: 802-6.
15. Gündoğar M, Özyürek T. Cyclic Fatigue Resistance of OneShape, HyFlex EDM, WaveOne Gold, and Reciproc Blue Nickel-titanium Instruments. J Endod 2017; 43: 1192-6.
16. WaveOne Gold brochure. Available at: https://www.dentsply.com/content/dam/ dentsply/pim/manufacturer/Endodontics/Obturation/Gutta_Percha_Points/WaveOne_   Gold_Gutta_Percha_Points/W1G_Brochure_EN.pdf. Accessed March 10, 2021.
17. Caldwell JL. Change in working length following instrumentation of molar canals. Oral Surg Oral Med Oral Pathol 1976; 41: 114-8.
18. Vahid A, Roohi N, Zayeri F. A comparative study of four rotary NiTi instruments in preserving canal curvature, preparation time and change of working length. Aust Endod J 2009; 35: 93-7.
19. Khurana P, Nainan MT, Sodhi KK, Padda BK. Change of working length in curved molar root canals after preparation with different rotary nickel-titanium instruments. J Conserv Dent 2011; 14: 264-8.

20. Kumar R, Khambete N, Patil S, Hoshing U, Medha A, Shetty R. Working length changes in curved canals after coronal flaring by using rotary files and hand file: An in vitro study. J Conserv Dent 2013; 16: 399-403.

21. Pak JG, White SN. Pain prevalence and severity before, during, and after root canal treatment: a systematic review. J Endod 2011; 37: 429-38.

22. Berutti E, Chiandussi G, Paolino DS, Scotti N, Cantatore G, Castellucci A, Pasqualini D. Effect of canal length and curvature on working length alteration with WaveOne reciprocating files. J Endod 2011; 37: 1687-90.

23. Davis RD, Marshall JG, Baumgartner JC. Effect of early coronal flaring on working length change in curved canals using rotary nickel-titanium versus stainless steel instruments. J Endod 2002; 28: 438-42.

24. Zhao D, Shen Y, Peng B, Haapasalo M. Root canal preparation of mandibular molars with 3 nickel-titanium rotary instruments: a micro-computed tomographic study. J Endod 2014; 40: 1860-4.

Original Article / Özgün Araştırma

The Role of Triglyceride-HDL Ratio and Triglyceride-glucose Index in Estimating Glycemic Control in Patients with Type 2 Diabetes Mellitus

Tip 2 Diabetes Mellitus Hastalarında Glisemik Kontrol Tahmininde Trigliserit-HDL Oranı ve Trigliserit-glukoz İndeksinin Rolü

● Özgür Altun, ● Semih Kalyon

University of Health Sciences Turkey, Prof. Dr. Cemil Taşcıoğlu City Hospital, Clinic of Internal Medicine, İstanbul, Turkey

Address for Correspondence/Yazışma Adresi:
Özgür Altun MD,
University of Health Sciences Turkey, Prof. Dr. Cemil Taşcıoğlu City Hospital, Clinic of Internal Medicine, İstanbul, Turkey
Phone : +90 505 798 41 42
E-mail : drozguraltun@yahoo.com
ORCID ID: orcid.org/0000-0003-1810-7490

Abstract

Objective: Our aim is to evaluate blood glucose regulation in patients with diabetes using the triglyceride glucose index and the triglyceride to high density lipoprotein (HDL) ratio.

Materials and Methods: The data of our retrospective study were obtained from the database of our hospital. The study was conducted with 340 type 2 patients with diabetes. Demographic data of the patients, glucose, hemoglobin A1c (HbA1c), high density lipoproteine-cholesterol (HDL-C), triglyceride, alanine aminotransferase (ALT) and creatinine values were recorded. The value obtained from the study was evaluated with the SPSS 21 version program.

Results: Of the participants, 202 were female and 138 were male. Patients were divided into groups according to the percentage of HbA1cs. In the group with HbA1c ≥7%, triglyceride/HDL value, triglyceride-glucose index, fasting glucose, diabetes age, triglyceride values were statistically significantly higher, while HDL cholesterol was lower. We found a significant correlation between Triglyceride/HDL value, triglyceride glucose index with both glucose and HbA1c. The cut-off value of triglyceride-glucose (TyG) was 9.38.

Conclusion: TG/HDL and TyG index values are related with HbA1c. In particular, the TyG index, whose cut-off value determined between these two parameters, is a parameter that can be used to evaluate good glycemic control instead of HbA1c in cases where HbA1c cannot be measured.

Öz

Amaç: Amacımız, diyabetli hastalarda kan şekeri regülasyonunu trigliserit glikoz indeksi ve trigliseritin yüksek yoğunluklu lipoprotein'e (HDL) oranını temel alarak değerlendirmektir.

Gereç ve Yöntemler: Retrospektif çalışmamızın verileri hastanemizin veri tabanından elde edilmiştir. Çalışma 340 tip 2 diyabet hastası ile yapılmıştır. Hastaların demografik verileri, glukoz, hemoglobin A1c (HbA1c), HDL-kolesterol, trigliserit, alanin aminotransferaz (ALT), kreatinin değerleri kayıt edildi. Çalışmadan elde edilen veriler SPSS 21 versiyon program ile değerlendirildi.

Bulgular: Katılımcıların 202'si kadın, 138'i erkekti. Hastalar HbA1c yüzdelerine göre gruplara ayrıldı. HbA1c ≥%7 olan grupta trigliserit/HDL değeri, trigliserit-glukoz indeksi, açlık glukozu, diyabet yaşı, trigliserit değerleri istatistiksel olarak anlamlı daha yüksek iken HDL kolesterol daha düşüktü. Trigliserit/HDL değeri ve trigliserit

glukoz indeksi ile hem glukoz ve hem HbA1c arasında anlamlı bir ilişki saptandı. Kötü glisemik kontrollü (HbA1c ≥%7) hastalar için trigliserit-glukoz indeksi için cut-off değer 9,38 olarak saptandı.

Sonuç: Trigliserit/HDL ve trigliserit-glikoz (TyG) indeks değerleri HbA1c ile ilişkilidir. Özellikle bu iki parametre arasından cut-off değeri belirlenen TyG indeksi, HbA1c bakılamayan durumlarda HbA1c yerine iyi glisemik kontrolü değerlendirmek için kullanılabilecek bir parametredir.

Introduction

In type 2 diabetes (T2DM) insulin resistance occurs in peripheral tissues. Insulin resistance occurs in peripheral tissues. Insulin levels decrease due to decreased pancreatic beta cell function over time (1). In the presence of insulin resistance, glucose and free fatty acid levels rise. This causes an increase in very low density lipoprotein (VLDL), the triglyceride (TG) transporter. The increase in VLDL levels is also due to decreased clearance owing to the decreased lipoprotein lipase activity and decreased hepatic uptake. An increase in hypertriglyceridemia increases cholesterol ester transfer protein enzyme activity, resulting in decreased high density lipoprotein-cholesterol (HDL-C) and increased Low Density Lipoprotein-cholesterol (LDL-C) levels (1).

The decrease in pancreatic beta cell reserve may develop due to many factors, and exposure of beta cells to increased free fatty acids is one of these reasons. Increasing free fatty acids may cause insulin resistance via oxidative pathways (2).

The complications of diabetes can be microvascular or macrovascular and due to these complications, diabetes is an increasing cause of morbidity and mortality. Prevention of these complications of diabetes is only possible with intense treatment. The most important laboratory test used to evaluate the success of treatment is hemoglobin A1c (HbA1c), which is the level of glycosylated hemoglobin over 3 months. Keeping HbA1c below 7% is necessary to prevent complications (3).

However, since HbA1c analysis cannot be performed in every hospital laboratory and it can be affected by anemia, kidney failure, and some drugs, a requirement has arisen to find an indicator that shows glycemic control well, can be easily performed in every laboratory, is cheaper, and that will not be affected by other variables (4).

Diabetes mellitus is accompanied by an increase in TG. In addition, low HDL-C levels have highlighted these two parameters in previous years. As a matter of fact, previous studies in the literature have shown that TG/HDL ratio very well reflects atherogenicity due to endothelial dysfunction, which poses a risk for metabolic syndrome and cardiovascular diseases (5-7). It has been revealed that the TG/HDL ratio also reflects insulin resistance (8). It has been determined by studies that the triglyceride-glucose (TyG) index also shows insulin resistance well (9,10). It has also been previously shown that the TyG index may indicate an increased risk of cardiovascular disease in Type 2 diabetics without symptoms (11). Studies have reported a strong correlation between the TyG index and the development of diabetes (12,13).

In previous studies, we found only one study on the relationship between diabetes mellitus, TG-HDL ratio and TyG index (14). Our study aims to decide whether the TG/HDL and TyG index in our patient population are useful in predicting glycemic control in diabetic patients.

Materials and Methods

Research permission was taken from the Ethics Committee of our hospital before starting the study with the approval number 259, dated 01.07.2021. İndividual with a diagnosis of T2DM who applied to the internal medicine outpatient clinic of our hospital were retrospectively searched from our hospital database.

All patients over 18 years of age followed up with T2DM participated in the study. First of all, those younger than eighteen years of age, those with chronic kidney failure and/or chronic liver disease, pregnant or breastfeeding women, those using statins, fenofibrate or fish oil, those with malignancy and thyroid disease were excluded from the study. In the second elimination stage, the patients with the missing data were excluded. The fasting blood glucose, HbA1c, lipid profile, alanine aminotransferase (ALT), and creatinine values were simultaneously checked with the body mass index (BMI). The remaining 340 patients after two eliminations were accepted as the study group.

Glucose, HbA1c, ALT, creatinine, and lipid values were studied using the colorimetric method in an auto-analyzer (Beckman Coulter Brand, AU5800, USA).

The TG-HDL formula: TG/HDL (15)

Calculated in BMI kg/m² (16).

For the TyG index, previous studies were guided, and values were obtained with the formula Ln [fasting triglyceride value (mg/mL) X fasting glucose value (mg/mL)/2] (16).

Patients were defined as two groups according to their HbA1c values as those with diabetes under control (<7%) and those with uncontrolled diabetes (<7%) (17).

Statistical Analysis

SPSS version 21 program was used. Normally distributed data were given as mean ± standard deviation, non-normally distributed data as median and interquartile range (25th and 75th percentiles). Compliance of quantitative data with normal distribution was determined by Kolmogorov-Smirnov test. Chi-square test was performed to compare categorical data If the distribution was homogeneous, Student t-test was performed. If not, the Mann-Withney U test was used. Pearson was used if the distribution was homogeneous in the correlation analysis. If not homogeneous, Spearman was applied. P<0.05 is significant.

Results

Two hundred two women and 138 men took part in the study. There were 153 patients with HbA1c levels <7 and 187 patients with ≥7. In the group with HbA1c ≥7, TG/HDL value and TyG index were statistically higher than the group with HbA1c <7 (p=0.010, p<0.001). In the group with HbA1c ≥7, glucose, diabetes duration, and triglyceride levels were statistically higher than the group with HbA1c <7 (Table 1).

We found a significant correlation between triglyceride/HDL value and both glucose and HbA1c. (r=0.207, p<0.001; r=0.261 p<0.001). There was positive correlation between TyG index and HbA1c and glucose levels (r=0.576, p<0.001; r=0.710 p<0.001). TyG index had a negative correlation with HDL (r=-0.356 p< 0.001). TyG index was positively correlated with LDL (r=0.134 p=0.016) (Table 2).

Cases with HbA1c ≥7% were considered poorly controlled diabetic. The receiver operating characteristic (ROC) curve was made. The cut-off value of the TyG index was 9.38 (sensitivity 69%, specificity 73%, AUC= 0.759) (Table 3) (Figure 1). However, a cut-off value could not be determined for TG/HDL.

Table 1. Comparison of demographic and laboratory data			
	HbA1c <%7 (n=153)	HbA1c ≥%7 (n=187)	p-value
Age (year)	59.50±10.50	60.40±10.5	0.407
BMI (kg/m²)	30.70±4.79	30.50±5.25	0.685
Female, n (%)	90 (59)	112 (60)	0.842[a]
Male, n (%)	63 (41)	75 (40)	
HbA1c (%)	6.30±0.44	9±1.72	<0.001
Glucose (mg/dL)	117(103-117)	198(143-242)	<0.001[b]
Diabetes duration	7.50±5	10±6.90	<0.001
HDL-C (mg/dL)	47±11	45±11	0.034
TG (mg/dL)	159 (111.5-201)	168 (121-249)	0.046[b]
LDL-C (mg/dL)	121 (101-121)	125 (106-147)	0.307[b]
ALT (IU/mL)	19 (15-25)	22 (16-27)	0.055[b]
Creatinine (mg/dL)	0.75±0.20	0.72±0.20	0.271
TG/HDL	3.42 (2.2-4.9)	3.9 (2.5-6.4)	0.010[b]
TyG index	9.09±0.54	9.73±0.75	<0.001
BMI: Body mass index, HbA1c: Hemoglobin A1c, HDL-C: High density lipoprotein cholesterol, TG: Triglyceride, LDL-C: Low density lipoprotein cholesterol, ALT: Alanine aminotransferase, Student t-test, [a]Chi-square test, [b]Mann Whitney-U test			

Table 2. Correlation between indexes values and data		Age (years)	Diabetes duration (years)	BMI (kg/m²)	Glucose (mg/dL)	HbA1c (%)	TG (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)
TGA/HDL	r	-0.084	-0.070	0.069	0.261	0.207	0.916	-0.667	0.032
	p	0.123	0.203	0.207	<0.001	<0.001	<0.001	<0.001	0.567
TyG index	r	-0.069	0.026	0.028	0.710	0.576	0.824	-0.356	0.134
	p	0.207	0.639	0.609	<0.001	<0.001	<0.001	<0.001	0.016

TGA: Thermal degradation, HDL: High density lipoprotein, TyG: Triglyceride-glucose, *Pearson and Spearman correlation tests were applied according to the distribution

Table 3. Cut-off data for TyG index value according to HbA1c <7 and ≥7					
	AUC (95%)	Cut-off	Sensitivity (%)	Specificity (%)	p-value
TyG index	0.759 (0.709-0.810)	9.38	69	73	0.001

TyG: Triglyceride-glucose
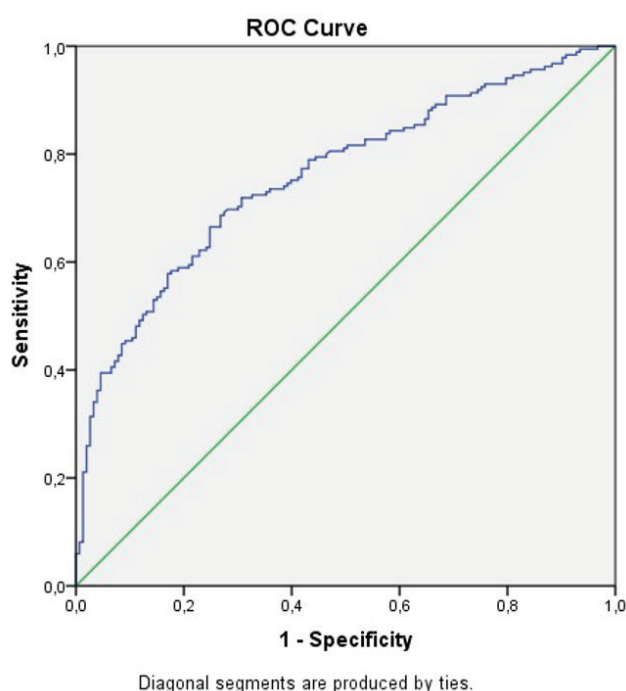
Figure 1. ROC curve of TyG index
ROC: Receiver operating characteristic, TyG: Triglyceride-glucose

Discussion

T2DM is espacially related with increased cardiovascular complications. Diabetes, hyperlipidemia, and obesity are among the metabolic syndrome parameters and each of them should be dealt with a separate treatment and follow-up program.

In our study, we found that the patients with poorly controlled diabetes (HbA1c ≥7) had higher TG-HDL ratio and TyG index. At the same time, both TG-HDL and TyG index were correlated with glucose and HbA1c. It is known that high TG and low HDL-C levels are related with insulin resistance and T2DM and atherosclerotic diseases (18,19). Since triglycerides and HDL cholesterol levels are affected by hyperinsulinemia, their levels may be affected by glycemici control in patients with diabetes (1). When we examine the previous studies in the literature; similar to our study, TG/HDL and TyG index were found to be correlated with HbA1c (14,20). Additionally, Babic et al. (14) It was concluded that TG/HDL and TyG index values are useful as an indicator of glycemic control. Zonszein et al. (21) They found that the triglyceride-HDL ratio is an easily predictable and practical biomarker in determining insulin-resistant patients and glycemic control in diabetic patients. T2DM patients have dyslipidemia characterized by predominance of LDL-C particles, increased triglyceride and decreased HDL-C levels (1). Dyslipidemia together with type 2 DM constitute the main risk factors for cardiovascular diseases. It has been reported that TG/HDL value and TyG index are related with atherosclerotic diseases (11,15). In our study, TyG index showed a positive correlation with LDL-C and an opposite correlation with HDL-C. Similar to our findings, the TyG index was found to be related with dyslipidemia in T2DM (14). Therefore, we can say that the TyG index predicts dyslipidemia.

Possible potential mechanisms to explain the relationship between TG/HDL and TyG index and

Hba1c could be; First, islet beta cell failure and insulin resistance are the main pathological features of T2DM. Increased glucose and free fatty acid levels have toxic effects on beta cells (2,22). Increased triglyceride levels can cause free fatty acids to rise, thereby increasing the flow of free fatty acids to the tissues. This may affect insulin resistance and glucose metabolism. Studies have shown that high triglyceride levels affect glucose metabolism in the liver and muscle (23). Second; recently, it has been shown that HDL can also directly affect glucose metabolism (24). Recombinant HDL infusion has been reported to be beneficial on glucose metabolism in T2DM (25). All these mechanisms point to the role of triglycerides and HDL-cholesterol in the pathogenesis of Type 2 DM. Therefore, TG/HDL value and TyG index are good options that can be used in the evaluation of regulation in T2DM.

In our study, when we performed ROC analysis, we found that the TyG index had a larger AUC than TG/HDL to identify poor diabetics. Accordingly, if the TyG index is above 9.38, which we assume as the cut-off value, the probability of the patient's HbA1c being over 7 is high.

The study has limitations. First of all, our study is retrospective and cross-sectional. Secondly, the relationship of TG/HDL ratio and TyG index with poor and well-controlled diabetes was examined, but their relationship with insulin resistance was not examined. Studies evaluated with the HOMA-IR levels may provide further information.

Conclusion

According to the results of our study, we can say the following; TG/HDL value and TyG index may be useful both in predicting dyslipidemia in Type 2 DM and in predicting diabetes regulation due to a positive relationship with HbA1c. Especially in cases where TyG index hba1c is not performed, it can be preferred instead of Hba1c Our study will shed light on similar studies to be done in the future.

Ethics

Ethics Committee Approval: The present cohort study was designed as a survey and was approved by the local Ethical Committee of İstanbul Prof. Dr. Cemil Taşçıoğlu City Hospital (decision no: 259, date: 02.07.2021)

Informed Consent: Retrospective study.
Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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

Financial Disclosure: The authors declared that this study received no financial support.

References

1. Quispe R, Martin SS, Jones SR. Triglycerides to high-density lipoprotein-cholesterol ratio, glycemic control and cardiovascular risk in obese patients with type 2 diabetes. Curr Opin Endocrinol Diabetes Obes 2016; 23: 150-6.

2. Zhou M, Zhu L, Cui X, Feng L, Zhao X, He S, et al. The triglyceride to high-density lipoprotein cholesterol (TG/HDL-C) ratio as a predictor of insulin resistance but not of β cell function in a Chinese population with different glucose tolerance status. Lipids Health Dis 2016; 15: 104.

3. American Diabetes Association. 6. Glycemic Targets: Standards of Medical Care in Diabetes-2021. Diabetes Care 2021; 44: S73-S84.

4. Saha S, Schwarz PEH. Impact of glycated hemoglobin (HbA1c) on identifying insulin resistance among apparently healthy individuals. J Public Health 2017; 25(Suppl 1): 505-12.

5. Keles N, Aksu F, Aciksari G, Yilmaz Y, Demircioglu K, Kostek O, et al. Is triglyceride/HDL ratio a reliable screening test for assessment of atherosclerotic risk in patients with chronic inflammatory disease? North Clin Istanb 2016; 3: 39-45.

6. Kim JH, Lee DY, Park SE, Park CY, Lee WY, Oh Ki-Won, et al. Triglyceride glucose index predicts coronary artery calcification better than other indices of insulin resistance in Korean adults: the Kangbuk Samsung Health Study. Precis Future Med 2017; 1: 43-51.

7. Zhang S, Du T, Zhang J, Lu H, Lin X, Xie J, et al. The triglyceride and glucose index (TyG) is an effective biomarker to identify nonalcoholic fatty liver disease. Lipids Health Dis 2017; 16: 15.

8. Ren X, Chen ZA, Zheng S, Han T, Li Y, Liu W, et al. Association between Triglyceride to HDL-C Ratio (TG/HDL-C) and Insulin Resistance in Chinese Patients with Newly Diagnosed Type 2 Diabetes Mellitus. PLoS One 2016; 11: e0154345.

9. Er LK, Wu S, Chou HH, Hsu LA, Teng MS, Sun YC, et al. Triglyceride Glucose-Body Mass Index Is a Simple and Clinically Useful Surrogate Marker for Insulin Resistance in Nondiabetic Individuals. PLoS One 2016; 11: e0149731.

10. Li X, Li G, Cheng T, Liu J, Song G, Ma H. Association between triglyceride-glucose index and risk of incident diabetes: a secondary analysis based on a Chinese cohort study : TyG index and incident diabetes. Lipids Health Dis 2020; 19: 236.

11. Lee EY, Yang HK, Lee J, Kang B, Yang Y, Lee SH, et al. Triglyceride glucose index, a marker of insulin resistance, is associated with coronary artery stenosis in asymptomatic subjects with type 2 diabetes. Lipids Health Dis 2016; 15: 155.

12. Zhang M, Wang B, Liu Y, Sun X, Luo X, Wang C, et al. Cumulative increased risk of incident type 2 diabetes mellitus with increasing triglyceride glucose index in normal-weight people: The Rural Chinese Cohort Study. Cardiovasc Diabetol 2017; 16: 30.

13. Low S, Khoo KCJ, Irwan B, Sum CF, Subramaniam T, Lim SC, et al. The role of triglyceride glucose index in development of Type 2 diabetes mellitus. Diabetes Res Clin Pract 2018; 143: 43-9.

14. Babic N, Valjevac A, Zaciragic A, Avdagic N, Zukic S, Hasic S. The Triglyceride/HDL Ratio and Triglyceride Glucose Index as Predictors of Glycemic Control in Patients with Diabetes Mellitus Type 2. Med Arch 2019; 73: 163-8.

15. Masson W, Siniawski D, Lobo M, Molinero G, Huerín M. Association between triglyceride/HDL cholesterol ratio and carotid atherosclerosis in postmenopausal middle-aged women. Endocrinol Nutr 2016; 63: 327-32.

16. Zheng R, Mao Y. Triglyceride and glucose (TyG) index as a predictor of incident hypertension: a 9-year longitudinal population-based study. Lipids Health Dis 2017; 16: 175.

17. Qaseem A, Wilt TJ, Kansagara D, Horwitch C, Barry MJ, Forciea MA, et al. Hemoglobin A1c Targets for Glycemic Control With Pharmacologic Therapy for Nonpregnant Adults With Type 2 Diabetes Mellitus: A Guidance Statement Update From the American College of Physicians. Ann Intern Med 2018; 168: 569-76.

18. Bonora E, Kiechl S, Willeit J, Oberhollenzer F, Egger G, Targher G, et al. Prevalence of insulin resistance in metabolic disorders: the Bruneck Study. Diabetes 1998; 47: 1643-9.

19. Sun GZ, Li Z, Guo L, Zhou Y, Yang HM, Sun YX. High prevalence of dyslipidemia and associated risk factors among rural Chinese adults. Lipids Health Dis 2014; 13: 189.

20. Hameed EK. TyG index a promising biomarker for glycemic control in type 2 Diabetes Mellitus. Diabetes Metab Syndr 2019; 13: 560-3.

21. Zonszein J, Lombardero M, Ismail-Beigi F, Palumbo P, Foucher S, Groenewoud Y, et al. Triglyceride High-Density Lipoprotein Ratios Predict Glycemia-Lowering in Response to Insulin Sensitizing Drugs in Type 2 Diabetes: A Post Hoc Analysis of the BARI 2D. J Diabetes Res 2015; 2015: 129891.

22. Robertson RP, Harmon J, Tran PO, Poitout V. Beta-cell glucose toxicity, lipotoxicity, and chronic oxidative stress in type 2 diabetes. Diabetes 2004; 53 Suppl 1: S119-24.

23. Parhofer KG. Interaction between Glucose and Lipid Metabolism: More than Diabetic Dyslipidemia. Diabetes Metab J 2015; 39: 353-62.

24. Drew BG, Rye KA, Duffy SJ, Barter P, Kingwell BA. The emerging role of HDL in glucose metabolism. Nat Rev Endocrinol 2012; 8: 237-45.

25. Drew BG, Duffy SJ, Formosa MF, Natoli AK, Henstridge DC, Penfold SA, et al. High-density lipoprotein modulates glucose metabolism in patients with type 2 diabetes mellitus. Circulation 2009; 119: 2103-11.

Original Article / Özgün Araştırma

Associations Between Glycosylated Hemoglobin (HbA1c) Level and Central Corneal and Macular Thickness in Diabetic Eyes Without Retinopathy

Retinopatisi Olmayan Diyabetik Gözlerde Glikolize Hemoglobin (HbA1c) Düzeyi ile Merkezi Korneal ve Maküler Kalınlık Arasındaki İlişki

● Burcu Kazancı[1], ● Dilek Özçelik Soba[1], ● Çağrı İlhan[2], ● Yasemin Özdamar Erol[1]

[1]University of Health Sciences Turkey, Ulucanlar Eye Training and Research Hospital, Clinic of Ophthalmology, Ankara, Turkey
[2]Hatay Training and Research Hospital, Clinic of Ophthalmology, Hatay, Turkey

Address for Correspondence/Yazışma Adresi:
Burcu Kazancı MD,
University of Health Sciences Turkey, Ulucanlar
Eye Research and Education Hospital, Clinic of
Ophthalmology, Ankara, Turkey
Phone : +90 505 266 84 86
E-mail : drburcus@hotmail.com
ORCID ID: orcid.org/0000-0002-0147-3841

Abstract

Objective: The comparison of a central corneal (CCT) and central foveal thickness (CMT) of type 2 patients with diabetes with a healthy control group and determine the difference according to fasting blood glucose (FBG), hemoglobin a1c (HbA1c) levels and time of diabetes.

Materials and Methods: Fifty patients with diabetes without retinopathy and 47 healthy controls of similar age and gender were compared. The mean of three consecutive corneal thickness measurements at the central cornea was obtained by the Pentacam HR imaging system (Oculus Optikgeräte GmbH, Wetzlar, Germany) and macular thickness was obtained by Heildenberg optical coherence tomography (OCT). The fiber Bragg grating (FBG) and HbA1c levels of all participants were evaluated. The analysis was performed on the right eye.

Results: Groups were statistically similar in terms of age and gender (p>0.05). Patients with diabetes had significantly higher FBG and HbA1c values than controls and the percentage of patients with HbA1c values less than 8% in Type 2 diabetes mellitus was significantly lower than healthy controls (p<0.001). Visual acuity logarithm of the minimum resolution angle of patients with diabetes was significantly lower than that of controls (p=0.034). However, similar results were obtained in both groups in terms of CCT and CMT values (p>0.05).

Conclusion: In our study, diabetic participants without DR had no difference at CCT and CMT between non-diabetic controls. This result shown that the macula and corneal thickness cannot be used as indicators of retinopathy and keratopathy.

Öz

Amaç: Tip 2 diyabetik hastaların santral kornea (SKK) ve maküler foveal kalınlıklarının (SMK) sağlıklı bir kontrol grubu ile karşılaştırılması ve açlık kan şekeri (AKŞ), hemoglobin A1c (HbA1c) düzeyleri ve diyabet süresine göre farkın belirlenmesi amaçlanmıştır.

Gereç ve Yöntemler: Retinopatisi olmayan 50 diyabetik hasta ve benzer yaş ve cinsiyet özelliği gösteren 47 sağlıklı kontrol karşılaştırıldı. SKK'de art arda üç kornea kalınlığı ölçümünün ortalaması Pentacam HR ımaging system (Oculus Optikgeräte GmbH, Wetzlar, Germany) ile ve maküla kalınlığı Heildenberg optik koherens tomografi (OKT) ile elde edildi. Tüm katılımcıların AKŞ ve HbA1c seviyeleri değerlendirildi. Analiz sağ göze yapıldı.

Bulgular: Yaş ve cinsiyet açısından gruplar istatistiksel olarak benzerlik göstermekteydi (p>0,05). Diyabetik hastaların AKŞ ve HbA1c değerleri sağlıklı kontrollere göre anlamlı olarak yüksek ve HbA1c değerleri %8'in altında olan tip 2 diabetes mellitus hastalarının yüzdesi sağlıklı kontrollerden anlamlı olarak düşüktü (p<0,001). Diyabetik hastaların görme keskinliği (LogMAR), sağlıklı kontrollere göre anlamlı derecede düşüktü (p=0,034). Öte yandan SKK ve SMK değerleri açısından her iki grupta da benzer sonuçlar elde edildi (p>0,05).

Sonuç: Çalışmamızda retinopatisi olmayan diyabetik katılımcılarda, diyabetik olmayan kontroller arasında SKK ve SMK açısından fark yoktu. Bu sonuç maküla ve kornea kalınlığının retinopati ve keratopatinin bir göstergesi olarak kullanılamayacağını göstermiştir.

Introduction

Diabetes mellitus (DM) is a multisystemic disease as a result of microvascular and macrovascular pathologies. Ocular complications of DM are anterior ischemic neuropathy, glaucoma, cataract, retinal vessel occlusions, and retinopathy (1,2). Although retinal involvement is very common in DM, it is expected that the cornea will be affected (3,4). It can be seen in corneal pathologies such as corneal epithelial defects, recurrent epithelial erosion, basement membrane disorders such as keratitis or ulcer development, epithelial disorders, endothelial cell number and morphology disorders, and decreased corneal sensitivity (5).

Glycosylated hemoglobin (HbA1c) level is a marker of glycemic control and it a critical indicator of the management of DM (6). Optical coherence tomography (OCT) is extensively used to measure retinal thickness and the Pentacam HR is an anterior segment tomography and it is used to evaluate non-contact corneal thickness (7).

In this study, we aimed to evaluate the central cornea and macular thickness in diabetic eyes without retinopathy and keratopathy according to the effect of HbA1c level of DM duration.

Materials and Methods

This study which was a cross sectional, prospective study, conducted in the University of Health Sciences Turkey, Ulucanlar Eye Training and Research Hospital between December 2019 and June 2020. The study was approved by the Institutional Ethics Committee (Ankara Training and Research Hospital, no: E-19-42, date: 26.12.2019) and in accordance with the principles of the Declaration of Helsinki. A signed informed consent form was obtained from all volunteers. There were 50 participants with type 2 DM patients without retinopathy and 47 non-diabetic age-sex matched control subjects. We divided the diabetic patients into two groups according to the HbA1c levels (≥8% or <8%) and disease duration.

The mean of three consecutive central corneal thickness (CCT) measurements was obtained by the Pentacam HR Imaging System and central macular thickness (CMT) was obtained by Heildenberg OCT in all participants.

Patients who were followed up in the retina department of in the hospital, met the study criteria and accepted to participate in the study were invited to the study. Patients with DM type 2 without retinopathy were older than 18 years and younger than 80 years of age, without previous eye surgery, no history of glaucoma, intraocular inflammation or infection and chronic topical drug use. The history of intraocular surgery, focal or pan-retinal photocoagulation and periocular or intravitreal injection use of contact lens, regular eye drop usage, spherical and cylindrical refractive errors >±3.00 D were excluded. Macular disease such as clinically significant macular edema, proliferative diabetic retinopathy and any retinal or retinal vascular disease were not included in the study. We included only the right eyes in our study.

Best-corrected visual acuity (BCVA), intraocular pressure measurement, detailed biomicroscopic and fundus examination with +90 D condensing lens were undergone in all subjects. The BCVA measurements were made on a Snellen chart and the data was transformed to logarithm of the minimum resolution angle (LogMAR). Venous blood samples were taken to determine the level of HbA1c. Fasting blood glucose (FBG) was measured simultaneously with HbA1c. Macular thickness measurements were performed using the Heildenberg OCT by trained the same technician after pupil dilatation with tropicamide 1% and cyclopentolate HCL 1%. The Pentacam Scheimpflug imaging system (Pentacam HR, Oculus, Wetzlar, Germany) provides two-dimensional images

that obtain the three-dimensional structure of the cornea by software analysis with using rotating cameras for detailed analysis through the optic axis from the epithelial surface of the cornea to the posterior capsule of the lens. In this system, CCT is obtained from the measurement difference in the anterior and posterior elevation maps (8).

Statistical Analysis

We evaluated research data on the computer via "SPSS for Windows 22.0 (SPSS Inc, Chicago, IL)". Visual (histogram and probability plots) and analytical tests such as Kolmogorov-Smirnov/Shapiro-Wilk were used for analysis for normal distribution variables, while student's t-test was used for analysis of non-normal distributions. Pearson's chi-square test and Fisher's Exact test was used for categorical variables. The Mann-Whitney U test was preferred for the analysis of two dependent groups. In order for the p value to be significant, it had to be below 0.05.

Results

A total of 97 individuals, 50 of whom were diagnosed as Diabetus mellitus type-2 and 47 healthy control subjects were examined. In Table 1, where the descriptive characteristics of the study groups are given, a similar distribution was observed in terms of age and gender (p>0.05) and HbA1c and FBC parameters were statistically higher in type 2 DM group (p<0.001 for both). Visual acuity values of diabetic group were (mean ± standard deviation) 0.93±0.18 (minimum-maximum: 0.2-1.0) and

0.98±0.09 (0.5-1.0) in control group and statictically significantly lower in the diabetic group (p=0.034). We classified the diabetic group according to HbA1c levels and duration of diabetes (Table 2, 3). There was no statistically significant difference between the groups with HbA1c levels ≥8% or <8% group in terms of age, gender, HbA1c and FBG levels (Table 2). At the same time, between subgroups of HbA1c levels, no difference was found in terms of CCT, CMT, and BCVA (Table 2). When we classified the diabetes group according to the duration of DM, 16 (34%) of 47 patients were diagnosed with DM over 10 years. The average duration of DM was 9,8 years (2-25 years) in HbA1c ≥8% group and 9,6 years (1-25 years) in HbA1c levels >%8 group. There was no difference in age, gender, fasting blood sugar and HbA1c levels between groups with diabetes duration (Table 3).

CCT values were 544.7±24.5 (507-623) µm in diabetic group and 543.2±34.6 (444-600) µm in control group. CMT values were 275.1±45.9 (214-443) µm in diabetic group and 269.6±24.8 (232-352) µm in control group. CCT and CMT values were not statistically significant different between the groups (p>0.05). In addition, it was observed that DM duration and level of HbA1c were not effective on BCVA, CCT and BMT values (p>0.05) (Table 4, 5).

Discussion

Multiple organ failure in diabetic patients were as a result of microvascular and macrovascular pathologies which are caused by hyperglycemia.

Table 1. Distribution of some descriptive characteristics between the study groups			
	Type 2 DM (n=50)	**Control (n=47)**	**p**
Age (year), mean ± SD (min-max)	57.5±9.2 (35-75)	54.3±9.6 (34-76)	0.099a
Gender, n (%)			
Female	14 (28.0)	21 (44.7)	0.087b
Male	36 (72.0)	26 (55.3)	
FBG (g/dL), mean ± SD (min-max)	173.3±74.2 (80-372)	101.8±15.0 (76-148)	<0.001c**
HbA1c (%), mean ± SD (min-max)	8.1±2.0 (5.7-13.3)	5.8±0.4 (5.0-6.5)	<0.001c**
HbA1c group, n (%)			
<6%	4 (8.0)	33 (70.2)	
6-8%	30 (60.0)	14 (29.8)	<0.001b**
>8%	16 (32.0)	0	
n: Number of individuals, %: Percentage, SD: Standard deviation, FBG: Fasting blood glucose, DM: Diabetes mellitus, Min-max: Minimum-maximum, aStudent's t-test, bPearson chi-square test, cMann-Whitney U test, *p<0.05, **p<0.01			

Table 2. Distribution of age, sex and FBG between HbA1c groups of patients with type-2 DM

	Total (n=50)	HbA1c		p-value
		<8% (n=33)	≥8% (n=17)	
Age (year), mean ± SD (min-max)	57.5±9.2 (35-75)	57.4±8.9 (35-75)	57.7±10.2 (37-75)	0.920[a]
Gender, n (%)				
Female	14 (28.0)	8 (24.2)	6 (35.3)	0.511[b]
Male	36 (72.0)	25 (75.8)	11 (64.7)	
DM duration (year), mean ± SD (min-max)	9.7±5.9 (1-25)	9.8±6.2 (2-25)	9.6±5.6 (1-25)	0.918[c]
DM duration group, n (%)				
<10 year	34 (68.0)	23 (69.7)	11 (64.7)	0.720[d]
≥10 year	16 (32.0)	10 (30.3)	6 (35.3)	
FBG (g/dL), mean ± SD (min-max)	173.3±74.2 (80-372)	147.5±49.8 (83-340)	223.4±88.8 (80-372)	0.006[c]**

n: Number of individuals, %: Percentage, SD: Standard deviation, FBG: Fasting blood glucose, DM: Diabetes mellitus, Min-max: Minimum-maximum, [a]s: Student's t-test, [b]Fisher's Exact test, [c]Mann-Whitney U test, [d]Pearson chi-square test, *p<0.05; **p<0.01

Table 3. Distribution of age, sex and fasting blood sugar between DM duration groups of patients with type 2 DM

	Total (n=97)	DM duration		p-value
		<10 year (n=34)	≥10 year (n=16)	
Age (year) mean ± SD (min-max)	57.5±9.2 (35-75)	56.6±9.2 (35-70)	59.4±9.2 (42-75)	0.320[a]
Gender n (%)				
Female	14 (28.0)	12 (35.3)	2 (12.5)	0.175[b]
Male	36 (72.0)	22 (64.7)	14 (87.5)	
HbA1c (%) mean ± SD (min-max)	8.1±2.0 (5.7-13.3)	7.9±1.9 (5.7-12.1)	8.6±2.2 (6.5-13.3)	0.248[c]
FBG (g/dL) mean ± SD (min-max)	173.3±74.2 (80-372)	163.4±73.2 (80-372)	194.2±74.3 (110-340)	0.094[c]

n: Number of individuals, %: Percentage, SD: Standard deviation, FBG: Fasting blood glucose, min-max: Minimum-maximum, DM: Diabetes mellitus, [a]Student's t-testi, [b]Fisher's Exact test, [c]Mann-Whitney U test

Table 4. Distribution of visual acuity, central corneal and macular thickness between HbA1c groups of patients with type 2 DM

	Total (n=50)	HbA1c		p-value
		<8% (n=33)	≥8% (n=17)	
	Mean ± SD (min-max)	Mean ± SD (min-max)	Mean ± SD (min-max)	
BCVA (LogMAR)	0.93±0.18 (0.2-1.0)	0.94±0.16 (0.3-1.0)	0.90±0.22 (0.2-1.0)	0.724[a]
CCT (μm)	544.7±24.5 (507-623)	543.9±25.6 (507-623)	546.2±22.7 (507-588)	0.757[b]
CMT (μm)	275.1±45.9 (214-443)	275.8±51.2 (214-443)	273.7±34.8 (235-391)	0.674[a]

n: Number of individuals, SD: Standard deviation, BCVA: Best-corrected visual acuity, CCT: Central corneal thickness, CMT: Central macular thickness, min-max: Minimum-maximum, LogMAR: Logarithm of the minimum resolution angle, [a]Mann-Whitney U test, [b]Student's t-test

Ocular complications of DM are the primary reason for progressive blindness in the world (9,10). Recently, 4,2 million humans were affected by diabetes which is a vital and progressive reason for blindness (11). Cornea and retina are the most affected ocular tissues by diabetes regulation and diabetes duration. Hence,

in this study, we planned to evaluate cornea and macula in eyes without diabetic retinopathy.

Calvo-Maroto et al. (12) found no significant difference in CCT between the diabetic group, which was classified according to the period of diabetes, regardless of the presence of retinopathy and

Table 5. Distribution of visual acuity, central corneal and macular thickness between HbA1c groups of patients with type 2 DM

	Total (n=97)	DM duration		p-value
		<10 year (n=34)	≥10 year (n=16)	
	Mean ± SD (min-max)	Mean ± SD (min-max)	Mean ± SD (min-max)	
BCVA (LogMAR)	0.93±0.18 (0.2-1.0)	0.95±0.12 (0.5-1.0)	0.88±0.26 (0.2-1.0)	0.269[a]
CCT (μm)	544.7±24.5 (507-623)	545.5±25.7 (507-623)	543.0±22.2 (507-585)	0.737[b]
CMT (μm)	275.1±45.9 (214-443)	270.2±40.1 (224-442)	285.6±56.4 (214-443)	0.204[a]

n: Number of individuals, SD: Standard deviation, BCVA: Best-corrected visual acuity, CCT: Central corneal thickness, CMT: Central macular thickness, min-max: Minimum-maximum, LogMAR: Logarithm of the minimum resolution angle, [a]Mann-Whitney U Test; [b]Student's t-test

the healthy control group. Similarly, we could not find difference between study groups in terms of CCT. Choo et al. (13) revealed that HbA1c level and severity of retinopathy had no effected to CCT, but they found endothelial differences. Besides, the same study reported that the HbA1c level did not affect endothelial morphology. Inoue et al. (14) similarly, they did not detect any difference in corneal thickness even at different stages.

However, Gao et al. (15) found that diabetes duration (<5, 5-10,>10 years) had a significant increase on CCT in 360 eyes of 180 patients. We thought that the most important reason for the difference between the study groups in this study is the high number of patients with more than a decade of diabetes duration (50% of patients). As the duration of diabetes increases, the rate of retinopathy increases. Since we especially preferred patients without retinopathy, the number of patients with diabetes duration more than 10 years was less in the study.

In a study of Toygar et al. (16), diabetic patients were classified based on the existence and severity of retinopathy. They reported that CCT of all diabetic was higher than a control group, but these results did not show any correlation with the presence of retinopathy. Similar to our data, there was no difference in CCT between study groups.

Lee et al. (17) noticed that in diabetic group with normal fundus or background diabetic retinopathy had higher CCT values compared to the control group and also was found that the disease duration >10 years was significantly higher from those less than 10 years. They emphasized that the duration of diabetes is effective on the corneal thickness.

In our study, there was no difference in CCT in both study group and HbA1c levels-disease duration subgroups. It can be interpreted that there will be no significant change in the corneal thickness in eyes without retinopathy. Furthermore, HbA1c levels and disease duration did not affect changes of cornea thickness in these eyes. It is not clear which mechanism about CCT is more prominet in eyes without retinopathy.

In Srinivsan's study, 60% of patients with non-proliferative diabetic retinopathy (NPDR) with only microaneurysms and the others who had mild NPDR were crosschecked with healthy group. They could not detect any difference between the groups in terms of CCT and CMT, supporting our data (18). Yeung et al. (6) found no statistically significant difference regarding the macular thickness between the diabetic patient group without retinopathy and the diabetic patient group with retinopathy without macular edema.

Dai included a large number of patients, no difference was found between groups with early DR and without retinopathy related to macular thickness (19). However, the macula was found to be significantly thicker in patients with moderate or worse retinopathy. This situation supports the result we achieved in our working group. We included diabetic patients without retinopathy to study whether macular thickness would be a marker without retinopathy in the macula, and we attributed the absence of a significant difference.

De Clerck et al. (20) who compared pre-diabetics groups, diabetes without retinopathy with a control group, found that macula thickness of pre-diabetic patients decreases prominently, besides, it is higher when diabetes becomes obvious clinically, they attributed this to neurodegenerative changes.

In a study conducted in Singapore which the patients with diabetic retinopathy at different stages, macular thicknesses in patients with no or mild diabetic retinopathy were not found different from

the control group. This situation shows the effect of retinopathy stage on macular thickness and is similar to our results. They also found no significant difference in macular thickness between moderate and severe DR patients without macular edema. In this study, no relation was found between macular thickness and HbA1c level. We included patients without retinopathy to determine whether macular thickness was a marker in the early period, and similarly, we revealed that the macula had no early effect (21).

Yolcu et al. (22) when the central macular thickness of type 1 DM patients with a diabetes duration of 6.1±2.8 years and without retinopathy and the control group were compared, was found no differences. This study, which is similar to us in terms of the duration of diabetes and retinopathy of its participants, supports our results.

There are many studies on the effects of diabetes on corneal thickness and macular thickness. As mentioned above, different results have been reported in these studies. We think that this difference is due to the variability of the retinopathy stages in diabetic patients. In addition, it should be considered that DM may have a complex effect on tissues. With the developing technology over time, the macula can be evaluated in more detail and a clearer result can be obtained.

When we examine the studies in the literature and evaluate our data, we think that the most important factors in corneal and macular thickness in diabetic retinopathy are the advanced stage of retinopathy and the duration of diabetes >10 years. The fact that our diabetes group is less than 10 years old and that we include advanced retinopathy groups are the most important limiting factors of our study. As a result of the study, although there was no significant difference between the diabetic group without retinopathy and maculopathy and the control group in terms of CCT and CMT, the significant difference in visual acuity makes us think that there are some changes in terms of microstructure. In a study, the visual acuity of the diabetic group without clinical retinopathy was found to be lower than that of the control group, and a difference was found in the level of capillary plexus flow between the groups examined by OCT angiography, even though there was no difference between the foveal avascular zones (23).

To the best of our knowledge, we did not find any study evaluating the effect of both macular and corneal thickness. We compared that type 2 DM patients with no retinopathy and healthy control group considering the duration of HbA1c and diabetes. In the results of study, no effect was found between macular and corneal thicknesses of HbA1c level and diabetes duration.

Conclusion

In light of the findings of this study, it can be assumed that DM may not cause changes in the thickness of macula and cornea in eyes without retinopathy. In conclusion, DM has different effects on each ocular tissue as macular and corneal thickness. It should not be used as an indicator of retinal and corneal involvement. Studies with more detailed examinations of the microstructure will be more illuminating.

Ethics

References

1.   Jeganathan VS, Wang JJ, Wong TY. Ocular associations of diabetes other than diabetic retinopathy. Diabetes Care 2008; 31: 1905-12.

2.   Writing Team for the Diabetes Control and Complications Trial/ Epidemiology of Diabetes Interventions and Complications Research Group. Effect of intensive therapy on the microvascular complications of type 1 diabetes mellitus. JAMA 2002; 287: 2563-9.

3. Memon AF, Mahar PS, Memon MS, Mumtaz SN, Shaikh SA, Fahim MF. Age-related cataract and its types in patients with and without type 2 diabetes mellitus: A Hospital-based comparative study. J Pak Med Assoc 2016; 66: 1272-6.

4. Negi A, Vernon SA. An overview of the eye in diabetes. J R Soc Med 2003; 96: 266-72.

5. Bikbova G, Oshitari T, Tawada A, Yamamoto S. Corneal changes in diabetes mellitus. Curr Diabetes Rev 2012; 8: 294-302.

6. Yeung L, Sun CC, Ku WC, Chuang LH, Chen CH, Huang BY, et al. Associations between chronic glycosylated haemoglobin (HbA1c) level and macular volume in diabetes patients without macular oedema. Acta Ophthalmol 2010; 88: 753-8.

7. Huang D, Swanson EA, Lin CP, Schuman JS, Stinson WG, Chang W, et al. Optical coherence tomography. Science 1991; 254: 1178-81.

8. Viswanathan D, Kumar NL, Males JJ, Graham SL. Comparative analysis of corneal measurements obtained from a Scheimpflug camera and an integrated Placido-optical coherence tomography device in normal and keratoconic eyes. Acta Ophthalmol 2015; 93: e488-94.

9. Vieira-Potter VJ, Karamichos D, Lee DJ. Ocular Complications of Diabetes and Therapeutic Approaches. Biomed Res Int 2016; 2016: 3801570.

10. Shih KC, Lam KS, Tong L. A systematic review on the impact of diabetes mellitus on the ocular surface. Nutr Diabetes 2017; 7: e251.

11. American Diabetes Association, Data from the National Diabetes Statistics Report, 2014, http://www.diabetes.org/diabetes-basics/statistics/.

12. Calvo-Maroto AM, Cerviño A, Perez-Cambrodí RJ, García-Lázaro S, Sanchis-Gimeno JA. Quantitative corneal anatomy: evaluation of the effect of diabetes duration on the endothelial cell density and corneal thickness. Ophthalmic Physiol Opt 2015; 35: 293-8.

13. Choo M, Prakash K, Samsudin A, Soong T, Ramli N, Kadir A. Corneal changes in type II diabetes mellitus in Malaysia. Int J Ophthalmol 2010; 3: 234-6.

14. Inoue K, Kato S, Inoue Y, Amano S, Oshika T. The corneal endothelium and thickness in type II diabetes mellitus. Jpn J Ophthalmol 2002; 46: 65-9.

15. Gao F, Lin T, Pan Y. Effects of diabetic keratopathy on corneal optical density, central corneal thickness, and corneal endothelial cell counts. Exp Ther Med 2016; 12: 1705-10.

16. Toygar O, Sizmaz S, Pelit A, Toygar B, Yabaş Kiziloğlu Ö, Akova Y. Central corneal thickness in type II diabetes mellitus: is it related to the severity of diabetic retinopathy? Turk J Med Sci 2015; 45: 651-4.

17. Lee JS, Oum BS, Choi HY, Lee JE, Cho BM. Differences in corneal thickness and corneal endothelium related to duration in diabetes. Eye (Lond) 2006; 20: 315-8.

18. Srinivasan S, Pritchard N, Sampson GP, Edwards K, Vagenas D, Russell AW, et al. Retinal thickness profile of individuals with diabetes. Ophthalmic Physiol Opt 2016; 36: 158-66.

19. Dai W, Tham YC, Cheung N, Yasuda M, Tan NYQ, Cheung CY, et al. Macular thickness profile and diabetic retinopathy: the Singapore Epidemiology of Eye Diseases Study. Br J Ophthalmol 2018; 102: 1072-6.

20. De Clerck EEB, Schouten JSAG, Berendschot TTJM, Goezinne F, Dagnelie PC, Schaper NC, et al. Macular thinning in prediabetes or type 2 diabetes without diabetic retinopathy: the Maastricht Study. Acta Ophthalmol 2018; 96: 174-82.

21. Sng CC, Cheung CY, Man RE, Wong W, Lavanya R, Mitchell P, et al. Influence of diabetes on macular thickness measured using optical coherence tomography: the Singapore Indian Eye Study. Eye (Lond) 2012; 26: 690-8.

22. Yolcu U, Çağıltay E, Toyran S, Akay F, Uzun S, Gundogan FC. Choroidal and macular thickness changes in type 1 diabetes mellitus patients without diabetic retinopathy. Postgrad Med 2016; 128: 755-60.

23. Meshi A, Chen KC, You QS, Dans K, Lin T, Bartsch DU, et al. ANATOMICAL AND FUNCTIONAL TESTING IN DIABETIC PATIENTS WITHOUT RETINOPATHY: Results of Optical Coherence Tomography Angiography and Visual Acuity Under Varying Contrast and Luminance Conditions. Retina 2019; 39: 2022-31.

Original Article / Özgün Araştırma

Is Outpatient ERCP Safe for Choledocholithiasis?

Koledok Taşı için Ayaktan ERCP Güvenli midir?

● İsmail Taşkıran[1], ● Bülent Ödemiş[2], ● Hakan Yıldız[3], ● Erkan Parlak[4]

[1]Aydın Adnan Menderes University Faculty of Medicine, Department of Gastroenterology, Aydın, Turkey
[2]University of Health Sciences Turkey, Ankara City Hospital, Clinic of Gastroenterology, Ankara, Turkey
[3]İstanbul Yeniyüzyıl University Gaziosmanpaşa Hospital, Clinic of Gastroenterology, İstanbul, Turkey
[4]Hacettepe University Faculty of Medicine, Department of Gastroenterology, Ankara, Turkey

Address for Correspondence/Yazışma Adresi:
İsmail Taşkıran Ph.D.,
Aydın Adnan Menderes University Faculty of
Medicine, Department of Gastroenterology,
Aydın, Turkey
Phone : +90 505 441 93 41
E-mail : dr_istaskiran@hotmail.com
ORCID ID: orcid.org/0000-0001-5450-5133

Abstract

Objective: This study aimed to compare the complications of outpatient and inpatient endoscopic retrograde cholangiopancreatography (ERCP) procedures in patients with bile duct stones and to investigate whether it is a safe approach to perform outpatient ERCP or not.

Materials and Methods: This prospective study consisted of 203 patients who had undergone ERCP for the first time with a diagnosis of choledocholithiasis between January 2013-July 2013.

Results: Of the patients included in the study, 102 had undergone outpatient and 101 had undergone inpatient ERCP. Complications following ERCP occurred in 9.8% of the outpatient group (pancreatitis in 5.9%, hemorrhage in 1.9%, cholangitis in 1%, perforation in 1%), while they occurred in the 11.9% of the inpatient group (pancreatitis in 6.9%, bleeding in 1%, cholangitis in 2%, perforation in 2%) (p=0.230, p=0.386, p=0.386, p=0.333, respectively; total complication comparison p=0.618). An amylase level below 150 U/L at the 4th hour after ERCP had a negative predictive value of 99.4% for pancreatitis to be negative. When the 4th hour amylase value of 300 U/L was taken as a limit, it had a positive predictive value of 52.1% in terms of pancreatitis positive. Additionally, the outpatient procedure was more cost-effective when the two groups were compared in terms of the cost [873.6±272.2 Turkish lira (TL) vs 1389.7±612.6 TL, p=0.001].

Conclusion: Our study demonstrated that outpatient ERCP was safe and cost-effective in selected cases with bile duct stones. The 4th hour amylase level below 150 U/L after ERCP showed that patients can be safely sent home and that the amylase value above 3-fold at the 4th hour can be considered the limit value for admission to the hospital for pancreatitis.

Öz

Amaç: Bu çalışmada safra yolu taşı olan hastalara ayaktan ve yatırılarak uygulanan endoskopik retrograd kolanjiyopankreatografi (ERCP) prosedürünün komplikasyonları karşılaştırılarak, ayaktan ERCP işleminin emniyetli bir yaklaşım olup olmadığı araştırılmıştır.

Gereç ve Yöntemler: Çalışmaya, 2013 Ocak-Temmuz ayları arasında safra yolu taşı tanısı almış olup ilk defa ERCP uygulanan 203 vaka prospektif olarak alınmıştır.

Bulgular: ERCP yapılan vakaların 102'si ayaktan, 101'i yatırılarak uygulanan hastalardı. Ayaktan grupta ERCP sonrası komplikasyon toplam %9,8 (pankreatit %5,9, kanama %1,9, kolanjit %1, perforasyon %1), yatan grupta %11,9 (pankreatit %6,9, kanama %1, kolanjit %2, perforasyon %2) olarak saptandı (sırasıyla p=0,230, p=0,386, p=0,386, p=0,333 toplam komplikasyon kıyaslaması p=0,618). ERCP

sonrası 4. saat amilaz ölçümünün 150 U/L'den düşük olması pankreatit olmama açısından %99,4 oranında negatif prediktif değere sahipti. 4. saat amilaz değerinin sınırı 300 kabul edildiğinde pankreatit olma açısından %52,1 oranında pozitif prediktif değere sahipti. Aynı zamanda iki grup maliyet açısından da kıyaslandığında ayaktan grup daha avantajlıydı [873,6±272,2 Türk lirası (TL) vs 1389,7±612,6 TL, p=0,001].
Sonuç: Bu çalışma safra yolu taşı olan seçilmiş hastalarda ayaktan ERCP işleminin emniyetli ve maliyet açısından daha etkin olduğunu, ERCP sonrası 4. saat amilaz ölçümünün 150 U/L'den düşük olması hastaları emniyetli bir şekilde taburcu edebileceğimizi aynı zamanda 4.saat amilaz ölçümünün 3 katın üzerinde olmasının pankreatit açısından hastaneye yatış için limit değer olabileceğini göstermiştir.

Introduction

The endoscopic retrograde cholangiopancreatography (ERCP) procedure is a relatively complex endoscopic procedure that requires special equipment and a long learning period. Although it can be seen as a safe procedure in experienced hands, it may cause more serious complications with higher rates than other standard endoscopic techniques. The rate of all complications of ERCP have been reported in the literature to be between 9.8-11.6%, with a mortality rate of 0.4% (1-5). For this reason, the ERCP procedure is generally performed as an inpatient procedure. Since some of the ERCP-related complications are noticed and intervened during the procedure and most of them are recognized during the first 2-6 hours post-procedure, outpatient ERCP has been increasingly studied in selected cases over the last 20 years and has become a reasonable option by experienced centers to reduce bed occupation and decrease costs (5-7). Bleeding and perforation during ERCP are often complications that can be recognized during the procedure. Early diagnosis of pancreatitis, which is the most common complication after ERCP, is very important. Hyperamylasemia after ERCP is known to peak between 90 minutes and 4 hours. Therefore, the amylase value at the 4-h has an important role in predicting pancreatitis (8). The aim of this study was to determine the reliability of outpatient ERCP procedure in patients with choledocholithiasis and to ensure that patients with 4-h amylase values lower than 150 units/L can be discharged safely in terms of the risk of pancreatitis, which is one of the complications of the procedure.

Materials and Methods

The ethics committee approval for the study was obtained from the Yüksek İhtisas Training and Research Hospital Non-Interventional Clinical Research Ethics Committee (decision no: 312, date: 05.12.2013).

This prospective study consists of 203 patients diagnosed with choledocholithiasis who received ERCP for the first time between January 2013 and July 2013 in a tertiary hospital where more than 2000 ERCP procedures are performed each year.

Prior to the ERCP procedure, age, sex, drug use, allergy to the contrast agent, comorbid diseases, American Society of Anesthesiologist (ASA) assesment scores and the cholecystectomy status of all patients were evaluated. Written informed consent was obtained from all patients, which described the ERCP procedure and provided information about possible complications. Complete blood count (CBC), coagulation parameters and biochemical profiles (renal functions, liver tests, amylase) were measured before the procedure in all patients. All patients had undergone at least one imaging technique (ultrasonography, computed tomography, magnetic resonance cholangiopancreatography, endosonography) before the procedure.

Before the each ERCP procedure, rectal non-steroidal anti-inflammatory drugs (NSAIDs) were routinely administered to patients.

The exclusion criteria of the patients were determined as follows; previous sphinceterotomy, patients <18 years of age, patients with cholangitis, patients with biliary pancreatitis, patients who received anticoagulation other than aspirin (clopidogrel, warfarin etc.), those with coagulation disorders (international normalized ratio >1.5, platelet <50,000/ mm3), ASA score of 4 and above for inpatients, ASA score of 3 or above for outpatients, and patients with altered gastointestinal system anatomy (Billroth-2 gastrectomy, Roux-en-Y hepaticojejunostomy, etc.). Patients with these criteria were not included in the study. The selection of the patients is summarized in Figure 1.

All patients were treated at least 8 hours after fasting. The outpatients were followed up post-opeatively for at least 4 hours in the 2-bed follow-up

unit, which was allocated to the gastroenterology department in the emergency unit of our hospital. All patients were fasted for the first 4 hours after ERCP procedure. Amylase and CBC values were measured at the 4-h after the procedure. The patients were evaluated by the gastroenterology fellow at the end of the 4-h. For outpatients, amylase levels of <150 units/L, normal hemoglobin and leukocyte levels and absence of epigastric tenderness and abdominal pain on the abdominal examination were accepted as the criteria for discharge. The patients were advised to follow an only-liquid diet during the first 24 hours of discharge. If the 4-h amylase value was >150 units/L in the outpatients, regardless of the presence of symptoms, the oral intake of the patient was not initiated and 24-h follow-up and control of the 24-h amylase values were planned. The 24-h amylase value of lower than 300 units/L and/or absence of any symptoms was accepted as a criterion for discharge. The outpatients who developed complications in the first 4 hours were admitted to the inpatient ward or the intensive care unit without delay. The inpatients were also evaluated by the gastroenterology fellow at the end of the 4-h. The oral intake of the patient was initiated with a liquid diet regime when the 4-h amylase value was <150 units/L and abdominal examination was normal, and the regime was turned to a normal diet when there was no additional problem at the end of 24 hours. In case when the 4th hour amylase value was >150

units/L, the amylase value was measured at the 24-h before the oral intake of the patient was initiated. The oral intake of the patient was initiated when the 24-h amylase value was below 300 units/L and/or in the absence of symptoms. Figure 2 summarizes how to manage patients according to their 4-h amylase value and complaints.

Support was received from the financial unit of our hospital in terms of cost calculation for outpatient and inpatient groups.

ERCP-specific complications (pancreatitis, cholangitis, bleeding, perforation) were defined and graded according to some special classifications (1,9,10).

Statistical Analysis

Statistical analysis of the data was performed using the Statistical Package for Social Sciences (SPSS) version 18 (SPSS Inc., Chicago, IL, United States). The difference between the groups in terms of categorical variables in outpatients and inpatients was evaluated using the chi-square or the Fisher's tests (where the values observed in the cells did not meet the chi-square test assumptions), and the t-test was used for the numerical data showing normal distribution and the Mann-Whitney U test was used for the data that were not normally distributed. A p value lower than 0.05 was considered statistically significant.

Results

Of the patients, 102 had undergone outpatient and 101 had undergone inpatient ERCP. 65 (63.7%) of the patients who had undergone outpatient ERCP were female and 37 (36.3%) were male. 56 patients (55.6%) of the patients who had undergone inpatient ERCP were female and 45 (44.6%) were male (p=0.220). The mean age of the outpatient group was 53.9±18.1
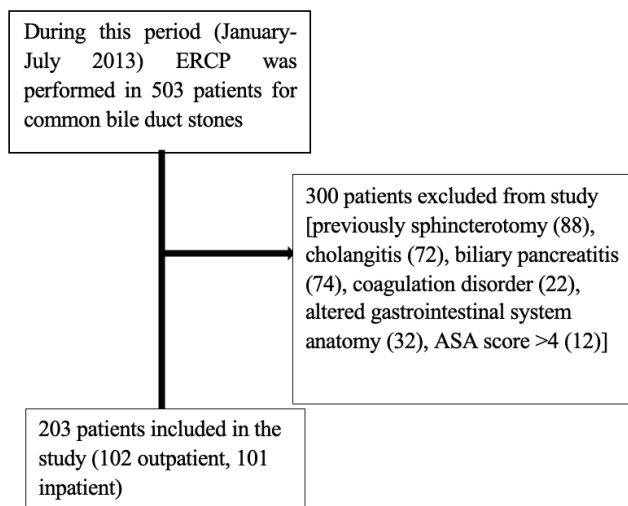
Figure 1. Patient selection
ERCP: Endoscopic retrograde cholangiopancreatography, ASA: American Society of Anesthesiologist
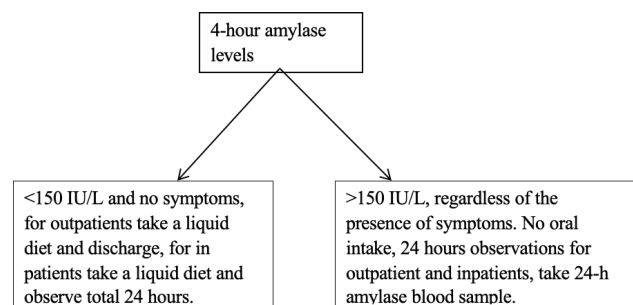
Figure 2. Patient management according to the 4-hour amylase level

years and that of the inpatient group was 65.6±16.6 years (p=0.001).

When the groups were compared according to the ASA score, 69 (67.6%) patients with an ASA score of 1 and 33 (32.4%) patients with an ASA score of 2 were identified in the outpatient group. In the inpatient group, 37 patients (36.6%) with ASA score of 1, 54 patients (53.4%) with a score of 2, and 10 patients with a score of 3 (10%) were identified. The ASA scores were found to be different between the groups (p=0.001).

No significant difference was found between the outpatient and the inpatient groups in terms of imaging techniques performed for suspicion of bile duct stone (p=0.07). The therapeutic procedures performed during the ERCP procedure were found to have a similar distribution in both groups (p=0.091). The baseline demographic data of the patients have been presented in Table 1.

When the baseline laboratory values were compared between the two groups, it was found that parameters other than creatinine and platelet levels were not different between the two groups (Table 2).

In the outpatient group, 8 (7.8%) patients had 10 ERCP-related complications (1 patient had pancreatitis+cholangitis, the other 1 had pancreatitis + bleeding), and the patients were followed up with hospitalization. In the inpatient group, 11 (10.8%) patients had 12 complications (pancreatitis + cholangitis were observed together in 1 patient). No statistically significant difference was observed between the two groups in terms of complications (p=0.618) (Table 3).

When the outpatient and the inpatient groups were compared in terms of cost, the outpatient group was found to have lower cost and this was found to be statistically significant [873.6±272.2 Turkish lira (TL) vs 1389.7±612.6 TL p=0.001].

No statistically significant difference was observed between the outpatients and the inpatients with similar ASA scores in terms of complications (Table 4).

Eighty five patients were determined with 4-h amylase values below 150 U/L in the outpatient group (n=102). Only 1 of these patients was admitted to the hospital again after 24-h and was diagnosed with pancreatitis+cholangitis. Again in this group, there were 7 patients with 4-h amylase values between 150-300 U/L and none of them developed pancreatitis.

Table 1. Patients demographics			
	Outpatient group (n=102) n (%)	Inpatient group (n=101) n (%)	p-value
Age	53.9±18.8	65.6±16.6	0.001
Female/male	65 (63.7)/37 (36.3)	56 (55.4)/45 (44.6)	0.22
Comorbidity	39 (37.5)	65 (62.5)	0.001
Cholecystectomy (+)	23 (22.5)	15 (14.8)	0.4
Aspirin (+)	18 (17.6)	28 (27.7)	0.161
NSAID (+)	19 (18.6)	20 (19.8)	0.831
Cannulation type	82 (79.4)/20 (19.6)	85 (84.2)/16 (15.8)	0.482
Normal/preincision			
Bile duct size (mm)	10±2.3	11.2±3.7	0.337
ASA score			
1	69 (67.6)	37 (36.6)	
2	33 (32.4)	54 (53.4)	0.001
3	0	10 (%10)	
Therapeutic procedure			
Sphincterotomy	102 (100)	101 (100)	
Stent	10 (9.8)	10 (9.9)	
Mechanical lithotripsy	3 (3)	5 (5)	0.091
Dilatation (TPBD)	5 (4.9)	14 (13.8)	
Pancreatic stent	5 (4.9)	0	
Diagnostic tool			
USG	71 (69.6)	82 (81.2)	
EUS	15 (14.7)	14 (13.9)	
MRCP	14 (13.7)	5 (5)	0.07
CT	2 (2)	0 (0)	
Cost, TL	873.6±272.2	1389.7±612.6	0.001

NSAID: Non-steroidal anti-inflammatory drugs, ASA: American Society of Anesthesiologist, USG: Ultrasonography, MRCP: Magnetic resonance cholangiopancreatography, CT: Computed tomography, TL: Turkish lira

Pancreatitis developed in 5 out of 10 patients with 4-h amylase values above 300 U/L.

In the inpatient group (n=101), 87 patients with 4-h amylase values below 150 U/L were determined and none of the patients developed pancreatitis. Pancreatitis did not develop in any of the 6 patients with 4-h amylase values between 150-300 U/L in the same group. The remaining 8 patients had 4-h

Table 2. Laboratory parameters

	Outpatient group (n=102) n (%)	Inpatient group (n=101) n (%)	p-value
Hgb (g/dL)	13.7±1.6	13.3±1.6	0.267
WBC (x10³/uL)	7.945±3.3	7.000±3.6	0.08
Plt (x10³/uL)	287.069±84.3	247.604±69.9	0.001
INR	1.07±0.7	1.2±0.8	0.054
ALT (U/L)	180±93	154±83	0.082
AST, median (U/L)	83.5 (12-644)	48 (10-770)	0.106
GGT (U/L)	313±275	336±313	0.907
Total bilirubin, median (mg/dL)	1.47 (0.2-14.3)	1.3 (0.3-29.5)	0.685
ALP (U/L)	243±141	258±209	0.522
Creatinin (mg/dL)	0.8±0.2	0.9±0.4	0.044
Amylase-base line (U/L)	79.3±46.2	79.8±44.5	0.765
Amylase-4-h, median (U/L)	83.5 (21-1836)	82 (24-2819)	0.858

Hgb: Hemoglobin, WBC: White blood cell, Plt: Platelet, INR: International normalized ratio, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, GGT: Glutamyl transferase, ALP: Alkaline phosphatase, *The median value is used because AST, total bilirubin, amylase 4-h did not have normal distribution

Table 3. Distribution of complications

Complication	Outpatient group (n=102) n (%)	Inpatient group (n=101) n (%)	p-value
Bleeding	2	1	0.386
Mild	1	0	-
Moderate	1	1	-
Severe	0	0	-
Pancreatitis	6	7	0.230
Mild	5	6	-
Moderate	1	1	-
Severe	0	0	-
Cholangitis	1	2	0.386
Mild	1	1	-
Moderate	0	1	-
Severe	0	0	-
Perforation	1	2	0.333
Mild	1	2	-
Moderate	0	0	-
Severe	0	0	-
Total post-ERCP complications	10 (9.8)	12 (11.9)	0.618

ERCP: Endoscopic retrograde cholangiopancreatography, *n shows the number of complications

amylase levels of over 300 U/L and 7 developed pancreatitis.

In the light of these data, the 4-h amylase value lower than 150 U/L stood out with a 99.4% negative predictive value for the absence of pancreatitis. An amylase level of up to 300 U/L in this analysis was also found to be safe. An amylase value of 300 U/L or higher was found to be an increased positive predictive value for pancreatitis (Table 5).

Discussion

In studies conducted on outpatient ERCP, it is recommended that outpatient ERCP be performed in selected cases by experienced centers, since most of the complications occur during the procedure or during the follow-up periods ranging from 2-6 hours.

In our study, the overall complication rate after ERCP was determined as 9.8% in the outpatient group and as 11.9% in the inpatient group. There was no difference between the two groups in terms of complications. When the literature was examined, it is seen that there was a similarity in terms of total and individual complications. In our study, ERCP-related mortality was not observed. In studies conducted by Cotton et al. (1) in 1991, Freeman et al. (2) in 1996, Johanson et al. (3) in 2002, and Mallery et al. (4) in 2003, complications after ERCP were reported to be between 9.8-11.6% and the mortality as 0.4%. In a study by Rábago et al. (5) in 2010, the total complication rate after ERCP was determined as 12.1%.

In our study, 10 (9.8%) complications were detected in 8 patients in the outpatient group. Eight of these complications (7.8%) were recognized during the first 4 hours of follow-up and these patients were hospitalized. Twelve (11.9%) complications were determined in 11 patients in the inpatient group. Ten (9.9%) of these complications were recognized during the first 4 hours of follow-up. Most of the complications of the patients occurred during the procedure or during the first 4 hours of the follow-up period.

In Hui et al.'s (6) study in 2004, the rate of complications in outpatient ERCP patients was similar to the rate of complications in patients who were hospitalized for at least one day after ERCP. Most complications occurred in the first 6 hours.

Table 4. Distribution of the number of patients who developed complications according to ASA 1 and ASA 2 scores in both groups

	Outpatient group n=102 (%)	Inpatient group n=91 (%)	p-value
Number of ASA 1 patients/number of patients with complications	69 (67.6)/4 (5.8)	37 (40.6)/5 (13.5)	0.189
Number of ASA 2 patients/number of patients with complications	33 (32.4)/4 (12.1)	54 (59.4)/6 (11.1)	0.910
ASA: American Society of Anesthesiologist			

Table 5. Four h amylase level cut-off values and test performance

Amylase levels	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
100 (normal)	100	64	18	100
150 (1.5x normal)	92.3	87.3	33.3	99.4
300 (3x normal)	92.3	94.2	52.1	99.4

The study of Katsinelos et al., (7) which was performed in 2011, shows similarity to our study. In this study, the outpatient group was found to be cost effective. Similarly, in our study, when the two groups were compared in terms of cost, it was seen that the outpatient group was found to be less costly.

When the outpatient and the inpatient groups were compared in terms of age and ASA scores, a difference was found between the two groups (age and ASA scores were higher in the inpatient group). This is due to the fact that patients with ASA score of 3 had not undergone an outpatient ERCP procedure. When the patients with ASA score of 3 were excluded from the inpatient group and the two groups were compared according to the similar ASA score, no difference was found between the two groups in terms of complications.

The most common complication after ERCP is pancreatitis. Bleeding and perforation are often recognized during the procedure. For this reason, amylase levels and the presence of abdominal pain in the outpatient group are the most frequently used parameters to be safely discharged and patients are discharged accordingly. In studies conducted for this purpose, the amylase levels obtained at the 2nd or 4th hours without waiting for 24 hour, can be a marker for pancreatitis.

In the study conducted by Thomas et al. (8), the 4-h amylase levels of lower than 150 U/L excluded pancreatitis with a 100% negative predictive value. A serum amylase level of above 3-fold was accepted as the limit value for hospital admission with a positive predictive value of 36.8%. If the amylase level were between 1.5-3.0 times, evaluation of additional risk factors for clinical findings and pancreatitis have been proposed.

In our study, the 4-h serum amylase level of below 150 U/L was found to have a rate of 99.4% negative predictive value for the absence of pancreatitis after ERCP. This showed that we can safely discharge patients with a 4-h amylase value below 150 U/L after ERCP. When the 4-h serum amylase level of 3 times above the normal value was taken as the cut-off value, it was found to have a positive predictive value of 52.1% in terms of pancreatitis. This value was considered to be a cut-off level for admission to hospital for pancreatitis.

In a study by Gottlieb et al. (11), the 2nd hour amylase level of lower than 276 U/L is reported to exclude pancreatitis with a high negative predictive value (97%).

In the European Society of Gastrointestinal Endoscopy guidelines, it was stated that the 4th hour serum amylase level of 1.5 times below normal values after ERCP eliminates pancreatitis after ERCP. It has also been stated that the same situation can be accepted as a criterion for the safe discharge of patients (12-14).

Up to the present, outpatient ERCP studies have conducted with heterogenous group patients (5-8,11,15). For the first time, our study has shown that

it is safe to perform ERCP on an outpatient basis in selected patients (choledocholithiasis).

There were some limitations in our study. These limitations were: working with a low-risk group for pancreatitis after ERCP (choledocholithiasis), the fact that all procedures were performed by experienced endoscopists (not reflecting the general population), use of preventive measure (rectal NSAIDs), use of amylase instead of lipase and incomplete randomization in patient selection.

Conclusion

Our study showed that performing outpatient ERCP was safe and cost-effective in selected cases with choledocholithiasis; patients with 4-h amylase levels of lower than 150 U/L can be safely sent to their homes, patients with 4-h amylase levels above 3-fold can be admitted to the hospital for pancreatitis and this value can be used as a cut-off value.

Ethics

Ethics Committee Approval: The ethics committee approval for the study was obtained from the Yüksek İhtisas Research and Training Hospital Non-Interventional Clinical Research Ethics Committee (decision no: 312, date: 05.12.2013).

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

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: İ.T., B.Ö., H.Y., E.P., Concept: İ.T., B.Ö., Design: İ.T., B.Ö., H.Y., E.P., Data Collection or Processing: İ.T., H.Y., Analysis or Interpretation: İ.T., B.Ö., H.Y., E.P., Literature Search: İ.T., Writing: İ.T.

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

Financial Disclosure: The authors declared that this study received no financial support.

References

1. Cotton PB, Lehman G, Vennes J, Geenen JE, Russell RC, Meyers WC, et al. Endoscopic sphincterotomy complications and their management: an attempt at consensus. Gastrointest Endosc 1991; 37: 383-93.

2. Freeman ML, Nelson DB, Sherman S, Haber GB, Herman ME, Dorsher PJ, et al. Complications of endoscopic biliary sphincterotomy. N Engl J Med 1996; 335: 909-18.

3. Johanson JF, Cooper G, Eisen GM, Freeman M, Goldstein JL, Jensen DM, et al. Quality assessment of ERCP. Endoscopic retrograde cholangiopacreatography. Gastrointest Endosc 2002; 56: 165-9.

4. Mallery JS, Baron TH, Dominitz JA, Goldstein JL, Hirota WK, Jacobson BC, et al. American Society for Gastrointestinal Endoscopy. Complications of ERCP. Gastrointest Endosc 2003; 57: 633-8.

5. Rábago L, Guerra I, Moran M, Quintanilla E, Collado D, Chico I, et al. Is outpatient ERCP suitable, feasible, and safe? The experience of a Spanish community hospital. Surg Endosc 2010; 24: 1701-6.

6. Hui CK, Lai KC, Wong WM, Yuen MF, Ng M, Chan CK, et al. Outpatients undergoing therapeutic endoscopic retrograde cholangiopancreatography: six-hour versus overnight observation. J Gastroenterol Hepatol 2004; 19: 1163-8.

7. Katsinelos P, Kountouras J, Chatzimavroudis G, Zavos C, Terzoudis S, Pilpilidis I, et al. Outpatient therapeutic endoscopic retrograde cholangiopancreatography is safe in patients aged 80 years and older. Endoscopy 2011; 43: 128-33.

8. Thomas PR, Sengupta S. Prediction of pancreatitis following endoscopic retrograde cholangiopancreatography by the 4-h post procedure amylase level. J Gastroenterol Hepatol 2001; 16: 923-6.

9. Banks PA, Bollen TL, Dervenis C, Gooszen HG, Johnson CD, Sarr MG, et al. Classification of acute pancreatitis--2012: revision of the Atlanta classification and definitions by international consensus. Gut 2013; 62: 102-11.

10. Wada K, Takada T, Kawarada Y, Nimura Y, Miura F, Yoshida M, et al. Diagnostic criteria and severity assessment of acute cholangitis: Tokyo Guidelines. J Hepatobiliary Pancreat Surg 2007; 14: 52-8.

11. Gottlieb K, Sherman S, Pezzi J, Esber E, Lehman GA. Early recognition of post-ERCP pancreatitis by clinical assessment and serum pancreatic enzymes. Am J Gastroenterol 1996; 91: 1553-7.

12. Dumonceau JM, Andriulli A, Deviere J, Mariani A, Rigaux J, Baron TH, et al. European Society of Gastrointestinal Endoscopy (ESGE) Guideline: prophylaxis of post-ERCP pancreatitis. Endoscopy 2010; 42: 503-15.

13. Dumonceau JM, Andriulli A, Elmunzer BJ, Mariani A, Meister T, Deviere J, et al. Prophylaxis of post-ERCP pancreatitis: European Society of Gastrointestinal Endoscopy (ESGE) Guideline - updated June 2014. Endoscopy 2014; 46: 799-815.

14. Dumonceau JM, Kapral C, Aabakken L, Papanikolaou IS, Tringali A, Vanbiervliet G, et al. ERCP-related adverse events: European Society of Gastrointestinal Endoscopy (ESGE) Guideline. Endoscopy 2020; 52: 127-49.

15. Tryliskyy Y, Bryce GJ. Post-ERCP pancreatitis: Pathophysiology, early identification and risk stratification. Adv Clin Exp Med 2018; 27: 149-54.

Original Article / Özgün Araştırma

The Validity and Reliability of the Turkish Pet Attitude Scale

Türkçe Evcil Hayvan Tutum Ölçeğinin Geçerlik ve Güvenirliği

 Rahşan Çevik Akyıl[1], Seyhan Çitlik Sarıtaş[2], Ayşegül Kahraman[1], Musa Şamil Akyıl[3], Filiz Abacıgil[4]

[1]Aydın Adnan Menderes University Faculty of Nursing, Department of Internal Medicine Nursing, Aydın, Turkey
[2]İnönü University Faculty of Nursing, Department of Nursing, Malatya, Turkey
[3]Aydın Adnan Menderes University Faculty of Dentistry, Department of Prosthetic Dentistry, Aydın, Turkey
[4]Aydın Adnan Menderes University Faculty of Medicine, Department of Internal Medicine, Aydın, Turkey

Address for Correspondence/Yazışma Adresi:
Rahşan Çevik Akyıl Prof. MD,
Aydın Adnan Menderes University Faculty of Nursing, Aydın, Turkey
Phone : +90 505 575 55 62
E-mail : rahsanakyil@hotmail.com
ORCID ID: orcid.org/0000-0002-4746-7463

Abstract

Objective: This study tests the Turkish validity and reliability of the Pet Attitude Scale (PAS) and adapt it to Turkish society.

Materials and Methods: The study population consisted of students in the departments of health at a university in Western Turkey. No sampling method was used; and the entire population was included in the study. Data were collected using a questionnaire on the participants' sociodemographic characteristics and the PAS, which consisted of 18 items scored between 1 and 7 under three factors: love and interaction, pets in the home, and joy of pet ownership. The construct validity of the scale was evaluated by Exploratory Factor Analysis (EFA) and Confirmatory Factor Analysis (CFA). The reliability of the PAS was assessed by Cronbach's alpha internal consistency coefficient, the item-total correlation, and the test-retest analysis.

Results: The scale factors' Cronbach's alpha reliability coefficient ranged between 0.45 and 0.92, the items' factor loads ranged between 0.51 and 0.85, and the total item correlation coefficients ranged between 0.31 and 0.77. The CFA supported the three-factor structure yielded by the EFA and showed good fit indices.

Conclusion: The scale was reliable for Turkish society compared to the original PAS. The factor structure resembled the results of a reevaluation of PAS. Thus, it can be suggested that the PAS is valid and reliable for application in Turkish society.

Öz

Amaç: Bu çalışmanın amacı Pet Tutum Ölçeği'nin (PTÖ) Türkçe geçerlik ve güvenirliğini test etmek ve Türk toplumuna uyarlamaktır.

Gereç ve Yöntemler: Araştırmanın evrenini Batı Türkiye'deki bir üniversitenin sağlık bölümlerinde okuyan öğrenciler oluşturdu. Örnekleme yöntemi kullanılmadı ve tüm popülasyon çalışmaya dahil edildi. Veriler, katılımcıların sosyo-demografik özelliklerine ilişkin bir anket ve 1 ile 7 arasında puanlanan 18 maddeden oluşan Evcil Hayvan Tutum Ölçeği kullanılarak üç faktör altında toplanmıştır: sevgi ve etkileşim, evde evcil hayvanlar ve evcil hayvan sahiplenme sevinci. Yapı geçerliği ölçeğin özellikleri açımlayıcı faktör analizi (AFA) ve doğrulayıcı faktör analizi (DFA) ile değerlendirilmiştir. Evcil hayvan tutum ölçeğinin güvenirliği Cronbach alfa iç tutarlılık katsayısı, madde-toplam korelasyonu ve test-tekrar test analizi ile değerlendirilmiştir.

Bulgular: Ölçek faktörlerinin Cronbach alfa güvenirlik katsayısı 0,45-0,92, maddelerin faktör yükleri 0,51-0,85 ve toplam madde korelasyon katsayıları 0,31-0,77 arasında değişmektedir. DFA, AFA tarafından elde edilen üç faktörlü yapıyı destekledi ve iyi uyum indeksleri gösterdi.

Sonuç: Ölçek, orijinal PTÖ ile karşılaştırıldığında Türk toplumu için güvenilirdi. Faktör yapısı, PTÖ'nün yeniden değerlendirilmesinin sonuçlarına benziyordu. Böylece evcil hayvan tutum ölçeğinin Türk toplumunda uygulanması için geçerli ve güvenilir olduğu söylenebilir.

Introduction

Animal-assisted interventions are interventional programs that constitute integral parts of targeted treatment procedures. They are supportive methods for ameliorating physical, psychological, and social problems of individuals (1). Studies have shown that being with pets regulates the hemodynamic parameters such as heart rate, increases neurohormonal activity, and reduces pain (2-4).

Although the relationship between having a pet and psychological health could not be shown, most studies have reported that people with a pet have better mental health (4,5). Various studies have shown that supporting positive moods affects empathy and socialization, and reduces the sense of loneliness (6-9).

Pets affect the general health of weaker populations such as elderly people, children, and those with psychiatric disorders, and even our relationships with other people (10). It was found in the elderly that social and cardiovascular health was positively affected by the support of pets (11). It was also reported that support of pets was effective on metal, physical, and social health and wellness of children and that it was recommended to be applied for educational and health purposes in children with special needs (12). A study reported that a positive approach towards pets encourages people to approach other people more positively (13).

The literature includes few studies on people-animal relationships in Turkey (14,15). The number of pets in homes has significantly increased in Turkey in the last decade. In addition, many pets on the streets often lead to contact between humans and animals. Along with the increase in the number of house pets in urban areas, various opinions have also come forth in society regarding the relationship between humans, pets, and houses. Institutional and social awareness has also increased on animal rights, welfare, and even animal health (16). This suggests that this topic will become a popular and required research field in Turkey soon.

Various measurement tools exist to assess the quality of pet owners' relationships (17-21). It was determined that the Pet Attitude Scale (PAS) was the commonly used measurement method (13,22-24) and the same scale using the form in English was applied to individuals in different cultures (25).

Bringing the scales on which knowledge had accumulated and recognized in international publications into Turkish language and using them shortens the time that an investigator would spend for preparing a new scale and provides the investigator ease of communication and information comparable to the results obtained in different communities. In addition, adapting the scales to Turkish might not facilitate researchers who do not have enough accumulation of knowledge and experience to develop a scale (26).

Römpke, while implementing the adaptation of PAS to the German language, stated that testing individuals with measurement tools in a language appropriate for their own culture would be more reliable, and more precise results might be gotten with better understanding the survey questions asked in their native language (27).

The fact that no scale had been developed in Turkey on this subject was the determinative factor in planning our study. With the suggestion that it would be a preliminary reference work for initiation of research on human-animal relationships, this study aimed to test the validity and reliability of the PAS and to implement its adaptation to the Turkish society.

Materials and Methods

Aim

This study aims to adapt the PAS into Turkish and test its validity and reliability.

Study Design and Participants

This was a methodological study. Students who were receiving education and training in various health departments of a university located in Western Turkey constituted the study group. A sample size of

300 participants was required to find a test's factor structure (28). The research was completed with 304 students who filled the forms completely. For gathering research data, the questionnaire, which was prepared by the investigators and containing sociodemographic characteristics of students, and PAS were used.

Ethical Consideration

Permission was obtained via e-mail for the adaptation of the PAS, which was developed by Templer et al. (29), into Turkish. In addition, the approval of the Faculty of Dentistry, Clinical Research Ethics Committee was obtained with number 2018/026 to conduct the study. The participants were informed about the study and that their personal information would be kept confidential. Those who volunteered were included in the study.

Data Collection

The data was collected with the students in the departments of health at a university in Western Turkey on weekdays during the course breaks between May 14 and June 5, 2018. The polls were distributed to the students, who were asked to complete the questionnaires. A second interview was carried out with 77 of the students three weeks later. The data collection process took approximately 12 to 15 minutes for each student.

The Pet Attitude Scale

PAS was modified by Templer et al. (29) in 2011 and had 18 items as a 7-point Likert type scale. Each item was scored between 1 and 7. The scale had three subscales that indicated individuals' attitudes towards pets, which were Love and Interaction (items 1, 2, 3, 5, 7, 8, 10, 11, 14, 16, 18), Pets in the Home (items 6, 9, 12, 13, 15) and Joy of Pet Ownership (items 4,17). The minimum and maximum scores were 18 and 126, respectively. The PAS was completed in 7 to 10 minutes. Templer et al. (29) found Cronbach's α coefficient to be 0.92 for PAS (29).

Language Validity

First, the PAS was translated from English into Turkish to test language validity. Then, the Turkish version was back-translated into English by two linguists. The researchers reviewed the English back-translation by comparing it to the original scale. A preliminary study was conducted with 18 students using the PAS. No item was removed from PAS.

Statistical Analysis

Data were analyzed using the Cronbach's α reliability coefficient, the Pearson Product-Moment Correlation Coefficient, factor analysis, the Barlett test, the Kaiser-Meyer Olkin test, correlations, numbers, and percentages. The p<0.05 was considered significant.

Results

The sociodemographic characteristics of the students are shown in Table 1. The average age of the students was 22. Of them, 62.8% were female, 99% were single, 72.4% studied nursing, 21.7% studied medicine, and 5.9% studied dentistry.

Validity

An EFA was applied using varimax rotation to determine the dimensional structure of the scale. The KMO value was used to test the suitability of the items for factor analysis, and the value obtained was determined to be 0.911. All values for the measure of sampling adequacy (MSA) were determined to be greater than 0.5 (all MSA >0.872). The Bartlett test for sphericity confirmed that the data was suitable for factor analysis (χ^2=2778.40, df=153, p<0.001). In the first attempt, a 3-factor structure with an eigenvalue greater than 1 was obtained. The first factor consisted of the items 1, 2, 3, 5, 7, 8, 10, 11, 14, 16 and 18, whereas the second factor consisted of the items 6, 9, 12, 13, and 15 and the third factor consisted of the

Table 1. The sociodemographic characteristics of the students (n=304)

		Mean	SD
Age		22.7	1.2
		n	%
Gender	Female	191	62.8
	Male	113	37.2
	Total	304	100.0
Marital status	Single	301	99.0
	Married	3	1.0
	Total	304	100.0
School	Medical faculty	66	21.7
	Faculty of dentistry	18	5.9
	School of health - department of nursing	220	72.4
	Total	304	100.0
SD: Standard deviation			

items 4 and 17. These three factors were determined to explain 57.4% of the total variance. The CFA performed for this structure revealed χ=467.614, df=132 (p<0.001), χ2/df=3.54, RMSEA=0.075, GFI=0.86, CFI=0.88, and IFI=0.89. The values obtained were determined to be outside the acceptable levels, even though close. Additionally, when the levels of internal consistency were analyzed, it was found to be 0.92 for the first factor, 0.76 for the second factor, and 0.45 for the third factor; the internal consistency was not at an acceptable level for the third factor. When the results of CFA and the problems related to the internal consistency level of the third factor were considered, it was concluded that such a 3-factor structure was not suitable, and the suitability of a 2-factor structure was tested.

The evaluation revealed that the first factor consisted of items 1, 2, 3, 5, 7, 8, 10, 11, 14, and 16, whereas the second factor consisted of items 4, 6, 9, 12, 13, 15, and 17. Therefore, the first factor was observed to consist of items related to positive behaviors, whereas the second factor consisted of items related to negative behaviors. The two factors were determined to explain 53.7% of the total variance. The factor loads of the items in the first factor were found to range from 0.569 to 0.883, whereas the factor loads of the items in the second factor from 0.592 to 0.832. CFA performed for this structure revealed χ^2=279.891, df=128 (p<0.001), χ^2/df=2.19, RMSEA=0.063, GFI=0.905, CFI=0.949, IFI=0.950 and SRMR=0.049. The results confirmed that this 2-factor structure had an acceptable level of suitability. The CFA Path Diagram of PAS-TR following the construction of the second CFA model was shown in Figure 1.

Reliability

Cronbach's α was used to determine the internal consistency level of PAS-TR. The internal consistency level was found to be 0.925 for the first factor, 0.824 for the second factor, and 0.906 for the entire scale. When the corrected item-total correlation levels were analyzed, it was determined that the items with the highest level were the items 5 and 8 (0.772 and 0.733, respectively) and those with the lowest level were the items 9 and 12 (0.313 and 0.370, respectively).

The test-retest results of the PAS revealed that the intraclass correlation coefficient (ICC) level was 0.738 (good) for the first factor, 0.776 (excellent) for
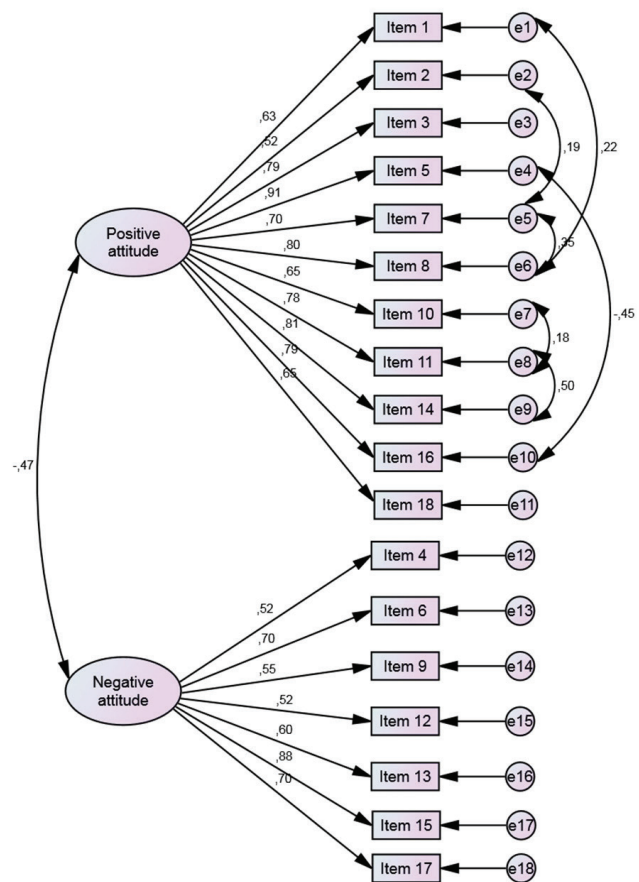
Figure 1. The path diagram of the Pet Attitude Scale

the second factor, and 0.773 (excellent) for the entire scale (30).

Discussion

In this study, PAS, which was modified by Templer et al. (29) in 2011, was adapted into Turkish. The psychometric features were tested on students in the departments of health in Turkey.

Validity

EFA and CFA were performed to determine the structural validity of PAS-TR. It was found that the scale was grouped under two factors in the Turkish version like the original scale and explained 53.7% of the total variance. A variance ratio ranging from 40% to 60% determined in the analysis of the scale has been considered sufficient (31). It has been shown in studies that items with a factor load below 0.30 must be not including during evaluation (32,33). It was determined in our study that the factor loads of PAS-TR ranging from 0.559-0.853 (Table 2); thus, no one

Table 2. Item-total score correlation coefficients, factor loadings of Pet Attitude Scale (n=304)					
	3-factor structure			2-factor structure	
	Factor 1	Factor 2	Factor 3	Factor 1	Factor 2
Item 1	0.602	-0.003	-0.377	0.632	-0.212
Item 2	0.637	-0.272	0.350	0.569	-0.063
Item 3	0.788	-0.121	-0.148	0.788	-0.189
Item 4	-0.118	0.355	0.592	-0.161	0.592
Item 5	0.856	-0.110	-0.167	0.853	-0.210
Item 6	-0.113	0.636	0.110	-0.200	0.730
Item 7	0.764	-0.234	0.114	0.729	-0.138
Item 8	0.826	-0.160	-0.090	0.815	-0.200
Item 9	0.001	0.519	0.289	-0.024	0.656
Item 10	0.675	0.028	-0.217	0.708	-0.063
Item 11	0.768	0.046	-0.432	0.818	-0.164
Item 12	-0.112	0.703	0.043	-0.087	0.617
Item 13	-0.034	0.607	-0.027	-0.081	0.669
Item 14	0.785	-0.035	-0.384	0.826	-0.193
Item 15	-0.253	0.580	0.448	-0.224	0.832
Item 16	0.769	-0.091	-0.124	0.777	-0.127
Item 17	-0.316	0.192	0.636	-0.299	0.678
Item 18	0.669	-0.146	-0.085	0.661	-0.165

the scale items were removed. The factor structure of PAS-TR determined by factor analysis was found to have construct validity.

CFA supported the 2-factor structure of the scale obtained as the result of EFA. The evaluation did not reveal good compliance regarding the X^2/df ratio and the RMSEA value. It has been stated in the literature that χ2/df<3 indicates a perfect fit, and the acceptable RMSEA value is ≤8 (34,35). The confirmatory factor analysis for this structure revealed the results as χ2=279.891, df=128 (p<0.001), χ2/df=2.19, RMSEA=0.063, GFI=0.905, CFI=0.949, IFI=0.950 and SRMR=0.049. These results confirmed the suitability of the 2-factor structure.

Reliability

Reliability was assessed using Cronbach's α internal consistency coefficient, item-total correlation, and rest-retest analysis. Cronbach's α consistency coefficient was calculated as an indicator of inner consistency and homogeneity of the PAS-TR. The scale is classified as quite reliable between 0.60 and 0.79, and highly reliable between 0.80 and 1.00 (36).

Cronbach's alpha coefficient was determined as 0.92-0.93 in the validity and reliability study of the original scale (29). In this study, Cronbach's α coefficient was used for determining the internal consistency of the PAS. The internal consistency level was calculated as 0.925 for the first factor, 0.824 for the second factor, and 0.906 for the entire scale. When the corrected item-total correlation levels were investigated, it was found that the items with the highest level were items 5 and 8 (0.772 and 0.733, respectively), whereas those with the lowest level were items 9 and 12 (0.313 and 0.370, respectively). The internal consistency of PAS-TR was high. PAS-TR is a reliable scale having internal consistency.

The items of the scale having low coefficients are considered not reliable enough. When the correlation coefficient of the item is under 30, it is suggested that insufficient reliability is present (37). The item-total correlation scores of PAS-TR were 0.313-0.772. The total score correlation coefficients of entire items in the scale were above 0.30. From this aspect, the total correlation values of PAS-TR were at the suitable reliability level.

When PAS-TR was done 77 students with an interval of three weeks for test-retest analysis, high-level positive statistically significant relationships were found to be present regarding the entire scale and all its sub-dimensions. Consistency of PAS-TR with time was determined. This result revealed that the internal consistency of the scale was high and that reliable results could be obtained in more than one application of the scale (31).

Conclusion

The results of this study in which we had measured the validity and reliability of the PAS revealed that the PAS adapts well to Turkish society.

Ethics

Ethics Committee Approval: The present cohort study was designed as a survey and was approved by the Clinical Research Ethics Committee of Adnan Menderes University Faculty of Dentistry (decision no: 98318678-020, date: 28.03.2018).

Informed Consent: Consent was obtained from the questionnaire participants.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: R.Ç.A., Design: R.Ç.A., Data Collection or Processing: A.K., M.Ş.A., Analysis or Interpretation: S.Ç.S., F.A., Literature Search: R.Ç.A., A.K., M.Ş.A., Writing: R.Ç.A.

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

Financial Disclosure: The authors declared that this study received no financial support.

References

1. Matuszek S. Animal-facilitated therapy in various patient populations: systematic literature review. Holist Nurs Pract 2010; 24: 187-203.

2. Beetz A, Uvnäs-Moberg K, Julius H, Kotrschal K. Psychosocial and psychophysiological effects of human-animal interactions: the possible role of oxytocin. Front Psychol 2012; 3: 234.

3. Marcus DA, Bernstein CD, Constantin JM, Kunkel FA, Breuer P, Hanlon RB. Impact of animal-assisted therapy for outpatients with fibromyalgia. Pain Med 2013; 14: 43-51.

4. Bert F, Gualano MR, Camussi E, Pieve G, Voglino G, Siliquini R. Animal assisted intervention: A systematic review of benefits and risks. Eur J Integr Med 2016; 8: 695-706.

5. Straede CM, Gates GR. Psychological health in a population of Australian cat owners. Anthrozoös 1993; 6: 30-42.

6. Serpell J. Beneficial effects of pet ownership on some aspects of human health and behaviour. J R Soc Med 1991; 84: 717-20.

7. Odendaal JS. Animal-assisted therapy - magic or medicine? J Psychosom Res 2000; 49: 275-80.

8. Parello CAK, Wesley Y, Campbell M. Examining pet attitude in relationship to loneliness and parenthood motivation in pet-owning adults. Health 2014; 6: 598-606.

9. Jacobson KC, Chang L. Associations Between Pet Ownership and Attitudes Toward Pets With Youth Socioemotional Outcomes. Front Psychol 2018; 9: 2304.

10. Amiot CE, Bastian B. Toward a psychology of human-animal relations. Psychol Bull 2015; 141: 6-47.

11. Krause-Parello CA, Kolassa J. Pet Therapy: Enhancing Social and Cardiovascular Wellness in Community Dwelling Older Adults. J Community Health Nurs 2016; 33: 1-10.

12. Min M, Zaw CC. A review on animal-assısted therapy and actıvıtıes for healthcare and teaching of children. Journal of Education and Social Scınces 2016; 5: 40-6.

13. Guthrie MF, PH Marshall, Hendrick SS, Hendrick C, Logue E. Human love styles and attitudes toward pets. Anthrozoös 2018; 31: 41-60.

14. Özen A, Özen R, Yaşar A, Armutak A, Bayrak S, Gezman A, Şeker İ. Attitudes of Turkish veterinary students and educators towards the moral status of animals and species rating. Kafkas Üniv Vet Fak Derg 2009; 15: 111-8.

15. Doğan Ö, Özkul T, Özen A. The attitudes and views of Firat and Uludag University Faculty of Veterinary Medicine students on the subject of dog psychology. Ankara Üniv Vet Fak Derg 2011; 58: 85-91.

16. Sıddıq AB, Erdem Ç, Şanlı Ş. The Scopes of Anthrozoology, the Study of Human-Animal Relationships in Turkey. İnsan ve Toplum Bilimleri Araştırmaları Dergisi 2018; 7: 805-26.

17. Wilson CC, Netting FE, New JC. The pet attitude inventory. Anthrozoös 1987; 1: 76-84.

18. Johnsons TP, Garrity TF, Stallones L. Psychometric evaluation of the Lexington attachment to pets scale (LAPS). Anthrozoös 1992; 5: 160-75.

19. Staats S, Miller D, Carnot MJ, Rada K, Turnes J. The Miller-Rada commitment to pets scale. Anthrozoös 1996; 9: 88-94.

20. Lago D, Kafer R, Delenay M, Connell C. Assessment of favorable attitudes toward pets: Development and preliminary validation of self-report pet relationship scales. Anthrozoös 1988; 1: 240-54.

21. Wilson CC, Netting FE. The status of instrument development in the human-animal interaction field. Anthrozoös 2012; 25: 11-55.

22. Fiocco AJ, Hunse AM. The Buffer Effect of Therapy Dog Exposure on Stress Reactivity in Undergraduate Students. Int J Environ Res Public Health 2017; 14: 707.

23. Dixon-Preylo B, Arikawa H. Comparison of vegetarians and non-vegetarians on pet attitude and empathy. Anthrozoös 2008; 21: 387-95.

24. Williams J, Lawrence A, Muldoon J. Children and their pets: exploring the relationships between pet ownership, pet

attitudes, attachments to pets, and emphaty. Education and Health 2009; 28: 12-5.

25. Al- Fayez G, Awadalla A, Templer DI, Arikawa H. Companion animal attitude and its family pattern in Kuwait. Society and Animals 2003; 11: 17-28.

26. Karaçam Z. Adaptation of Scales to Turkish. Ebelik ve Sağlık Bilimleri Dergisi. 2019; 2: 28-37.

27. Römpke AK. Adaptation of the Pet Attitude Scale to German. Society& Animals 2019; 27: 11-24.

28. ITC. Guidelines for Translating and Adapting Tests (Second Edition). Indernational Journal of Testing 2018; 18: 101-34. Doi: 10.1080/15305058.2017.1398166

29. Templer DI, Arikawa H. The pet attitude scale. In Blazina C, Boyraz G, Shen-Miller D (Eds.)," in The psychology of the human-animal bond: A resource for clinicians and researchers. New York: NY, Springer; 2011.p. 335-59.

30. Cicchetti DV. Guidelines, criteria, and rules of thumb for evaluating normed and standardized assessment instruments in psychology. Psychological Assessment 1994; 6: 284-90.

31. Şencan H. Sosyal ve Davranışsal Ölçümlerde Güvenilirlik ve Geçerlilik. Ankara: Seçkin Yayıncılık; 2005.

32. Burns N, Grove SK. The practice of nursing research appraisal, synthesis, and generation of evidence. St Louis: MO: Saunders 2009.

33. Streiner DL, Kottner J. Recommendations for reporting the results of studies of instrument and scale development and testing. J Adv Nurs 2014; 70: 1970-9.

34. Klem L. Reading and understanding MORE multivariate statistics. Structural equation modeling, Grimm LG, Yarnold PR, Eds., Washington D. C.: American Psychological Association 2000.

35. Alpar R. Çok Değişkenli İstatistiksel Yöntemler (3. Baskı). Ankara: Detay Yayıncılık; 2011.

36. Polit DF, Beck CT. Essentials of nursing research: appraising evidence for nursing practice (7th ed.). Philadelphia, PA: Lippincott Williams & Wilkins; 2010.

37. Akgül A. Tıbbi Araştırmalarda İstatiksel Analiz Teknikleri-SPSS Uygulamaları, Ankara: Emek Ofset Ltd. Şti.; 2005.

Biochemical and Histopathological Investigation of Liver Tissues on High Fat Diet Fed Rats

Yüksek Yağ İçerikli Diyetle Beslenmiş Sıçanların Karaciğer Dokularının Histokimyasal ve Biyokimyasal İncelenmesi

 Tuğba Çelik Samancı[1], Alpaslan Gökçimen[2], Tuncay Kuloğlu[3], Murat Boyacıoğlu[4], Yurdun Kuyucu[5], Sait Polat[5]

[1]Recep Tayyip Erdoğan University Faculty of Medicine, Department of Histology and Embryology, Rize, Turkey
[2]Aydın Adnan Menderes University Faculty of Medicine, Department of Histology and Embryology, Aydın, Turkey
[3]Fırat University Faculty of Medicine, Department of Histology and Embryology, Elazığ, Turkey
[4]Aydın Adnan Menderes University Faculty of Veterinary, Department of Pharmacology and Toxicology, Aydın, Turkey
[5]Çukurova University Faculty of Medicine, Department of Histology and Embryology, Adana, Turkey

Abstract

Objective: Hepatic steatosis is a common cause of liver disease and has become more prevalent recently. This study was conducted to investigate the biochemical and histopathological changes in the liver tissue high fat diet (HFD)-induced steatosis in rats.

Materials and Methods: In this study, 16 male Wistar-albino rats (160±30 g in weight) were used and divided into two groups: The normal fed diet group fed with a standard diet and the HFD group fed with a HFD for 10 weeks. At the end of the experiment, the liver sections and blood from the heart underwent biochemical and histological investigations. The sections obtained from the liver were examined by light and electron-microscopy and the sections stained immunohistochemically to determine the iNOS activity. The antioxidant activities in liver samples and the alanine aminotransferase (ALT), aspartate aminotransferase (AST), triglyceride (TG) levels in serum were measured.

Results: Because of light-microscopy and electron-microscopic investigations, histopathological changes caused the steatosis were observed in the HFD group. The histopathological damage observed in the liver was confirmed biochemically with the increase in the ALT, AST, and TG levels. While the malondialdehyde activity increased, the superoxide dismutase and catalase activities decreased in the HFD group. iNOS enzyme activity increased in the HFD group.

Conclusion: In this study, it was observed that steatosis developed in the liver tissue of rats fed with the HFD at 10 weeks and oxidative damage was triggered with the influence of inflammation and activation of the antioxidant defense system.

Öz

Amaç: Karaciğer yağlanması kronik karaciğer hastalıklarının en yaygın sebeplerinden birisidir ve son yıllarda oldukça artmıştır. Bu çalışmada yüksek yağ içerikli diyetle (HFD) sıçanlarda oluşturulan yağlanma modelinin karaciğer dokularındaki etkilerinin yapısal ve biyokimyasal olarak araştırılması amaçlanmıştır.

Gereç ve Yöntemler: Çalışmada 16 adet Wistar albino cinsi erkek sıçan (160±30 g) kullanıldı. Sıçanlar rasgele iki gruba ayrıldı. Normal beslenen grup standart sıçan yemi ile ve HFD ile 10 hafta boyunca beslendi. Deney sonunda biyokimyasal ve yapısal incelemeler için karaciğer ve kalpten kan örnekleri alındı. Karaciğer

dokusundan elde edilen kesitler ışık mikroskobu ve elektron mikroskobu ile incelendi ve iNOS enzim aktivitesini belirlemek amacıyla immünohistokimyasal olarak boyandı. Karaciğer dokularında malondialdehit (MDA), süperoksit dismutaz (SOD), CAT, glutatyon enzim aktiviteleri ve serum örneklerinde ALT, AST ve trigliserit (TG) seviyeleri ölçüldü.

Bulgular: Işık mikroskobu ve elektron mikroskobik incelemeler sonucunda HFD diyetle beslenen grupta yağlanmaya sebep olan yapısal değişiklikler gözlendi. HFD grupta gözlenen ALT, AST ve TG değerlerindeki artış ile karaciğerde gözlenen yapısal hasar biyokimyasal olarak da doğrulandı. HFD grupta MDA aktivitesi artarken SOD ve CAT aktivitelerinde düşüş gözlendi. HFD grupta iNOS enzim aktivitesi de anlamlı şekilde arttı.

Sonuç: Bu çalışmada 10 hafta boyunca yüksek yağ içerikli diyetle beslenen sıçanların karaciğer dokularında yağlanmanın geliştiği ve bu sürece inflamasyonunda etki etmesiyle birlikte oksidatif hasarın tetiklenip antioksidan savunma sisteminin devreye girdiği gözlendi.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is a pathology that results from excessive accumulation of fat in the liver independent of alcohol intake. NAFLD, with a prevalence of 25%, is one of the most common chronic diseases worldwide, including liver damage that can progress from simple steatosis to steatohepatitis, fibrosis and cirrhosis (1). Pathologically, NAFLD is characterized by excessive hepatic lipotoxicity, oxidative stress and inflammation. Hepatic steatosis, the earliest stage of NAFLD, occurs as a result of accumulation of lipid droplets in the cytoplasm of hepatocytes. The damage caused by lipid deposition increases the sensitivity of the liver to many factors such as pro-inflammatory cytokines, lipid peroxidation, mitochondrial dysfunction and oxidative stress. Oxidative stress triggers structural damage in the liver, causing changes in the antioxidant enzyme activities.

Various animal models have recently been used to understand the of NAFLD mechanism. In the development of NAFLD models, genetically modified animal models such as obese mice (ob/ob) and dyslipidemic mice were used, as well as rodent models fed with diets of different content (2-4). High fat diet (HFD) is the most common in diet induced NAFLD models and this model is the closest model to NAFLD formation in humans. However, the HFD content and duration of feeding with HFD cause differences in NAFLD progression. In studies investigating the effects of therapeutic substances in NAFLD, it is of great importance to monitor the stage from which the NAFLD regresses.

The objective of this study was to investigate the biochemical, immunohistochemical and histopathological as well as the ultrastructural changes that may occur in the liver of male wistar rats fed with a HFD containing 45% fat for 10 weeks.

Materials and Methods

Experimental Design

In our study, 16 Wistar albino male rats (weight 160±30 g) were obtained from the Aydın Adnan Menderes University Laboratory of Experimental Animals (Aydın, Turkey). The research protocol was approved by Aydın Adnan Menderes University Animal Research Ethics Committee (no: 64583101/2014/058, date: 11.06.2014). During the experimental and adaptation processes, the rats were kept alive in optimum conditions (20-22 °C, humidity of 45-65% and 12-hour light, 12-hour dark). After the adaptation period, the rats were randomly divided into 2 groups (n=8). The control group [Normal fed diet (NFD) group] was fed with standard rat feed and the fattening group (HFD group) with a diet of high-fat content (HFD) for 10 weeks. During the experiment, the rats had unlimited access to drinking water and feed. The standard rat chow consumed by the control group was also obtained from Bil-Yem (Ankara, Turkey). The HFD diet, which contains fat (45%), carbohydrate (35%) and protein (20%), consumed by the HFD group, was prepared according to the content used in the experimental model created by Li et al. (5) in 2014. After the 10-week, the rats were sacrificed under anesthesia, the liver tissues were removed for biochemical and histopathological examinations, and blood samples were obtained from the heart tissues. The relative liver weights of rat were calculated using liver weights and final body weights [(liver weight/body weight]×100].

Histopathological Examination

For the light microscopic examinations, the liver tissues were fixed in formalin (10%, 24 h). 5-µm thick sections were taken from the paraffin-embedded tissues with a microtome. The liver sections were stained with Harris hematoxylin-eosin (H-E) (Merck,

Germany) for light microscobic investigation (BX50, Olympus, Japan) and photographed with a camera (DP21, Olympus, Japan).

For electron microscopic examinations, the liver tissues were fixed in glutaraldehyde (5%, 4h). Then, tissue samples washed with a Millonig phosphate buffer. Subsequently, the tissues detected with osmium tetroxide (OsO_4, 1%, 2h) were washed with the Millonig phosphate buffer. After the dehydration step, tissues treated with propylene oxide were embedded in araldite. Semi-thin sections obtained with ultramicrotome (Leica, Germany) were stained with toluidine blue stain. The thin liver sections were stained with lead citrate and uranyl acetate. The liver tissue samples were examined by electron microscopy (Zeiss E. M 10B). The fat content observed in hepatocytes with light microscopy and electron microscopy examinations was 0: (<5%), 1: (5% ~ 33%), 2: (34% ~ 66%) and 3: (>66%) relative to the reference (6).

Immunohistochemical Examination

For immunohistochemical examination, 5-μm sections obtained from paraffin embedded tissues were taken on polylysine slides. After the deparaffinization process, it was passed through graded alcohol series. Ultra-V block (Lab Vision, TA125UB, USA) was applied to the liver tissues treated with hydrogen peroxide (H2O2) to prevent background staining. Subsequently, 60-minutes incubation was performed using inducible nitric oxide synthase (iNOS) (NOS2, diluted 1: 500, Santa Cruz, Europe). The tissues were then treated with secondary antibody (TA-125-AF, Lab Vision Corporation, USA). AEC (TA125AF, Lab Vision Corporation, USA) and dropped on tissues and stained with Mayer's Hematoxylin to be examined. In the evaluation of the immunohistochemical staining, the immunoreactive prevalence (<25%=0.1, 26%-50%=0.4, 51%-75%=0.6, 76%-100%=0.9) and severity (none=0, very low=0.5, low=1, moderate=2, severe=3) were determined based on histoscore (Histoscore=prevalence x severity).

Biochemical Analysis

For biochemical analysis, blood samples were centrifuged for 15 min. The triglyceride (TG), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were analysed in serum samples. The liver samples obtained from the rats were homogenized in IKA Overhead Stirrer Homogenizer brand homogenizer (Yellow line) at 2000 rpm for 1 min. The homogenate was then centrifuged and the supernatant superoxide dismutase (SOD), malondialdehyde (MDA), glutathione peroxidase (GSH) and catalase (CAT) activities were measured (7-10).

Statistical Analysis

Statistical analysis of the data was performed using the SPSS 19.0 Software (IBM, USA), $p<0.05$ was considered significant. The normality of continuous variables was evaluated with the Kolmogorov-Smirnov test. The Student's t-test was used for the normally distributed data and the Mann-Whitney U test was used for the continuous variables that deviated from normality. Descriptive statistics were expressed as mean ± standard deviation for the normally distributed continuous variables, and as median (25-75%) for the skewed parameters.

Results

Rat Body Weights and Relative Liver Weights

During the experiment, the body weights of the rats were measured weekly and using the data obtained, the percentage change in the body weight of the rats was calculated [(final weight - initial weight)/ final weight x 100]. Although there was an increase in the body weight in the HFD group, no significant difference was observed in the percentage of changes in body weight (p=0.674) and the relative liver weight (p=0.782) (Table 1).

Histopathological Analysis

As a result of examining the tissues stained with H-E and toluidine blue under the light microscope,

Table 1. Representation of the rat body weights, relative liver weights and histoscore of iNOS immunoreactivity

Variable	NFD group (n=8)	HFD group (n=8)	p-value
Percentage change in body weight (mean ± SD)	56.31±4.27	65.68±4.82	0.674
Relative Liver Weight (g/100 g) (mean ± SD)	3.69±0.16	3.47±0.17	0.782
iNOS immunreactivity score median (25-75%)	0.30 (0.20-0.40)	0.41 (0.34-0.48)	0.021
NFD: Normal fed diet, HFD: High fat diet, SD: Standard deviation, iNOS: Inducible nitric oxide synthase			

the hepatocytes and normal-appearing sinusoids were observed to be radially arranged around the central vein in the NFD group. Microvesicular and macrovesicular lipid drops were observed in the cytoplasm of hepatocytes around the central vein in the HFD group with H-E staining. Diffuse microvesicular and occasional macrovesicular lipid droplets were seen in the cytoplasm of hepatocytes in semi-thin sections stained with toluidine blue of liver samples obtained from rats belonging to the HFD group. Grade III steatosis was detected in the hepatocytes in liver tissues of rats in the HFD group as a result of examination of liver samples stained with H-E and toluidine blue under the light microscope and scoring of steatosis (Figure 1).

In electron microscopic examinations, the nuclei, and the nucleoli of the hepatic cells in the NFD group and the mitochondria and endoplasmic reticula were of normal size and shape. In electron microscopic examination of liver tissues of rats in the HFD group, macrovesicular and microvesicular lipid droplets were observed in hepatocytes with normal-appearing nuclei and nucleoli contents. In addition, degeneration of the mitochondria in the hepatocyte cytoplasm was observed in the cristae and most of the mitochondria were hypertrophied (Figure 1).

Immunohistochemical Examination

In the immunohistochemical staining performed with iNOS antibody to determine the iNOS enzyme activity, positivity was seen as granules (brown) in the cytoplasm of hepatocytes, particularly around the central vein in regions where the iNOS activity was positive (Figure 1). When the data obtained by scoring iNOS immunohistochemical staining were compared with the NFD group, a significant increase in the number of iNOS positive cells in the HFD group was observed (p=0.021) (Table 1).

Biochemical Results

For biochemical analysis, serum ALT and AST activities increased in the HFD group compared to the NFD group (p=0.001) (Table 2). In addition, the TG levels were significantly increased in the HFD group (p=0.001) (Table 2). Compared to the NFD group, there was an increase in the MDA levels, which is an indicator of lipid peroxidation, in liver tissue samples in the HFD group (p=0.001). Compared to the NFD group, there was a decrease in antioxidant enzyme levels measured in liver tissues in the HFD group,

SOD (p=0.001) and CAT (p=0.005) activities, while no significant difference was observed in the GSH levels (p=0.644) (Table 2).

Discussion

NAFLD is a common cause of chronic liver disease, and its worldwide prevalence continues to increase. This situation triggers studies on the treatment and prevention of NAFLD (11). Experimental animal studies are very important to define the mechanisms that cause the progression of NAFLD to NASH. An
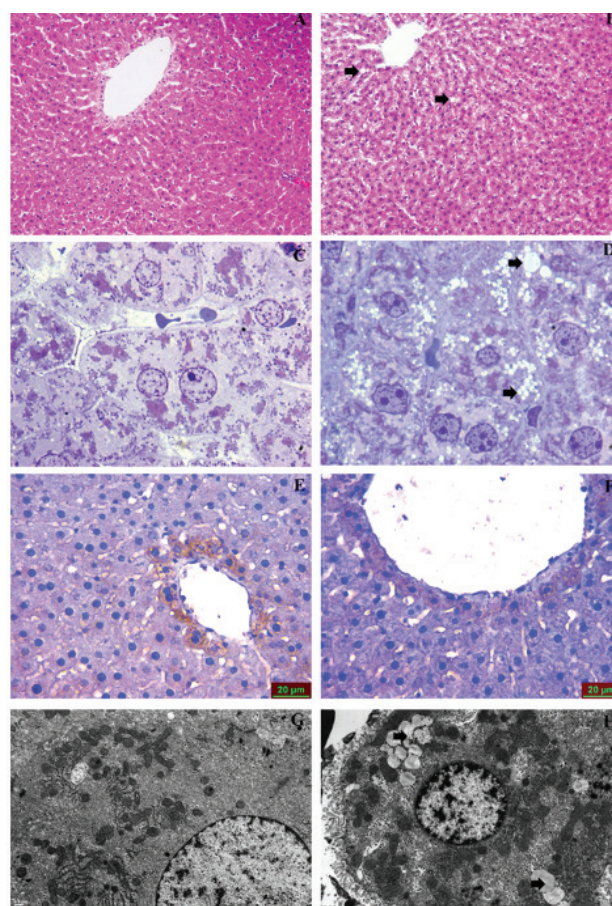
Figure 1. Photographic images of liver tissue from light and electron microdscopic examination. A: NFD group hematoxylin-eosin staining 100x, B: HFD group Hematoxylin eosin staining 100x, C: NFD group toluidine blue staining 1000x, D: HFD group toluidine blue staining 1000x, E: NFD group iNOS immunohistochemical staining 400x, F: HFD group iNOS immunohistochemical staining 400x, G: NFD group electron microscopy image, H: HFD group Electron microscopy image. Arrows: Macro and microvesicular oil droplets
NFD: Normal fed diet, HFD: High fed diet, iNOS: Inducible nitric oxide synthase

Table 2. Measurement of biochemical parameters in liver and blood samples

Variable	NFD group (n=8)	HFD group (n=8)	p-value
ALT (U/L) median (25-75%)	36.37 (35.10-37.20)	49.00 (42.02-54.50)	0.001
AST (U/L) median (25-75%)	105.16 (10402-106.20)	127.01 (125.62-128.39)	0.001
TG (mg/dL) median (25-75%)	74.35 (69.41-83.37)	128.18 (118.85-138.42)	0.001
MDA (nmol/mg) median (25-75%)	28.09 (23.11-30.35)	55.51 (44.51-61.25)	0.001
SOD (U/mg) median (25-75%)	4.14 (3.70-4.72)	2.66 (2.50-2.71)	0.001
CAT (k/mg) median (25-75%)	12.42 (10.17-17.83)	7.81 (6.03-8.82)	0.005
GSH (mg/kg) (mean ± SD)	15.11±2.38	12.02±1.80	0.644

NFD: Normal fed diet, HFD: High fed diet, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, TG: Trygliseride, MDA: Malondialdehyde, SOD: Superoxide dismutaz, CAT: Catalase, GSH: Glutation peroxidase

ideal experimental model would be expected to reflect the etiology, progression, and pathology of human NAFLD (12). HFD is one of the most important models reflecting the real picture of human NAFLD. In the current study, the NAFLD model was created using HFD, which is considered as one of the main causes of NAFLD in humans. We conducted this study as a pilot of a study we designed for HFD-induced fatty liver therapy. Furthermore, we examined the biochemical and histopathological changes in the liver tissue of rats fed with a diet containing 45% fat for 10 weeks.

Experimental models of fatty liver have been created in various ways (12). In our study, fatty liver was created in rats using a diet with high-fat content, which is considered one of the main causes of fatty liver in humans. The fatty patterns vary depending on different lipid contents and different durations. The HFD diet we applied to rats in our study was prepared according to the study by Li et al. (5). The duration of 10 weeks is used in nutrition studies with HFD as the time in which fatty formation is formed and treatment is begun. Nutrition with HFD for 10 weeks is used in studies as the time in which active substances are used to create fat (13).

Although the mechanisms of NAFLD have not been understood yet, previous studies have indicated the most important causes of NAFLD as oxidative stress and increased lipid peroxidation (14). The increase in ROS as a result of feeding with HFD triggers lipid peroxidation in liver tissue by causing steatosis. Increased lipid peroxidation causes changes in antioxidant levels. Lipid peroxidation and oxidative stress due to increased Ros may cause deterioration in liver parenchyma and organelle structures.

As a result of the experiment, no significant increase in the percentage change in body weight was observed in the NFD and HFD groups, consistent with the study of Lieber et al. (15). There was no significant difference in the relative liver weights of the two groups, consistent with the literature (16,17).

Liver tissues stained with H-E were examined under a light microscope and we observed that the liver in the NFD group had a normal histological appearance. In the HFD group, microvesicular and macrovesicular lipid droplets were observed, which constitute the third degree of fat. Various researchers reported that feeding with HFD caused microvesicular and macrovesicular adiposity in rats in their histopathological examinations (18,19).

As a result of electron-microscopic examinations, it was observed that the NFD group had a normal histology, while in the HFD group, mitochondria were more hypertrophic, and deterioration occurred in the mitochondrial cristae. In the study carried out by Wang et al. (20), in the thin structure investigations performed on rats fed with HFD for 12 weeks, they demonstrated that HFD had caused mitochondrial damage comprising swelling in the mitochondrial matrix. Studies have shown that HFD causes histopathological damage in liver tissue, but that there may be differences in the degree of damage depending on the fat content and the duration of application (5).

ALT and AST, which we studied in the serum samples, are biochemical markers of liver damage. The TG content studied in serum samples provides information about the severity of fat accumulation. Consistent with previous studies, the increase in ALT,

AST, and TG content in the HFD group biochemically confirms the histopathological damage (21,22).

ROS, which occurs during the fatty liver process, increases the nitric oxide production with the development of inflammation in the liver and causes a progression in the damage to the tissues (23). When the iNOS enzyme activity was evaluated by immunohistochemical examination, there was an increase in enzyme activity in the HFD group. This increase is considered a marker of inflammation in liver tissue. The ROS increase caused by oxidative stress due to excessive lipid accumulation in liver tissue triggers the activation of the antioxidant defense system. In this context, the level of MDA, which is a lipid peroxidation marker, is an indicator of the increase in lipid peroxidation developing due to fat in the HFD group and is in parallel with the literature (24). While the SOD and CAT activities decreased in the HFD group, there was no difference in the GSH activity between the groups. Echeverría et al. (25) also reported that HFD diet administration caused a decrease in CAT and SOD activities in rats. This is the effect of oxidative stress on antioxidant enzyme activities, which is demonstrated by the increase in iNOS and MDA activities.

Conclusion

In conclusion, this study shows that fatty liver disease induced by the HFD diet (containing 45% fat) causes histopathological and biochemical changes in liver tissue in rats. It was histopathologically and biochemically confirmed that the fatty model created for 10 weeks triggered oxidative stress in liver tissue. Furthermore, this study is a preliminary study for our study in which the treatment study of fatty liver is investigated and is a guide for studies to be conducted on the fatty model.

Ethics

Ethics Committee Approval: The research protocol was approved by Aydın Adnan Menderes University Animal Research Ethics Committee (no: 64583101/2014/058, date: 11.06.2014).

Informed Consent: Since the materials used in this study do not related with any patient, informed patient approval was not required.

Peer-review: Externally and internally peer-reviewed.

References

1. Adams LA, Angulo P, Lindor KD. Nonalcoholic fatty liver disease. CMAJ 2005; 172: 899-905.
2. Koteish A, Diehl AM. Animal models of steatosis. Semin Liver Dis 2001; 21: 89-104.
3. Iizuka K, Bruick RK, Liang G, Horton JD, Uyeda K. Deficiency of carbohydrate response element-binding protein (ChREBP) reduces lipogenesis as well as glycolysis. Proc Natl Acad Sci U S A 2004; 101: 7281-6.
4. Kulinski A, Vance DE, Vance JE. A choline-deficient diet in mice inhibits neither the CDP-choline pathway for phosphatidylcholine synthesis in hepatocytes nor apolipoprotein B secretion. J Biol Chem 2004; 279: 23916-24.
5. Li S, Liao X, Meng F, Wang Y, Sun Z, Guo F, et al. Therapeutic role of ursolic acid on ameliorating hepatic steatosis and improving metabolic disorders in high-fat diet-induced non-alcoholic fatty liver disease rats. PLoS One 2014; 9: e86724.
6. Brunt EM, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. Am J Gastroenterol 1999; 94: 2467-74.
7. Sun Y, Oberley LW, Li Y. A simple method for clinical assay of superoxide dismutase. Clin Chem 1988; 34: 497-500.
8. Aebi H. Catalase in vitro. Methods Enzymol 1984; 105: 121-6.
9. Tietze F. Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione: applications to mammalian blood and other tissues. Anal Biochem 1969; 27: 502-22.
10. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979; 95: 351-8.
11. Sumida Y, Yoneda M. Current and future pharmacological therapies for NAFLD/NASH. J Gastroenterol 2018; 53: 362-76.
12. Jahn D, Kircher S, Hermanns HM, Geier A. Animal models of NAFLD from a hepatologist's point of view. Biochim Biophys Acta Mol Basis Dis. 2019;943-953.
13. Chen XY, Cai CZ, Yu ML, Feng ZM, Zhang YW, Liu PH, et al. LB100 ameliorates nonalcoholic fatty liver disease via the AMPK/Sirt1 pathway. World J Gastroenterol 2019; 25: 6607-18.

14. Friedman SL, Neuschwander-Tetri BA, Rinella M, Sanyal AJ. Mechanisms of NAFLD development and therapeutic strategies. Nat Med 2018; 24: 908-22.

15. Lieber CS, Leo MA, Mak KM, Xu Y, Cao Q, Ren C, et al. Model of nonalcoholic steatohepatitis. Am J Clin Nutr 2004; 79: 502-9.

16. Wu Y, Wang C, Sun H, LeRoith D, Yakar S. High-efficient FLPo deleter mice in C57BL/6J background. PLoS One 2009; 4: e8054.

17. Park HJ, Lee JY, Chung MY, Park YK, Bower AM, Koo SI, et al. Green tea extract suppresses NFκB activation and inflammatory responses in diet-induced obese rats with nonalcoholic steatohepatitis. J Nutr 2012; 142: 57-63.

18. Chen X, Li L, Liu X, Luo R, Liao G, Li L, et al. Oleic acid protects saturated fatty acid mediated lipotoxicity in hepatocytes and rat of non-alcoholic steatohepatitis. Life Sci 2018; 203: 291-304.

19. Gårdebjer EM, Cuffe JSM, Ward LC, Steane S, Anderson ST, Dorey ES, et al. Effects of periconceptional maternal alcohol intake and a postnatal high-fat diet on obesity and liver disease in male and female rat offspring. Am J Physiol Endocrinol Metab 2018; 315: E694-704.

20. Wang Z, Li S, Wang R, Guo L, Xu D, Zhang T, et al. The protective effects of the β3 adrenergic receptor agonist BRL37344 against liver steatosis and inflammation in a rat model of high-fat diet-induced nonalcoholic fatty liver disease (NAFLD). Mol Med 2020; 26: 54.

21. Xie X, Yan D, Li H, Zhu Q, Li J, Fang YP, et al. Enhancement of Adiponectin Ameliorates Nonalcoholic Fatty Liver Disease via Inhibition of FoxO1 in Type I Diabetic Rats. J Diabetes Res 2018; 2018: 6254340.

22. Yang JM, Sun Y, Wang M, Zhang XL, Zhang SJ, Gao YS, et al. Regulatory effect of a Chinese herbal medicine formula on non-alcoholic fatty liver disease. World J Gastroenterol 2019; 25: 5105-19.

23. Ding X, Jian T, Li J, Lv H, Tong B, Li J, et al. Chicoric Acid Ameliorates Nonalcoholic Fatty Liver Disease via the AMPK/Nrf2/NFκB Signaling Pathway and Restores Gut Microbiota in High-Fat-Diet-Fed Mice. Oxid Med Cell Longev 2020; 2020: 9734560.

24. Yi HW, Zhu XX, Huang XL, Lai YZ, Tang Y. Selenium-enriched Bifidobacterium longum protected alcohol and high fat diet induced hepatic injury in mice. Chin J Nat Med 2020; 18: 169-77.

25. Echeverría F, Valenzuela R, Bustamante A, Álvarez D, Ortiz M, Soto-Alarcon SA, et al. Attenuation of High-Fat Diet-Induced Rat Liver Oxidative Stress and Steatosis by Combined Hydroxytyrosol-(HT-) Eicosapentaenoic Acid Supplementation Mainly Relies on HT. Oxid Med Cell Longev 2018; 2018: 5109503.

Original Article / Özgün Araştırma

Basophil and Eosinophil Counts May Give Clues in the Differential Diagnosis of Carotid Artery Disease and Vasculitis: A Comparative Study

Karotid Arter Hastalığı ve Vaskülitin Ayırıcı Tanısında Bazofil ve Eosinofil Sayıları İpuçları Verebilir: Karşılaştırmalı Bir Çalışma

 Ahmet Şair

Aydın Adnan Menderes University Faculty of Medicine, Department of Neurology, Aydın, Turkey

Address for Correspondence/Yazışma Adresi:
Ahmet Şair Ph.D.,
Adnan Menderes University Medicine Faculty,
Department of Neurology, Aydın, Turkey
Phone : +90 444 12 56-2606
E-mail : ahmetsair@gmail.com
ORCID ID: orcid.org/0000-0003-1384-6518

Abstract

Objective: This study aimed to investigate if there is any difference in terms of leukocyte and platelet counts between vasculitis and cerebral thromboembolism.

Materials and Methods: In this cross-sectional study, the records of patients who underwent diagnostic cerebral angiography at Aydın Adnan Menderes University Hospital between January 2017 and December 2020 were recorded retrospectively. One hundred fifty-nine patients were included in this study. One hundred twenty-nine patients have the diagnosis carotid artery disease (CAD group) in varying degrees and 30 patients have the diagnosis of vasculitis (vasculitis group).

Results: Fifty-five (34.6%) patients were women and 104 (65.4%) were men. The median (1st quartile-3rd quartile) age was 67 (56-75). In the atherosclerosis group, 14.7% minimal occlusion (occlusion level: 20-49%), 15.5% moderate occlusion (occlusion level: 50-70%), 69.8% severe occlusion (occlusion level: 75-100%) were detected. The age is significantly higher in the CAD group than in the vasculitis group (p<0.001). Also, eosinophil (p=0.028) and basophil (p<0.001) levels were significantly higher, platelet level was significantly lower in the atherosclerosis group than in the vasculitis group. The comparison is done according to gender. There are significantly higher eosinophil (p=0.007) and basophil (p=0.006) levels in male patients with atherosclerosis than in male patients with vasculitis but not in females.

Conclusion: Compared with vasculitis cases, basophil and eosinophil levels were higher and platelet levels were lower in CAD cases. In cases with suspected CAD, higher eosinophil and basophil levels, and lower thrombocyte levels may be clues in terms of diagnosis and may guide clinicians in early diagnosis.

Öz

Amaç: Bu çalışmanın amacı, vaskülit ve serebral tromboemboli arasında lökosit ve trombosit sayıları açısından fark olup olmadığını araştırmaktır.

Gereç ve Yöntemler: Bu kesitsel çalışmada, Aydın Adnan Menderes Üniversitesi Hastanesi'nde Ocak 2017-Aralık 2020 tarihleri arasında tanısal serebral anjiyografi yapılan hastaların kayıtları geriye dönük olarak kaydedildi. Bu çalışmaya 159 hasta dahil edildi. Yüz yirmi dokuz hastaya değişen derecelerde karotis arter hastalığı (KAH grubu), 30 hastaya vaskülit (vaskülit grubu) tanısı konmuştur.

Bulgular: Hastaların 55'i (%34,6) kadın, 104'ü (%65,4) erkekti. Ateroskleroz grubunda, ortanca (1. çeyrek-3. çeyrek) yaş 67 (56-75) idi. %14,7 minimal oklüzyon

(tıkanma seviyesi: %20 ila %49), %15,5 orta oklüzyon (oklüzyon seviyesi: %50 ila %70) ve %69,8 ciddi oklüzyon (oklüzyon seviyesi: %75 ila %100) tespit edildi. KAH grubunda yaş vaskülit grubuna göre anlamlı olarak daha yüksektir (p<0,001). Ayrıca ateroskleroz grubunda vaskülit grubuna göre eozinofil (p=0,028) ve bazofil (p<0,001) düzeyleri anlamlı olarak yüksek, trombosit düzeyi anlamlı olarak düşüktü. Karşılaştırma cinsiyete göre yapıldı. Aterosklerozlu erkek hastalarda eozinofil (p=0,007) ve bazofil (p=0,006) seviyeleri vaskülitik erkeklere göre anlamlı olarak daha yüksekti, ancak kadınlarda yoktu.

Sonuç: Vaskülit olguları ile karşılaştırıldığında, KAH vakalarında bazofil ve eozinofil seviyeleri daha yüksek ve trombosit seviyeleri daha düşüktü. KAH şüphesi olan olgularda eozinofil ve bazofil düzeylerinin artması, trombosit düzeylerinin düşmesi tanı açısından ipucu olabilir ve klinisyene erken tanıda yol gösterici olabilir.

Introduction

Stroke is a neurological disorder and the second leading cause of death. It is caused by impaired perfusion due to blockage of blood vessels to the brain. The most common reason for stroke is atherosclerosis which is a disease of arteries and characterized by abnormal accumulation of lipids, inflammatory cells, matrix deposits. Inflammation plays an important role in the process of plaque rupture and the formation of thrombosis (1). Similar to atherosclerosis, vasculitis involving large vessels such as giant cell arteritis affect the vascular endothelium, causing vessel narrowing, occlusion, and various clinical findings related to these effects. In particular, ischemia as a result of these diseases may be life-threatening (2). On the other hand, the incidence of atherosclerosis increases with age (3).

Eosinophils and basophils are involved in the pathogenesis of vasculitis (4). Eosinophils are specialized inflammatory cells that routinely measured as part of the complete blood cell count. Brain infarcts due to thromboembolism are said to be associated with the toxicity of eosinophils (5). Eosinophils promote atherosclerosis involving von Willebrand factor (VWF) exposure on endothelial cells and increased atherogenic platelet adhesion to the vessel wall. Once arterial thrombosis is triggered, eosinophils are rapidly recruited to the lesion site in an integrin-dependent manner and intensively interact with platelets resulting in propagation and stabilization of developing thrombi (6). Basophils are a small fraction of leukocytes and secrete heparin and histamine. There is a significant relationship between the low basophil count as well as the basophilic percent in the blood picture with common thrombotic problems namely the deep vein thrombosis (7). However, Nemec et al. (8) found an increase in basophil count with a higher dose of anticoagulant compared to low dose anticoagulant in dogs but this finding didn't reach significance.

In various studies, the use of imaging methods for the differential diagnosis of atherosclerosis and vasculitis has been examined, and it has been emphasized that these two diseases can be differentiated with various imaging methods (9). To the best of our knowledge, there is no study examining various biomarkers and presenting evidence-based results on this subject. The aim of this study was to investigate if there is any difference in terms of leukocyte and platelet counts between vasculitis and cerebral thromboembolism.

Materials and Methods

In this cross-sectional study, the records of patients who underwent diagnostic cerebral angiography at Aydın Adnan Menderes University Hospital between January 2017 and December 2020 were recorded retrospectively. Ethical approval of the study was obtained from the Aydın Adnan Menderes University Ethics Committee (protocol no: 2021/101, date: 10.06.2021).

Study Group

All cases meeting the inclusion criteria were included in the study by examining the cerebral angiographies performed on the relevant dates.

Inclusion criteria:

- Over the age of 18,

- Those with carotid artery disease (CAD) that is diagnosed as narrowing or near complete or complete blockage of vessels due to plaques during digital substraction angiography.

- Patients with small-medium sized vasculitis of central nervous system which is demonstrated by the presence of vessel stenoses or aneurysms during digital substraction angiography.

Exclusion criteria:

- Cases that did not meet the inclusion criteria,

- Presence of active infection, chronic inflammatory disease, severe allergy

Finally, 159 patients were included in this study. One hundred twenty-nine patients have the diagnosis CAD in varying degrees and 30 patients have the diagnosis of vasculitis.

Variables

In our study, the following parameters were examined

- Sociodemographic characteristics (gender, age),
- The result of angiography (vascular occlusion degree),
- Blood sample results (the count of leukocyte, neutrophil, lymphocyte, monocyte, eosinophil, basophil, platelet),
- Monocyte/lymphocyte ratio,
- Platelet/lymphocyte ratio,
- Neutrophil/lymphocyte ratio.

Procedure of the Study

The blood samples of cases who applied to our clinic and underwent cerebral angiography were retrospectively screened.

Statistical Analysis

All analyses were performed on SPSS v21 (SPSS Inc., Chicago, IL, USA). For the normality check, the Shapiro-Wilk test was used. Data are given as (interquartile range; IQR) for continuous variables according to the normality of distribution and frequency (percentage) for categorical variables. Non-normally distributed variables were analyzed with the Mann-Whitney U test. Categorical variables were evaluated using the chi-square tests. Two-tailed p-values of less than 0.05 were considered statistically significant.

Results

Fifty five (34.6%) patients were women and 104 (65.4%) were men. The median (IQR) age was 67 (56-75). In the atherosclerosis group, 14.7% minimal occlusion (occlusion level: 20% to 49%), 15.5% moderate occlusion (occlusion level: 50% to 70%), 69.8% severe occlusion (occlusion level: 75% to 100%) were detected. The age is significantly higher in the CAD group than in the vasculitis group (p<0.001). Also, eosinophil (p=0.028) and basophil (p<0.001) levels were significantly higher, platelet level was significantly lower in the atherosclerosis group than the vasculitis group. The comparison is done according to gender. There are significantly higher eosinophil (p=0.007) and basophil (p=0.006) levels in male patients with atherosclerosis than male patients with vasculitis but not in females (Table 1).

Table 1. Summary of patients' characteristics with regard to groups

	Carotid artery disease (n=129)	Vasculitis (n=30)	p-value
Gender			
Male	87 (67.4%)	17 (56.7%)	0.264
Female	42 (32.6%)	13 (43.63%)	-
Age (year)	68.5 (62-76)	45.5 (38-53)	<0.001
Vascular occlusion (%)	85 (65-95)	0 (0-0)	-
Leukocyte (x10³/mL)	8.75 (7.33-10.23)	8.60 (7.50-11.26)	0.528
Neutrophil (x10³/mL)	5.44 (4.47-7.29)	5.57 (4.46-8.26)	0.644
Lymphocyte (x10³/mL)	2.06 (1.54-2.61)	1.99 (1.44-2.86)	0.681
Monocyte (x10³/mL)	0.57 (0.44-0.72)	0.48 (0.41-0.63)	0.108
Eosinophil (x10³/mL)	0.14 (0.09-0.24)	0.10 (0.04-0.20)	0.028
Basophil (x10³/mL)	0.03 (0.02-0.05)	0.02 (0.02-003)	<0.001
Platelet (x10³/mL)	243 (194-290)	269.5 (247-310)	0.012
Moncyte/lymphocyte	0.26 (0.18-0.39)	0.24 (0.19-0.27)	0.202
Platelet/lymphocyte	121.68 (88.45-167.14)	132.06 (105.44-184.06)	0.509
Neutrophil/lymphocyte	2.92 (1.96-4.22)	3.04 (2.00-3.8)	0.925
Data are given as median (1st quartile-3rd quartile) for continuous variables and as frequency (percentage) for categorical variables			

Discussion

Atherosclerosis and vasculitis, which are diseases with inflammation in large and small vessels, may present with clinically similar symptoms. In this study, in which the clinical features of cases diagnosed with vasculitis and different degrees of CAD were compared, basophil and eosinophil cells levels were significantly higher and platelet levels were significantly lower in CAD cases when compared with vasculitis cases.

For years, eosinophils have been known as cells involved in parasitic infection and allergy. However, today it is emphasized that eosinophils have an important role in immunoregulation. Eosinophils continue to be examined for their specific roles in various studies (10,11). In this study, we determined that CAD cases had significantly higher eosinophil levels when compared to vasculitis cases. There are similar results on this subject in the literature. Madjid et al. (12) suggested that the development of cardiovascular events originating from atherosclerosis in the future can be predicted by the level of eosinophil. Tanaka et al. (13) reported a relationship between the level of eosinophils and the severity of coronary heart disease and vascular stenosis. Similarly, different studies have shown a correlation between eosinophil level and the risk of atherosclerotic disease (14,15). The reason for this relationship has not been fully revealed in the studies. In a study examining the reasons for the association of eosinophil level with atherosclerotic events and the development of thrombosis, Marx et al. (6) showed that an increase in eosinophil level increases the exposure of VWF and platelet adhesion in the endothelium. As a result of their studies, the authors underlined that eosinophil is a promising parameter in terms of atherosclerotic events and thrombosis risk. On the other hand, an increase in eosinophil level was also shown in vasculitis cases (7). There has been no previous study comparing eosinophil values between vasculitis and CAD cases. Therefore, the difference between the groups detected in our study could not be compared with the results of other studies.

Basophils, like eosinophils, are another type of leukocyte that has an important role in the immune system. Various studies have investigated the relationship between basophil level and atherosclerotic diseases and vasculitis. Pizzolo et al. (16) emphasized that basophil level is associated with factor II plasma coagulant activity in coronary artery disease and is important in the development of thrombosis. The relationship between atherosclerosis in the coronary artery and basophil level has also been shown in different studies (17). It has been suggested that basophils may play a role in the increase of extracellular DNA traps, polyphosphate, and platelet-activating factors, which can trigger coagulation and thrombosis. Studies focus on the fact that basophil can be effective in increasing thrombosis and coagulation through these pathways (18,19). Although there are studies on the role of basophil in the pathogenesis of vasculitis, this conclusion could not be reached clearly (20). In our study, basophil level was found to be higher in CAD cases when compared with vasculitis cases. In previous studies, basophil levels were not compared between vasculitis and atherosclerotic diseases. Therefore, we believe that the results of our study are valuable.

In the case of vascular damage, steps that result in platelet adhesion, activation, and aggregation are triggered. When the atherosclerotic vessel is mechanically damaged or the plaque ruptures, the blood encounters thrombogenic substances, and platelets are involved in this pathophysiological process (21). After vascular damage, platelets migrate to the area of endothelial damage and form a hemostatic plaque. The next process depends on the ability of the platelets to bind to the subendothelial matrix, and the rapid shape and biochemical changes of the platelets. Available data on the role of platelets in the development of human atherosclerosis indicate that platelet activation is increased in coronary artery disease, transplant vasculopathy, and CAD (22). It has been reported that platelet activation is associated with increased wall thickness in the carotid artery. In addition, some platelet-derived chemokines and growth factors have been found in atherosclerotic plaques. Antiplatelet drugs are thought to be ineffective in preventing the progression of atherosclerosis (22). It has been shown that the platelet level is significantly reduced in ischemic conditions (23). In addition, it has been emphasized that there may be a relationship between the prognosis of ischemic diseases and the platelet level (24). On the other hand, the relationship between vasculitis and platelet level was also investigated, and

it was found that platelet level may increase in active vasculitis involving small vessels (25). Based on the studies on vasculitis and atherosclerosis (although the platelet level was not compared between vasculitis and CAD cases in previous studies), we can say that we found lower platelet levels in vasculitis cases and higher in CAD cases in accordance with the literature.

This study has many limitations. The single-center nature of the study limits its generalizability. The retrospective collection of data limited the inclusion of additional variables in the study. Many parameters that may affect the presence of atherosclerosis, the presence of vasculitis and routine blood values (including basophils and eosinophils) are not examined. The effects of these parameters at different levels led us to misinterpret our results. Asymptomatic or undiagnosed atherosclerosis or vasculitis in the different vessels of the subjects are not evaluated. Pathologies found in different vessels may have an effect on the results. Additional diseases and drugs used are not recorded. Furthermore, healthy controls were not involved in this study. These situations may led us to interpret the results more negatively or positively. The vasculitis subtype was not recorded and subtype-specific analyzes are not performed. Eosinophil and basophil levels may differ in different vasculitis subtypes. Since the older results of the cases are not evaluated in our study, a cause-and-effect interpretation can not be made. The differences mentioned may occurred before or after the diseases.

Conclusion

Compared with vasculitis cases, basophil and eosinophil levels were higher and platelet levels were lower in CAD cases. In cases with suspected CAD, increased eosinophil and basophil levels, and decreased thrombocyte levels may be clues in terms of diagnosis and may guide clinicians in early diagnosis. In this way, clinicians can reduce the mortality and morbidity rates associated with this life-threatening disease by applying the necessary treatment in the earlier period. By examining more cases and more parameters in future studies, the role of basophil and eosinophil levels in the pathogenesis of CAD can be revealed in detail.

Ethics

Ethics Committee Approval: The present cohort study was designed as a survey and was approved by the Clinical Research Ethics Committee of Aydın Adnan Menderes University (protocol no: 2021/101, date: 10.06.2021).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Financial Disclosure: The author declared that this study received no financial support.

References

1. Bentzon JF, Otsuka F, Virmani R, Falk E. Mechanisms of plaque formation and rupture. Circ Res 2014; 114: 1852-66.

2. Jennette JC. Overview of the 2012 revised International Chapel Hill Consensus Conference nomenclature of vasculitides. Clin Exp Nephrol 2013; 17: 603-6.

3. Head T, Daunert S, Goldschmidt-Clermont PJ. The Aging Risk and Atherosclerosis: A Fresh Look at Arterial Homeostasis. Front Genet 2017; 8: 216.

4. Moyer CF, Jerome WG, Taylor R, Tulli H, Reinisch CL. Autoimmune vasculitis in MRL/Mp-lpr/lpr mice: orthochromatic basophils participate in the development of delayed-type hypersensitivity angiitis. Autoimmunity 1992; 12: 159-65.

5. Sarazin M, Caumes E, Cohen A, Amarenco P. Multiple microembolic borderzone brain infarctions and endomyocardial fibrosis in idiopathic hypereosinophilic syndrome and in Schistosoma mansoni infestation. J Neurol Neurosurg Psychiatry 2004; 75: 305-7.

6. Marx C, Novotny J, Salbeck D, Zellner KR, Nicolai L, Pekayvaz K, et al. Eosinophil-platelet interactions promote atherosclerosis and stabilize thrombosis with eosinophil extracellular traps. Blood 2019; 134: 1859-72.

7. Naser HA. Basophil count as predictor to the deep vein thrombosis and the thrombo-embolization. Muthanna Medical Journal 2018; 5: 95-106.

8. Nemec A, Drobnič–Košorok M, Butinar J. The effect of high anticoagulant k3-edta concentration on complete blood count and white blood cell differential counts in healthy beagle dogs. Slov Vet Res 2005; 42: 65-70.

9. Nienhuis PH, van Praagh GD, Glaudemans AWJM, Brouwer E, Slart RHJA. A Review on the Value of Imaging in Differentiating between Large Vessel Vasculitis and Atherosclerosis. J Pers Med 2021; 11: 236.

10. Liao W, Long H, Chang CC, Lu Q. The Eosinophil in Health and Disease: from Bench to Bedside and Back. Clin Rev Allergy Immunol 2016; 50: 125-39.

11. Hellmark T, Ohlsson S, Pettersson Å, Hansson M, Johansson ACM. Eosinophils in anti-neutrophil cytoplasmic antibody associated vasculitis. BMC Rheumatology 2019; 3: 1-12.

12. Madjid M, Awan I, Willerson JT, Casscells SW. Leukocyte count and coronary heart disease: implications for risk assessment. J Am Coll Cardiol 2004; 44: 1945-56.

13. Tanaka M, Fukui M, Tomiyasu K, Akabame S, Nakano K, Yamasaki M, et al. Eosinophil count is positively correlated with coronary artery calcification. Hypertens Res 2012; 35: 325-8.

14. Niccoli G, Ferrante G, Cosentino N, Conte M, Belloni F, Marino M, et al. Eosinophil cationic protein: A new biomarker of coronary atherosclerosis. Atherosclerosis 2010; 211: 606-11.

15. Niccoli G, Cosentino N. Eosinophils: a new player in coronary atherosclerotic disease. Hypertens Res 2012; 35: 269-71.

16. Pizzolo F, Castagna A, Olivieri O, Girelli D, Friso S, Stefanoni F, et al. Basophil Blood Cell Count Is Associated With Enhanced Factor II Plasma Coagulant Activity and Increased Risk of Mortality in Patients With Stable Coronary Artery Disease: Not Only Neutrophils as Prognostic Marker in Ischemic Heart Disease. J Am Heart Assoc 2021; 10: e018243.

17. Morshed M, Hlushchuk R, Simon D, Walls AF, Obata-Ninomiya K, Karasuyama H, et al. NADPH oxidase-independent formation of extracellular DNA traps by basophils. J Immunol 2014; 192: 5314-23.

18. Yousefi S, Morshed M, Amini P, Stojkov D, Simon D, von Gunten S, et al. Basophils exhibit antibacterial activity through extracellular trap formation. Allergy 2015; 70: 1184-8.

19. Cromheecke JL, Nguyen KT, Huston DP. Emerging role of human basophil biology in health and disease. Curr Allergy Asthma Rep 2014; 14: 408.

20. Badimon L, Padró T, Vilahur G. Atherosclerosis, platelets and thrombosis in acute ischaemic heart disease. Eur Heart J Acute Cardiovasc Care 2012; 1: 60-74.

21. Kaplan ZS, Jackson SP. The role of platelets in atherothrombosis. Hematology Am Soc Hematol Educ Program 2011; 2011: 51-61.

22. Gawaz M, Langer H, May AE. Platelets in inflammation and atherogenesis. J Clin Invest 2005; 115: 3378-84.

23. Ranjith MP, Divya R, Mehta VK, Krishnan MG, KamalRaj R, Kavishwar A. Significance of platelet volume indices and platelet count in ischaemic heart disease. J Clin Pathol 2009; 62: 830-3.

24. Zheng X, Guo D, Peng H, Zhong C, Bu X, Xu T, et al. Platelet counts affect the prognostic value of homocysteine in acute ischemic stroke patients. Atherosclerosis 2019; 285: 163-9.

25. Willeke P, Kümpers P, Schlüter B, Limani A, Becker H, Schotte H. Platelet counts as a biomarker in ANCA-associated vasculitis. Scand J Rheumatol 2015; 44: 302-8.

Original Article / Özgün Araştırma

Evaluation of the Knowledge Level, Behavior, and Attitudes of Obstetrics and Gynecology Specialists on the Relationship Between Periodontal Diseases and Pregnancy Outcomes

Periodontal Hastalıklar ve Hamilelik Sonuçları Arasındaki İlişki Üzerine Kadın Hastalıkları ve Doğum Doktorlarının Bilgi Düzeyi, Davranış ve Tutumlarının Değerlendirilmesi

◉ Gülbahar Ustaoğlu[1], ◉ Ülkü Mete Ural[2], ◉ Tuğçe Paksoy[3], ◉ Handan Ankaralı[4]

[1]Bolu Abant İzzet Baysal University Faculty of Dentistry, Department of Periodontology, Bolu, Turkey
[2]Bolu Abant İzzet Baysal University Faculty of Dentistry, Department of Obstetrics and Gynecology, Bolu, Turkey
[3]İstanbul Atlas University Faculty of Dentistry, Department of Periodontology, İstanbul, Turkey
[4]İstanbul Medeniyet University Faculty of Medicine, Department of Biostatistics and Medical Informatics, İstanbul, Turkey

Abstract

Objective: To investigate the knowledge and awareness level of obstetricians on the relationship between periodontal diseases (PDs) and adverse pregnancy outcomes, and to compare them regarding gender, age, professional experience and institution to identify the pertaining issues requiring improvement.

Materials and Methods: Data were collected by the administration of an online questionnaire. The link was sent to the e-mail addresses of 80 obstetricians (50 female, 30 men). The questionnaire consisted of 23 questions focused on the determination of the personal characteristics, attitudes, and the level of knowledge of obstetricians about the relationship between periodontal diseases and preterm labor.

Results: The presence of gingival disease (GD) was higher in females (p=0.001). Females were found to be more aware and sensitive about the relationship between the periodontal diseases and poor obstetric outcomes (p<0.05). Obstetricians in training and research institutions and obstetricians in the early postgraduate period were more likely to believe that there was a significant relationship between GD and obstetric outcomes (p<0.05).

Conclusion: The knowledge and awareness level of obstetricians on the relationship between PDs and adverse pregnancy outcomes may be influenced by gender, age, professional experience and institution. Active collaboration between periodontologists and obstetricians will provide benefits in monitoring and follow-up of the oral health of pregnant women in terms of prevention of poor obstetric outcomes.

Öz

Amaç: Bu çalışmanın amacı kadın hastalıkları ve doğum uzmanlarının periodontal hastalık ve olumsuz gebelik sonuçları arasındaki ilişki hakkındaki bilgi ve farkındalık seviyelerini araştırmak, cinsiyet, yaş, mesleki deneyim ve çalışılan kurum açısından karşılaştırmak ve iyileştirme gerektiren sorunları tespit etmektir.

Gereç ve Yöntemler: Toplam 80 (50 kadın, 30 erkek) kadın hastalıkları ve doğum uzmanına e-posta yoluyla gönderilen çevrim içi anket uygulanmıştır. Anket, uzmanlarının kişisel özellikleri, periodontal sağlık ile hamilelikte erken doğum, düşük doğum ağırlıklı bebek ve preeklampsi arasındaki ilişki hakkındaki bilgi düzeyleri ve tutumlarını değerlendiren 23 sorudan oluşmaktadır.

Bulgular: Gingival hastalık varlığı kadınlarda daha yüksekti (p=0,001). Periodontal/gingival enflamasyon ve kötü obstetrik sonuçlar arasındaki ilişki konusunda kadınların daha yüksek duyarlılığa sahip olduğu görüldü (p<0,05). Ağız sağlığının gebelik sonuçlarına etki ettiğini düşünen hekimlerin akademik personelde ve mezuniyet sonrası erken dönemde yer alan grupta daha fazla olduğu tespit edildi (p<0,05).

Sonuç: Kadın doğum uzmanlarının periodontal hastalıklar ile olumsuz gebelik sonuçları arasındaki ilişki hakkındaki bilgi ve farkındalık düzeyi cinsiyet, yaş, mesleki deneyim ve çalıştıkları kurumdan etkilenebilir. Periodontologlar ve kadın hastalıkları ve doğum uzmanlarının aktif iş birliği, gebelerin ağız sağlığının sorgulanması, tedavi ve takip için yönlendirilmesi, kötü obstetrik sonuçların önlenmesi açısından fayda sağlayacaktır.

Introduction

Periodontal diseases (PDs) are destructive, chronic, and multifactorial diseases with the primary etiological factor of microbial dental plaques, affecting the supportive tissues of the tooth (1-3). It is a well-recognized fact today that PDs are associated with systemic diseases such as cardiovascular, respiratory system diseases, diabetes, as well as with poor obstetrical outcomes, including premature birth, low birth weight, and pre-eclampsia (4). Increased estrogen and progesterone levels during pregnancy and developing immunosuppression may influence the biological and clinical characteristics of periodontal infections in the presence of microbial dental plaques and may aggravate the response to irritation (5,6). The number of *Prevotella intermedia*, *Bacteroides species*, and *Campylobacter rectus* increases in pregnancy. The increased availability of these pathogens enhances the susceptibility to periodontal damage by interfering with the periodontal microflora (5).

Several epidemiological studies are available on the adverse outcomes of periodontal infections in pregnancy, including abortions, premature birth, very premature birth, low birth weight, very low birth weight, and pre-eclampsia (7-9). Premature birth and low birth weight associated with PD are explained in the literature by the release of inflammatory products into the systemic circulation as a result of the maternal and fetal immunological responses against the hematogenous spread of oral bacteria (5,10). Periodontal disease is critical for both maternal and neonatal well-being, as several study reports support that adverse obstetric outcomes are prevented by treating this situation (11,12). However, pregnant women are not routinely referred to a dental examination during their follow-up (13,14).

The adequate level of knowledge of obstetricians on the association between the complications of pregnancy and dental and PDs is critically important in terms of reducing the negative pregnancy outcomes by questioning and referring the pregnant women to a periodontologist for the symptoms of PD (15). Datas from the studies demonstrate that there is knowledge and awareness of periodontal disease and its potential role as a pregnancy risk factor but suggest limited incorporation of dental care into clinical medical practice (16,17) and the attitudes of these obstetricians were not in agreement with their apparent knowledge regarding PDs and their possible repercussions (18).

Our study aimed to investigate the knowledge and awareness level of obstetricians on the relationship between PD and adverse pregnancy outcomes, to compare them according to age, gender, professional experience, and institution and to identify the pertaining issues requiring improvement.

Materials and Methods

The study protocol was approved by the Bolu Abant İzzet Baysal University Faculty of Medicine, Clinical Researches Ethics Committee (decision no: 2018-71, date: 12.04.2018). This cross-sectional study was conducted to evaluate the awareness on periodontal health during pregnancy and its association to delivery of preterm low birth weight infants by collecting information with an online questionnaire, the link of which was e-mailed to 80 obstetricians in Turkey. One-to-one interviews were made with obstetricians in the National Obstetrics and Gynecology Congress and their e-mail addresses were obtained after their consent. Participants were obstetricians or perinatologists who were dealing with

pregnants only. Specialists focusing on gynecology or general obstetrics were excluded from this study.

The questionnaire was comprised of 23 questions prepared with respect to previously published questionnaires (15-18). The questions were initially translated to Turkish by three lecturers with fluent English language skills. These translated questions were back translated to English by a native English speaker, and the translations which were more consistent with the original version were determined. These steps were taken by the method described by Beaton et al. (19) After checking the Turkish version of the questionnaire in terms of grammar, it was evaluated for validation, and necessary modifications were employed by a pilot study that was conducted on 10% of the total sample size. Those individuals who participated in the pilot study were excluded from the main study to prevent possible bias.

Information was gathered about the personal characteristics, attitudes, and the level of knowledge of obstetricians related to the relationship between PD and preterm labor.

Questions were presented in four sections: Personal data, experiences with oral health, awareness and knowledge of obstetricians on potential changes, which might occur during pregnancy, and on possible risk factors for preterm labor/low birth weight, and Physician behavior towards patients.

When the studies are examined, a power analysis was performed considering the effect of the professional experience in years (≤10 and >10 years) on the knowledge level, which is one of the primary outcomes of the study. Considering a similar study, the sample size was calculated considering Type I errors (0.05), targeted power (0.80), and it is concluded that there should be at least 40 people in each trial group (16).

Statistical Analysis

Descriptive values of the data obtained were expressed as numbers and percentage frequencies and tabulated. The internal consistency of the 10-item scale was evaluated with the Kuder-Richarson-20 (KR-20) coefficient. The relevance of answers to related questions was examined with the Pearson chi-square test or Fisher-Freeman-Halton test by evaluation of the percentage frequencies listed in the tables. The level of statistical significance was accepted as a p-value of less than 0.05. SPSS (version 23) program was used for statistical analyses.

Results

A total of 80 (50 female, 30 men) obstetricians, who were either residents or specialists participated in the study. Awareness of obstetricians on periodontitis and low birth weight premature infants were assessed using a scale, which included ten questions. The internal consistency of this scale was found to be 0.715 and was evaluated to be good.

The presence of PD was higher in females (p=0.001). The number of females answering "yes" to the following questions "May gingival/periodontal inflammation affect the outcomes of pregnancy?; Have your patients ever complained about gum bleeding or swelling or loose tooth during pregnancy?; Is PD a risk factor for premature births and low birth weight?; Can PD be safely treated during pregnancy?" was high (Table 1).

The presence of PDs was highest in the ≤45- year old group (p=0.001). The percentage of participants answering "yes" to the training history for oral health was higher in the >45- year old group (p=0.035). Answering "yes" to the taking care of the oral health of patients was found higher in the >45- year old group (p=0.004) (Table 2).

The number of answers given to the questions related to the time elapsed since the graduation from the medical school, and the number of PD cases treated were higher in dentists who graduated within less than ten years from medical faculty (p=0.010).

The frequency of PDs was highest in the participants with the duration of professional experience of ≤10 years in obstetrics and gynecology practice (p=0.039). The question about the history of gingival treatment was answered "yes" more commonly in obstetricians with an experience of ≤10 years in obstetrics and gynecology (p=0.004). Answering "yes" to the question "may gingival/periodontal inflammation affect the outcomes of pregnancy?" was found to be highest in the group with an experience of ≤10 years in obstetrics and gynecology (p=0.050) (Table 3).

Answering "≤1 year" to the question "When was the last time you visited a dentist for a control examination?" was higher in participants working at a private hospital (p=0.011). The presence of PDs treated was highest in participants working at a university hospital (p=0.016). Answering "yes" to the question "taking care of the oral health of your

Table 1. Comparison of genders in terms of answers to questions						
		Women		Men		p-value
		%	n	n	%	
When was the last time you visited a dentist for a control examination?	≤1 year	24	48.0[a]	21	70.0[b]	0.049
	>1 year	26	52.0[a]	9	30.0[b]	
Do you suffer from a gingival disease?	No	18	36.0[a]	23	76.7[b]	0.001
	Yes	32	64.0[a]	7	23.3[b]	
Have you ever been treated for gingival diseases?	No	32	64.0	23	76.7	0.321
	Yes	18	36.0	7	23.3	
Have you ever been trained for oral health?	No	29	58.0	17	56.7	0.907
	Yes	21	42.0	13	43.3	
Do you take care of the oral health of your patients?	No	21	42.0	13	43.3	0.907
	Yes	29	58.0	17	56.7	
Do you take anamnesis about oral health?	No	24	48.0	19	63.3	0.248
	Yes	26	52.0	11	36.7	
Do you think that pregnancy increases the likelihood of gingival inflammation?	No	1	2.0	2	6.7	0.553
	Yes	49	98.0	28	93.3	
Do you think that gingival/periodontal inflammation may affect the outcomes of pregnancy?	No	4	8.0[a]	7	23.3[b]	0.050
	Yes	46	92.0[a]	23	76.7[b]	
Have your patients ever complained about gum bleeding or swelling or loose tooth during pregnancy?	No	0	0.0[a]	3	10.0[b]	0.049
	Yes	50	100.0[a]	27	90.0[b]	
If your answer to the previous question is "Yes"; Have you advised your patient to visit a dentist?	No	0	0	0	0	-
	Yes	50	100.0	27	100.0	
Do you think that periodontal disease is a risk factor for preterm-low birth?	No	3	6.0[a]	6	20.7[b]	0.048
	Yes	47	94.0[a]	23	79.3[b]	
Do you think that treatment of periodontal diseases during pregnancy can reduce the risk of premature deliveries?	No	3	6.0	4	13.3	0.416
	Yes	47	94.0	26	86.7	
Does gum inflammation cause pre-eclampsia during pregnancy?	No	28	57.1	22	73.3	0.160
	Yes	21	42.9	8	26.7	
Are you aware of the term "pregnancy gingivitis"?	No	6	12.2	6	20.0	0.357
	Yes	43	87.8	24	80.0	
Do you think that progesterone and estrogen play an important role in the etiology of gingivitis during pregnancy?	No	8	16.3	4	13.3	0.719
	Yes	41	83.7	26	86.7	
Do you think that periodontal diseases can be safely treated during pregnancy?	No	4	8.0[a]	7	23.3[b]	0.050
	Yes	46	92.0[a]	23	76.7[b]	
Do you think that dental plaque scaling and root surface planing procedures can be performed to eliminate periodontal disease during pregnancy?	No	12	24.0	7	23.3	0.946
	Yes	38	76.0	23	76.7	
*Significant differences between the ratios in the rows were indicated by the letters, [a,b]Placed next to the ratio. If the ratios in the same row carry a completely different letter, it indicates that the difference between them is statistically significant						

Table 2. Comparison of age groups in terms of answers to questions						
	Age					p-value
	≤45 age		>45 age			
		n	%	n	%	
When was the last time you visited a dentist for a control examination?	≤1 year	35	58.3	10	50.0	0.606
	>1 year	25	41.7	10	50.0	
Do you suffer from a gingival disease?	No	23	38.3[a]	18	90.0[b]	0.001
	Yes	37	61.7[a]	2	10.0[b]	
Have you ever been treated for gingival diseases?	No	38	63.3	17	85.0	0.096
	Yes	22	36.7	3	15.0	
Have you ever been trained for oral health?	No	39	65.0[a]	7	35.0[b]	0.035
	Yes	21	35.0[a]	13	65.0[b]	
Do you take care of the oral health of your patients?	No	31	51.7[a]	3	15.0[b]	0.004
	Yes	29	48.3[a]	17	85.0[b]	
Do you take anamnesis about oral health?	No	35	58.3	8	40.0	0.198
	Yes	25	41.7	12	60.0	
Do you think that pregnancy increases the likelihood of gingival inflammation?	No	3	5.0	0	0.0	0.569
	Yes	57	95.0	20	100.0	
Do you think that gingival/periodontal inflammation may affect the outcomes of pregnancy?	No	8	13.3	3	15.0	0.851
	Yes	52	86.7	17	85.0	
Have your patients ever complained about gum bleeding or swelling or loose tooth during pregnancy?	No	1	1.7	2	10.0	0.153
	Yes	59	98.3	18	90.0	
If your answer to the previous question is "Yes"; Have you advised your patient to visit a dentist?	No	0	0	0	0	-
	Yes	59	100.0	18	100.0	
Do you think that periodontal disease is a risk factor for preterm-low birth?	No	7	11.9	2	10.0	0.821
	Yes	52	88.1	18	90.0	
Do you think that treatment of periodontal diseases during pregnancy can reduce the risk of premature deliveries?	No	5	8.3	2	10.0	0.819
	Yes	55	91.7	18	90.0	
Does gum inflammation cause pre-eclampsia during pregnancy?	No	37	61.7	13	68.4	0.786
	Yes	23	38.3	6	31.6	
Are you aware of the term "pregnancy gingivitis"?	No	7	11.7	5	26.3	0.148
	Yes	53	88.3	14	73.7	
Do you think that progesterone and estrogen play an important role in the etiology of gingivitis during pregnancy?	No	11	18.3	1	5.3	0.275
	Yes	49	81.7	18	94.7	
Do you think that periodontal diseases can be safely treated during pregnancy?	No	10	16.7	1	5.0	0.275
	Yes	50	83.3	19	95.0	
Do you think that dental plaque scaling and root surface planing procedures can be performed to eliminate periodontal disease during pregnancy?	No	17	28.3	2	10.0	0.132
	Yes	43	71.7	18	90.0	

*Significant differences between the ratios in the rows were indicated by the letters, [a,b]Placed next to the ratio. If the ratios in the same row carry a completely different letter, it indicates that the difference between them is statistically significant

Table 3. Comparing the results of professional experience in terms of the response to the questions of obstetricians		Age				p-value
		≤10 year		> 10 year		
			n	%	n	%	
When was the last time you visited a dentist for a control examination?	≤1 year	14	53.8	31	57.4	0.813
	>1 year	12	46.2	23	42.6	
Do you suffer from a gingival disease?	No	9	34.6[a]	32	59.3[b]	0.039
	Yes	17	65.4[a]	22	40.7[b]	
Have you ever been treated for gingival diseases?	No	12	46.2[a]	43	79.6[b]	0.004
	Yes	14	53.8[a]	11	20.4[b]	
Have you ever been trained for oral health?	No	16	61.5	30	55.6	0.638
	Yes	10	38.5	24	44.4	
Do you take care of the oral health of your patients?	No	13	50.0	21	38.9	0.469
	Yes	13	50.0	33	61.1	
Do you take anamnesis about oral health?	No	11	42.3	32	59.3	0.231
	Yes	15	57.7	22	40.7	
Do you think that pregnancy increases the likelihood of gingival inflammation?	No	2	7.7	1	1.9	0.245
	Yes	24	92.3	53	98.1	
Do you think that gingival/periodontal inflammation may affect the outcomes of pregnancy?	No	1	3.8[a]	10	18.5[b]	0.050
	Yes	25	96.2[a]	44	81.5[b]	
Have your patients ever complained about gum bleeding or swelling or loose tooth during pregnancy?	No	0	0.0	3	5.6	0.547
	Yes	26	100.0	51	94.4	
If your answer to the previous question is 'Yes'; Have you advised your patient to visit a dentist?	No	0	0	0	0	-
	Yes	26	100.0	51	100.0	
Do you think that periodontal disease is a risk factor for preterm-low birth?	No	4	16.0	5	9.3	0.453
	Yes	21	84.0	49	90.7	
Do you think that treatment of periodontal diseases during pregnancy can reduce the risk of premature deliveries?	No	2	7.7	5	9.3	0.816
	Yes	24	92.3	49	90.7	
Does gum inflammation cause pre-eclampsia during pregnancy?	No	14	53.8	36	67.9	0.320
	Yes	12	46.2	17	32.1	
Are you aware of the term "pregnancy gingivitis"?	No	6	23.1	6	11.3	0.194
	Yes	20	76.9	47	88.7	
Do you think that progesterone and estrogen play an important role in the etiology of gingivitis during pregnancy?	No	4	15.4	8	15.1	0.973
	Yes	22	84.6	45	84.9	
Do you think that periodontal diseases can be safely treated during pregnancy?	No	6	23.1	5	9.3	0.162
	Yes	20	76.9	49	90.7	
Do you think that dental plaque scaling and root surface planning procedures can be performed to eliminate periodontal disease during pregnancy?	No	9	34.6	10	18.5	0.160
	Yes	17	65.4	44	81.5	

*Significant differences between the ratios in the rows were indicated by the letters, [a,b]Placed next to the ratio. If the ratios in the same row carry a completely different letter, it indicates that the difference between them is statistically significant

patients" was found lower in participants working at public hospitals (p=0.001). Answering "yes" to the taking anamnesis about oral health was found higher in participants working at university hospital compared to those working at a public hospital (p=0.028). Answering "yes" to the question "Does gum inflammation cause pre-eclampsia during pregnancy?" found lower in participants working at private hospitals compared to those working either at public hospitals or university hospitals (p=0.036). The number of participants working either at private hospitals or university hospitals answering "yes" to the following questions was higher "Can PDs be safely treated during pregnancy?; Can dental plaque scaling and root surface planing procedures be performed to eliminate periodontal disease during pregnancy?" (Table 4).

Answering "yes" to the suffering from a gingival disease was found to be higher in specialists and academic personnel (p=0.006). Answering "yes" to the taking care of the oral health of your patients was observed to be the highest in the academic personnel group (p=0.003). Answering "yes" to the taking anamnesis about oral health was lowest in the specialists' group (p=0.003). Answering "yes" to the question, "Can dental plaque scaling and root surface planing procedures be performed to eliminate periodontal disease during pregnancy?" was found to be higher in academic personnel and director groups (p=0.007) (Table 5).

Discussion

A variety of studies have been conducted to evaluate the level of knowledge and behavioral patterns of obstetricians on the relationship between pregnancy and PD (17,18,20). Although the relationship between PDs and poor obstetric outcomes is known, the attitudes and knowledge on this subject are used in a limited manner in clinical medical practice. In our study, we found that among the participants, who have taken an anamnesis about the oral health of the patients were higher in academic personnel and >45 age, and who thought that dental procedures could be applied to eliminate periodontal disease during pregnancy were higher in academic personnel.

Fifty of the 80 participants in our study were female. Similar to our study, the proportion of females was between 40% and 60% in other studies (16,18).

75% of the participants in our study were in the ≤45 age, similar to the results of studies conducted in India and France (16,21).

When the experiences of the participants as specialists were categorized into 10-year intervals, it was observed that 32.5% of obstetricians had professional experience of 10 years or less. The study conducted in France reported that 39.5% of the participants had a professional experience of 10 years or less (16). The 48.75% of the participants reported that they had suffered from PD, however only 31.25% of them reported that they had received treatment. Cohen et al. (16) observed similar results to those of our study, reporting that 31.2% of obstetricians had a diagnosis of PD and 22.7% of them received treatment. As these findings demonstrated that treatment of PD could be neglected even in highly educated obstetricians, activities to raise awareness, and increase the level of knowledge should target all subgroups in the society.

According to our data, 57.5% of the participants reported that they did not receive any training on oral health. In this series, 53.75% of the participants reported that they did not take oral health history from their patients. Furthermore, 42.5% of the participants reported that they did not take care of the oral health of their patients. Other studies conducted in various countries reported lower rates as 26.3% in France and 49% in the United States (16,22). On the other hand, 96.25% of the participants in our study reported that pregnancy aggravated gingival inflammation, and 86.25% of them reported that PDs influenced pregnancy outcomes. Similar rates of impingement on pregnancy outcomes were reported by obstetricians from France and the United States as 84% and 74.7%, respectively (16,22). Despite the lower rates of participants receiving training on oral care and the lower rates of their taking care of oral health of their patients, their levels of knowledge were higher. This can be explained by the fact that theoretical knowledge has not been applied to clinical practice. Moreover, the percentage of obstetricians aged 45 years and over was higher in regards to being interested in the oral health of patients and in regards to taking anamnesis on oral health. A study in the literature reported that this situation might be related to a better level of knowledge and experience of obstetricians in the 45-50 years age group (17).

Table 4. Comparison of the results collected from participants working in different types of institutions								p-value
		Public hospital		University hospital		Private hospital		
		n	%	n	%	n	%	
When was the last time you visited a dentist for a control examination?	≤1 year	9	42.9[a]	14	45.2[a]	22	78.6[b]	0.011
	>1 year	12	57.1[a]	17	54.8[a]	6	21.4[b]	
Do you suffer from a gingival disease?	No	15	71.4[a]	10	32.3[b]	16	57.1[ab]	0.016
	Yes	6	28.6[a]	21	67.7[b]	12	42.9[ab]	
Have you ever been treated for gingival diseases?	No	16	76.2	18	58.1	21	75.0	0.288
	Yes	5	23.8	13	41.9	7	25.0	
Have you ever been trained for oral health?	No	13	61.9	18	58.1	15	53.6	0.877
	Yes	8	38.1	13	41.9	13	46.4	
Do you take care of the oral health of your patients?	No	17	81.0[a]	7	22.6[b]	10	35.7[b]	0.001
	Yes	4	19.0[a]	24	77.4[b]	18	64.3[b]	
Do you take anamnesis about oral health?	No	15	71.4[a]	11	35.5[b]	17	60.7[ab]	0.028
	Yes	6	28.6[a]	20	64.5[b]	11	39.3[ab]	
Do you think that pregnancy increases the likelihood of gingival inflammation?	No	0	0.0	3	9.7	0	0.0	0.113
	Yes	21	100.0	28	90.3	28	100.0	
Do you think that gingival/periodontal inflammation may affect the outcomes of pregnancy?	No	5	23.8	4	12.9	2	7.1	0.248
	Yes	16	76.2	27	87.1	26	92.9	
Have your patients ever complained about gum bleeding or swelling or loose tooth during pregnancy?	No	1	4.8	2	6.5	0	0.0	0.472
	Yes	20	95.2	29	93.5	28	100.0	
If your answer to the previous question is "Yes"; Have you advised your patient to visit the dentist?	No	0	0	0	0	0	0	-
	Yes	20	100.0	29	100.0	28	100.0	
Do you think that periodontal disease is a risk factor for preterm-low birth?	No	3	15.0	4	12.9	2	7.1	0.740
	Yes	17	85.0	27	87.1	26	92.9	
Do you think that treatment of periodontal diseases during pregnancy can reduce the risk of premature deliveries?	No	2	9.5	3	9.7	2	7.1	0.933
	Yes	19	90.5	28	90.3	26	92.9	
Does gum inflammation cause pre-eclampsia during pregnancy?	No	11	55.0[a]	16	51.6[a]	23	82.1[b]	0.036
	Yes	9	45.0[a]	15	48.4[a]	5	17.9[b]	
Are you aware of the term "pregnancy gingivitis"?	No	3	15.0	4	12.9	5	17.9	0.923
	Yes	17	85.0	27	87.1	23	82.1	
Do you think that progesterone and estrogen play an important role in the etiology of gingivitis during pregnancy?	No	3	15.0	4	12.9	5	17.9	0.923
	Yes	17	85.0	27	87.1	23	82.1	
Do you think that periodontal diseases can be safely treated during pregnancy?	No	8	38.1[a]	3	9.7[b]	0	0.0[b]	0.001
	Yes	13	61.9[a]	28	90.3[b]	28	100.0[b]	
Do you think that dental plaque scaling and root surface planing procedures can be performed to eliminate periodontal disease during pregnancy?	No	9	42.9[a]	5	16.1[b]	5	17.9[b]	0.050
	Yes	12	57.1[a]	26	83.9[b]	23	82.1[b]	

*Significant differences between the ratios in the rows were indicated by the letters, [a,b]Placed next to the ratio. If the ratios in the same row carry a completely different letter, it indicates that the difference between them is statistically significant

Table 5. Comparison of the answers to the questions according to academic position of the responders								
		Current position						
		Academic personnel		Director		Specialist		p-value
		n	%	n	%	n	%	
When was the last time you visited a dentist for a control examination?	≤1 year	17	51.5	1	20	27	64.3	0.171
	>1 year	16	48.5	4	80	15	35.7	
Do you suffer from a gingival disease?	No	11	33.3[a]	5	100[c]	25	59.5[ab]	0.006
	Yes	22	66.7[a]	0	0[c]	17	40.5[ab]	
Have you ever been treated for gingival diseases ?	No	19	57.6[a]	5	100[b]	31	73.8[ab]	0.104
	Yes	14	42.4[a]	0	0[b]	11	26.2[ab]	
Have you ever been trained for oral health?	No	17	51.5	2	40	27	64.3	0.417
	Yes	16	48.5	3	60	15	35.7	
Do you take care of the oral health of your patients?	No	7	21.2[a]	2	40[ab]	25	59.5[b]	0.003
	Yes	26	78.8[a]	3	60[ab]	17	40.5[b]	
Do you take anamnesis about oral health?	No	11	33.3[a]	2	40[ab]	30	71.4[b]	0.003
	Yes	22	66.7[a]	3	60[ab]	12	28.6[b]	
Do you think that pregnancy increases the likelihood of gingival inflammation?	No	3	9.1	0	0	0	0	0.161
	Yes	30	90.9	5	100	42	100	
Do you think that gingival/periodontal inflammation may affect the outcomes of pregnancy?	No	4	12.1	2	40	5	11.9	0.253
	Yes	29	87.9	3	60	37	88.1	
Have your patients ever complained about gum bleeding or swelling or loose tooth during pregnancy?	No	2	6.1	0	0	1	2.4	0.659
	Yes	31	93.9	5	100	41	97.6	
If your answer to the previous question is "Yes"; Have you advised your patient to visit the dentist?	No	0	0	0	0	0	0	-
	Yes	31	100	5	100	41	100	
Do you think that periodontal disease is a risk factor for preterm-low birth?	No	4	12.1	0	0	5	12.2	0.709
	Yes	29	87.9	5	100	36	87.8	
Do you think that treatment of periodontal diseases during pregnancy can reduce the risk of premature deliveries?	No	3	9.1	0	0	4	9.5	0.773
	Yes	30	90.9	5	100	38	90.5	
Does gum inflammation cause pre-eclampsia during pregnancy?	No	20	60.6	2	50	28	66.7	0.682
	Yes	13	39.4	2	50	14	33.3	
Are you aware of the term "pregnancy gingivitis"?	No	4	12.1	1	25.0	7	16.7	0.577
	Yes	29	87.9	3	75.0	35	83.3	
Do you think that progesterone and estrogen play an important role in the etiology of gingivitis during pregnancy?	No	6	18.2	0	0	6	14.3	0.876
	Yes	27	81.8	4	100	36	85.7	
Do you think that periodontal diseases can be safely treated during pregnancy?	No	3	9.1	0	0	8	19.0	0.424
	Yes	30	90.9	5	100	34	81.0	
Do you think that dental plaque scaling and root surface planning procedures can be performed to eliminate periodontal disease during pregnancy?	No	3	9.1[a]	0	0[a]	16	38.1[ab]	0.007
	Yes	30	90.9[a]	5	100[a]	26	61.9[ab]	

*Significant differences between the ratios in the rows were indicated by the letters, [a,b]Placed next to the ratio. If the ratios in the same row carry a completely different letter, it indicates that the difference between them is statistically significant

The majority of participants considered PD as a risk factor for premature birth and low birth weight and thought that treatment of PD in pregnancy could reduce the risk of premature births, and 36.25% of them reported that gingival inflammation could lead to pre-eclampsia. In a study conducted by Shah et al. (17) 87.9% of study participants reported that PDs posed a risk for low birth. The majority of obstetricians was aware of the term pregnancy gingivitis and reported that progesterone and estrogen levels in pregnancy cause gingivitis. In the literature, as current information about the effects of pregnancy on gingival inflammation, some studies concluded that increased estrogen and progesterone levels in pregnancy change subgingival microbiota and immunologic physiological mediators in periodontal tissue (23,24).

86.25% of the participants thought that PD could be safely treated and 76.25% of them thought that dental plaque scaling and root planning were possible during pregnancy. This difference can be explained by the fact that obstetricians may not have an adequate level of knowledge about the treatment procedures of PD. Similar to our results, several investigators reported that 84.6-97.4% of the obstetricians considered that dental/gingival treatments were applicable during pregnancy (16,25).

Conclusion

This study revealed that updating the periodontal disease and pregnancy information, the institution where the participants work, and their professional experiences affect the awareness of the obstetricians on the dental health of their patients. We think that conducting joint workshops and establishment of health care units enabling the active collaboration of periodontologists and obstetricians will provide benefits in monitoring oral health of pregnant women in terms of improving the dental approach and referral rates of obstetricians. Furthermore, questioning and referring pregnant women for the signs and symptoms of PD during routine pregnancy monitoring will reduce the risk of premature births and delivery of low birth weight infants, as well as decreasing the rates of perinatal mortality and morbidity, which will contribute to the national economy.

Ethics

Ethics Committee Approval: The present cohort study was designed as a survey and was approved by the Clinical Research Ethics Committee of Bolu Abant İzzet Baysal University (decision no: 2018-71, date: 12.04.2018).

Informed Consent: One-to-one interviews were made with obstetricians in the National Obstetrics and Gynecology Congress and their e-mail addresses were obtained after their consent.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: G.U., Ü.M.U., T.P., H.A., Concept: G.U., Ü.M.U., T.P., H.A., Design: G.U., Ü.M.U., T.P., H.A., Data Collection or Processing: G.U., Ü.M.U., T.P., H.A., Analysis or Interpretation: G.U., Ü.M.U., T.P., H.A., Literature Search: G.U., Ü.M.U., T.P., H.A., Writing: G.U., Ü.M.U., T.P., H.A.

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

Financial Disclosure: The authors declared that this study received no financial support.

References

1. Pihlstrom BL, Michalowicz BS, Johnson NW. Periodontal diseases. Lancet 2005; 366: 1809-20.

2. Carrizales-Sepúlveda EF, Ordaz-Farías A, Vera-Pineda R, Flores-Ramírez R. Periodontal Disease, Systemic Inflammation and the Risk of Cardiovascular Disease. Heart Lung Circ 2018; 27: 1327-34.

3. Puertas A, Magan-Fernandez A, Blanc V, Revelles L, O'Valle F, Pozo E, et al. Association of periodontitis with preterm birth and low birth weight: a comprehensive review. J Matern Fetal Neonatal Med 2018; 31: 597-602.

4. Igari K, Kudo T, Toyofuku T, Inoue Y, Iwai T. Association between periodontitis and the development of systemic diseases. Oral Biology and Dentistry 2014; 2: 4.

5. Armitage GC. Bi-directional relationship between pregnancy and periodontal disease. Periodontol 2000 2013; 61: 160-76.

6. Figuero E, Carrillo-de-Albornoz A, Martín C, Tobías A, Herrera D. Effect of pregnancy on gingival inflammation in systemically healthy women: a systematic review. J Clin Periodontol 2013; 40: 457-73.

7. Jarjoura K, Devine PC, Perez-Delboy A, Herrera-Abreu M, D'Alton M, Papapanou PN. Markers of periodontal infection and preterm birth. Am J Obstet Gynecol 2005; 192: 513-9.

8. Toygar HU, Seydaoglu G, Kurklu S, Guzeldemir E, Arpak N. Periodontal health and adverse pregnancy outcome in 3,576 Turkish women. J Periodontol 2007; 78: 2081-94.

9. Agueda A, Ramón JM, Manau C, Guerrero A, Echeverría JJ. Periodontal disease as a risk factor for adverse pregnancy outcomes: a prospective cohort study. J Clin Periodontol 2008; 35: 16-22.

10. Wiener RC, Wiener-Pla R. Literacy, pregnancy and potential oral health changes: the Internet and readability levels. Matern Child Health J 2014; 18: 657-62.

11. Gazolla CM, Ribeiro A, Moysés MR, Oliveira LA, Pereira LJ, Sallum AW. Evaluation of the incidence of preterm low birth weight in patients undergoing periodontal therapy. J Periodontol 2007; 78: 842-8.

12. Tarannum F, Faizuddin M. Effect of periodontal therapy on pregnancy outcome in women affected by periodontitis. J Periodontol 2007; 78: 2095-103.

13. Boggess KA, Urlaub DM, Massey KE, Moos MK, Matheson MB, Lorenz C. Oral hygiene practices and dental service utilization among pregnant women. J Am Dent Assoc 2010; 141: 553-61.

14. Vergnes JN, Pastor-Harper D, Constantin D, Bedos C, Kaminski M, Nabet C, et al. Perceived oral health and use of dental services during pregnancy: the MaterniDent study. Sante publique 2013; 25: 281-92.

15. Strafford KE, Shellhaas C, Hade EM. Provider and patient perceptions about dental care during pregnancy. J Matern Fetal Neonatal Med 2008; 21: 63-71.

16. Cohen L, Schaeffer M, Davideau JL, Tenenbaum H, Huck O. Obstetric knowledge, attitude, and behavior concerning periodontal diseases and treatment needs in pregnancy: influencing factors in France. J Periodontol 2015; 86: 398-405.

17. Shah HG, Ajithkrishnan C, Sodani V, Chaudhary NJ. Knowledge, attitude and practices among Gynecologists regarding Oral Health of expectant mothers of Vadodara City, Gujarat. Int J Health Sci (Qassim) 2013; 7: 136-40.

18. Rocha JM, Chaves VR, Urbanetz AA, Baldissera Rdos S, Rosing CK. Obstetricians' knowledge of periodontal disease as a potential risk factor for preterm delivery and low birth weight. Braz Oral Res2011; 25: 248-54.

19. Beaton DE, Bombardier C, Guillemin F, Ferraz MB. Guidelines for the process of cross-cultural adaptation of self-report measures. Spine (Phila Pa 1976) 2000; 25: 3186-91.

20. Rahman G, Asa'ad F, Baseer MA. Periodontal health awareness among gynecologists in Riyadh, Saudi Arabia. J Int Soc Prev Community Dent 2015; 5: 211-7.

21. Nutalapati R, Ramisetti A, Mutthineni RB, Jampani ND, Kasagani SK. Awareness of association between periodontitis and PLBW among selected population of practising gynecologists in Andhra Pradesh. Indian J Dent Res 2011; 22: 735.

22. Morgan MA, Crall J, Goldenberg RL, Schulkin J. Oral health during pregnancy. The journal of maternal-fetal & neonatal medicine 2009; 22: 733-9.

23. Carrillo-de-Albornoz A, Figuero E, Herrera D, Bascones-Martínez A. Gingival changes during pregnancy: II. Influence of hormonal variations on the subgingival biofilm. J Clin Periodontol 2010; 37: 230-40.

24. Wu M, Chen SW, Su WL, Zhu HY, Ouyang SY, Cao YT, et al. Sex Hormones Enhance Gingival Inflammation without Affecting IL-1β and TNF-α in Periodontally Healthy Women during Pregnancy. Mediators Inflamm 2016; 2016: 4897890.

25. Tarannum F, Prasad S; Muzammil, Vivekananda L, Jayanthi D, Faizuddin M. Awareness of the association between periodontal disease and pre-term births among general dentists, general medical practitioners and gynecologists. Indian J Public Health 2013; 57: 92-5.

Original Article / Özgün Araştırma

Psychological Effects of COVID-19 Pandemic in Dental Healthcare Workers

COVID-19 Pandemisinin Diş Sağlığı Çalışanlarının Psikolojileri Üzerine Etkileri

🆔 Gözde Kandemir Demirci[1], 🆔 Mustafa Melih Bilgi[2], 🆔 Esin Erdoğan[2], 🆔 İlknur Kaşıkçı Bilgi[3], 🆔 Mehmet Kemal Çalışkan[1]

[1]Ege University Faculty of Dentistry, Department of Endodontology, İzmir, Turkey
[2]University of Health Sciences Turkey, İzmir Bozyaka Training and Research Hospital, Clinic of Psychiatry, İzmir, Turkey
[3]Bornova Oral and Dental Health Center, İzmir, Turkey

Address for Correspondence/Yazışma Adresi:
Gözde Kandemir Demirci MD,
Ege University Faculty of Dentistry,
Department of Endodontology, İzmir, Turkey
Phone : +90 232 311 46 08
E-mail : dt.gozdekandemir@hotmail.com
ORCID ID: orcid.org/0000-0001-7327-1010

Abstract

Objective: This study aimed to evaluate the mental health status of Dental Health Care Workers (DHCWs) in a dental emergency university clinic treating urgent patients during the pandemic.

Materials and Methods: A hundred fifteen participants were selected from DHCWs who actively worked during the pandemic in a Dental Emergency Clinic of a university. Depression, anxiety and stress levels of participants were measured with the Depression-Anxiety-Stress Scale and their insomnia levels were assessed with the Insomnia Severity Index. Correlations between independent continuous and dependent variables tested with Spearman test. Mann-Whitney U and Kruskal-Wallis tests were used used to evaluate possible effects of independent variables. The psychological data of the aerosol-generating treatment group were compared to the rest of the participants using Mann-Whitney U tests. In all tests $\alpha=0.05$ significance level was set.

Results: The rates of DHCWs scored above the cut-off points were 54% for depression, 40% for anxiety, 36% for stress and 40% for insomnia. Feeling negative emotions before the pandemic significantly interacted with all psychometric measurements. Younger age, feeling anxious about changing working conditions and/or obtaining personal protective equipment was correlated positively with stress points (p=0.035, p=0.008, p=0.007, respectively). A significant percentage of DHCWs presented high scores on depression, anxiety, stress and insomnia in this study.

Conclusion: The authorities and healthcare executives must show programmed leadership and support for DHCWs during the COVID-19 outbreak. The integration of programs developed to mitigate stress among DHCWs recommended during the COVID-19 pandemic.

Öz

Amaç: Bu çalışmanın amacı, COVID-19 pandemisi sırasında acil hastaları tedavi eden bir diş hekimliği acil üniversite kliniğindeki Diş Sağlığı Çalışanlarının (DSÇ) ruh sağlığı durumunu değerlendirmektir.

Gereç ve Yöntemler: Bir üniversitenin acil diş kliniğinde pandemi sırasında aktif olarak çalışan DSÇ'lerden 115 katılımcı seçildi. Katılımcıların depresyon, anksiyete ve stres düzeyleri Depresyon-Anksiyete-Stres Ölçeği ile, uykusuzluk düzeyleri ise uykusuzluk şiddet indeksi ile değerlendirildi. Bağımsız sürekli ve bağımlı değişkenler

arasındaki korelasyonlar Spearman testi ile test edildi. Bağımsız değişkenlerin olası etkilerini değerlendirmek için Mann–Whitney U ve Kruskal–Wallis testleri kullanıldı. Aerosol oluşturan tedaviler grubunun psikolojik verileri, Mann–Whitney U testi kullanılarak diğer katılımcılarla karşılaştırıldı. Tüm testlerde α=0,05 anlamlılık düzeyi kabul edilmektedir.
Bulgular: Kesme puanının üzerinde puanlanan DSÇ'lerin oranı depresyon için %54, anksiyete için %40, stres için %36 ve uykusuzluk için %40 idi. Pandemiden önce olumsuz duygular hissetmek, tüm psikometrik ölçümlerle önemli ölçüde etkileşime girdi. Yaşın küçük olması, çalışma koşullarının değişmesi ve/veya kişisel koruyucu ekipman edinme konusunda endişeli olması stres puanları ile pozitif yönde ilişkili bulunmuştur (sırasıyla p=0,035, p=0,008, p=0,007). DHCW'lerin önemli yüzdeleri bu çalışmada depresyon, anksiyete, stres ve uykusuzluk puanlarında yüksek puan aldı.
Sonuç: Yetkililerin ve sağlık yöneticilerinin COVID–19 salgını sırasında DSÇ'lere programlı liderlik ve destek göstermeleri gerektiği sonucuna vardık. COVID–19 salgını sırasında DSÇ'ler arasında stresi azaltmak için geliştirilen programların entegrasyonu önerilmektedir.

Introduction

In late 2019, a novel coronavirus severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) which is an enveloped RNA virus that is contagious among humans was reported in China (1). SARS-CoV-2 is a beta-coronavirus belonging to the important "coronavirus" family (2). Two coronaviruses have caused severe epidemics in humans: the SARS-CoV occurred in China in 2002-2003 and the Middle East Respiratory Syndrome-Coronavirus (MERS-CoV) appeared in Saudi Arabia in 2012 (3,4). World Health Organization announced the coronavirus disease-2019 (COVID-19) as a pandemic on 11 March 2020.

Transmission of the virus is possible by direct contact with an area contaminated by saliva droplets or salivary secretions that are expelled during speech, sneezing or coughing attacks (5). SARS-CoV-2 virus survives on the dry inert surfaces only a few hours (6), but may live several days in aqueous surroundings (7). SARS-CoV-2 enters the body throughout the mucous membranes (oral, nasal or even conjunctival ocular) or the skin (through a wound) (8). It has been reported that virus shedding can also happen in the absence of clinical symptoms (9). Although the measures taken in all dental offices seem sufficient, dentists like whole other healthcare staff may be exposed to COVID-19 unintendedly due to asymptomatic and pre-symptomatic transmission of SARS-CoV-2 (9,10).

Dental health care workers (DHCWs) had the greatest COVID-19 risk of any other profession due to their close contact with patients (11). Dental procedures involve the use of aerosol-generating devices, in addition to working in close proximity with the oral mucosa and secretions. The high risk of COVID-19 cross infection can occur through inhalation of aerosol and droplets from infected persons or by direct interaction with mucous membranes, oral fluids and contaminated instruments and surfaces (12).

Additionally, it was reported that the higher expression of key receptor (angiotensin-converting enzyme 2) for COVID-19 in minor salivary glands than in lungs and salivary glands might present a reservoir for COVID-19 asymptomatic infection (13). The positive rate of SARS-CoV-2 in the saliva of patients can reach up to 100%, and live virus can cultivate in saliva samples (13,14), therefore the strong potential infectivity of saliva should be considered (14). All these specific conditions and high-risk factors make DHCWs more vulnerable to the SARS-CoV-2 infection.

Hitherto, many guidelines, reviews, articles and editorials including urgent dental treatment protocols for prevention or controlling the spread of SARS-CoV-2 were published (15,16). However, the number of confirmed and possible cases has continued to increase and deaths of health care workers (HCWs) during pandemic were reported. On the other hand, the workload and work pressure on DHCWs have also increased. Due to the risk of exposure to the virus, DHCWs fear of being infected and spreading the virus to family, friends or colleagues is increasing (17). It was reported that the increased level of depression, anxiety, insomnia and stress symptoms among medical staffs worked in epidemic prevention and control were as high as 50.7%, 44.7%, 36.1% and 73.4%, respectively (18). In March 2020, when coronavirus lockdowns and stay at home orders were enured to slow the spread of the COVID-19 outbreak, academic dental institutions also had to take measures quickly against the rapidly developing crisis in Turkey. Routine dental procedures were cancelled and only urgent dental treatment procedures were performed in order to prevent the transmission of COVID-19 to the community. Dental and oral surgery treatments

were limited to non-aerosol-generating procedures to decrease the risks of transmission of SARS-CoV-2. More specifically, the usage of dental drills and ultrasonic instruments were largely reduced and personal protective equipment (PPE) were provided to dentists and dental-staff working in the pandemic clinics. The aim of this study was to evaluate mental health status of DHCWs in Dental Emergency Clinic of a University in Turkey treating urgent dental patients during the COVID-19 outbreak. Primarily, depression, anxiety, stress and insomnia levels of all DHCWs' were measured. As a second outcome, the psychological characteristics of the dentists working on aerosol-generating treatments (endodontists and pedodontists) and their assisting dental staffs were compared to those of the other dental specialists in practice.

Materials and Methods

Participant Selection

The study protocol was reviewed and approved by the Local Ethical Committee (no: 65234543-050.06.04, date: 29.06.2020). All participants were selected from DHCWs who actively worked during the pandemic in a Dental Emergency Clinic of a university in Turkey, between 16th March and 31th May 2020. They were informed about the study in written form and only the individuals who signed the consent form were included. Face-to-face interviews were conducted with 115 participants by the same researcher during the pandemic. Study sample consists of 85 dentists (postgraduate students and post-master degree academics involved in clinical practice) and 30 dental staffs. Fifty-three participants in the whole group worked on dental emergency treatments involving the use of aerosol-generating devices. We reached nearly all target population, but only five dental staffs refused our invitation to the study. Cases with moderate or higher severity of psychological symptoms were referred to related branches for counselling and support.

Data Collection Form

This form included questions about sociodemographic variables (age, gender, marital status), family relations (distancing from family, fear of infecting family members) and social relations (stigmatization, negative manners from society),

encountering infection (infected by SARS-CoV-2, treating patients who were later diagnosed SARS-CoV-2), history of psychiatric treatment before and during pandemic, and occupational conditions (work experience, workload, cooperation with co-workers, management support, gaining PPE, satisfaction from work).

Psychometric Scales

Depression-Anxiety-Stress Scale (DASS-42) is a self-report four-point Likert type scale including 42 items reflecting depression, anxiety and stress levels of individuals at last week (19). In Turkish validity and reliability study of DASS-42, cut-off points for depression (9<), anxiety (7<) and stress (14<) have been reported previously (20).

Insomnia Severity Index (ISI) is a self-report five-point Likert type scale measuring severity of insomnia (0-28 points) by seven questions (21). Developers of ISI noted that scoring above ten points indicates insomnia and scoring higher than fifteen points means clinical insomnia. The validity and reliability of the Turkish form of ISI were studied formerly (22).

Statistical Analyses

All of the data violated normal distribution, so non-parametric tests were used in this study. Correlations between independent continuous variables and dependent variables were tested with Spearman test. Possible significant effects of independent categoric variables in the data collection form on psychometric parameters were analyzed with Mann-Whitney U Test for two levels. For multi-levels, Kruskal-Wallis Test were used. If any significant result was obtained with Kruskal-Wallis Test, then Mann-Whitney U Tests were performed for multiple comparisons between groups with Bonferroni correction. The psychological data of aerosol-generating treatments group were compared to the rest of the participants using Mann-Whitney U Test. In all tests $\alpha=0.05$ significance level was accepted.

Results

Sociodemographic variables, psychometric measurements and dichotomous variables regarding possible psychological effects of SARS-CoV-2 were shown in Table 1. Severity distribution of psychiatric symptoms according to the cut-off points were presented in Table 2. The other measures of data

collection form were demonstrated in Table 3 and Table 4. All psychometric measurements (depression, anxiety, stress, insomnia scores) were significantly and positively correlated with each other (p<0.001). Age was significantly and negatively correlated with stress scores (p=0.035).

There was a significant interaction between treated patient's number and stress scores (H=11.5 df=3 p=0.009). Subgroup analyses revealed that stress scores of DHCWs treating average 5-10 patients [n=35, med=15 (2-37)] were higher than those who were treating 10-15 patients in a day [n=12, med=6 (0-22), p=0.018] (Table 3).

Feeling anxious before pandemic was significantly correlated with all psychometric measurements (p<0.001 for stress and depression scores, p=0.001 for anxiety scores and p=0.017 for insomnia scores). Subgroup analyses for stress scores demonstrated that mild, moderate and severe level groups scored significantly higher than normal level group (p=0.001, p<0.001 and p=0.028, respectively). Regarding anxiety points, subgroup analyses showed that mild, moderate and severe level groups had significantly higher anxiety points compared to normal level group (p=0.016, p=0.02, p=0.032, respectively). When compared to the no anxiety group, feeling mild, moderate, severe and extremely severe anxious before pandemic groups were significantly more depressive (p=0.012, p=0.004, p=0.034 and p=0.39, respectively). Additionally, the only significant difference for insomnia points was between the severe level and normal level groups (p=0.038) (Table 3).

Feeling depressive before pandemic was significantly related with all psychometric measurements (p<0.001 for stress and depression scores, p=0.004 for anxiety scores and for insomnia scores). Subsequent analyses for stress points demonstrated that severe, moderate and mild level groups scored significantly higher than normal level group (p=0.002, p=0.004 and p=0.011, respectively). The only significant difference regarding anxiety points was between severe and normal level groups (severe>never p=0.007). Feeling depressive before pandemic presented significantly higher depression scores for severe and moderate groups compared to the never group (p=0.001 and p=0.03, respectively). Subgroup analyses for insomnia scores revealed that severe, moderate and mild level groups had significantly higher insomnia scores than the normal level group (p=0.039, p=0.016 and p=0.037, respectively) (Table 3).

Feeling anxious about variations in working conditions or increasing workload during or after pandemic was significantly interacted with stress points (p=0.016). Subgroup analyses explained this interaction with feeling severe level anxiety of DHCWs had higher stress points than feeling mild level ones (p=0.008) (Table 4). Feeling anxious about obtaining PPE during or after pandemic also was significantly related to stress points (p=0.021). Very severe level anxious group scored significantly higher stress points than mild level anxious group (p=0.007) (Table 3).

Table 1. Sociodemographic, psychometric and dichotomous variables

Variables (n=115)	Mean ± SD	(Median/ minimum- maximum)
Age	31.8±9	(27/23-60)
Working Experience (year)	8.3±9.6	(3/1-39)
DASS-42-Depression Score	11.2±8	(11/0-37)
DASS-42-Anxiety Score	7±5.9	(6/0-28)
DASS-42-Stress Score	12.6±7.6	(12/0-37)
ISI-Insomnia Score	6.9±4.7	(6/0-24)

DASS-42: Depression-Anxiety-Stress Scale, ISI: Insomnia Severity Index, SD: Standard Deviation

Table 2. Severity levels of psychometric scale results

Psychiatric measurements	Healthy (n %)	Mild (n %)	Moderate (n %)	Severe (n %)	Very severe (n %)
DASS-42-Depression Level	53 (46)	22 (19)	28 (24)	7 (6.4)	5 (4.6)
DASS-42-Anxiety Level	69 (60)	12 (10)	20 (18)	8 (7)	6 (5)
DASS-42-Stress Level	74 (64)	19 (17)	17 (15)	3 (2.6)	2 (1.4)
ISI-Insomnia Level	69 (60)	39 (34)	5 (4.6)	2 (1.4)	0 (0)

DASS-42: Depression-Anxiety-Stress Scale, ISI: Insomnia Severity Index

Gender, marital status, isolation from family, working frequency, psychiatric help before pandemic, psychiatric help during pandemic, SARS-CoV-2 infection of relative, SARS-CoV-2 related dead of relative, negative reaction of society, stigmatization, hiding job from society, support form colleagues, feeling anxious during dental treatment, fear of infecting relatives, satisfaction from decisions of

Table 3. Analysis of factors significantly associated with psychological stress of DCHWs					
Variables	n (%)	SS ± SD	AS ± SD	DS ± SD	IS ± SD
Treated patient's number in a day					
0-5	48 (41.7)	12.19±6.68	6.17±4.96	10.38±7.36	6.29±3.28
5-10	35 (30.4)	15.97±8.56*	8.74±7.04	14.31±9.05	8.03±5.98
10-15	12 (10.4)	8.17±6.97*	5.75±5.91	9.42±7.95	6.67±5.94
20<	20 (17.4)	10.20±5.78	6.40±5.21	9.05±6.43	6.45±4.27
p-value	-	0.009*	0.350	0.076	0.763
Feeling anxious before pandemic					
Normal	26 (22.6)	6.81±5.58	3.62±3.67*	6.77±6.05	4.62±3.15*
Mild	52 (45.2)	13.96±7.03	7.83±6.16*	12.54±8.55	7.79±5.09
Moderate	27 (23.5)	14.81±7.10	8.37±6.24*	12.89±6.29	6.67±4.08
Severe	6 (5.2)	18±9.82	10.17±4.26*	17.33±9.67	11±6.57*
Extremely severe	4 (3.5)	8.75±5.32	2.75±2.63*	3.25±2.06	5.25±2.5
p-value	-	0.000*	0.001*	0.000*	0.017*
Feeling depressive before pandemic					
Normal	31 (27)	8.06±5.54*	4.32±3.77*	7.35±6*	4.39±3.01*
Mild	44 (38.3)	13.55±6.97*	7.64±6.64	11.86±7.91	7.25±4.67*
Moderate	24 (20.9)	14.29±6.19*	7.67±5.40	12.75±6.84*	8.04±5.05*
Severe	12 (10.4)	18.92±10.62*	11.08±5.90*	18.42±9.86*	9.33±6.24*
Extremely severe	4 (3.5)	7.50±4.20*	3±2.31	4±2.94	8±4.24*
p-value	-	0.000*	0.004*	0.000*	0.004*
Feeling anxious about working conditions during/after pandemic					
Normal	8 (7)	11.63±6.57	5.62±3.46	11±7.05	5.88±7.47
Mild	26 (22.6)	8.42±6.97*	5.38±5.13	7.92±7.08	6.12±4.3
Moderate	30 (26.1)	12.37±5.72	6.47±4.71	11.10±6.05	5.93±3.86
Severe	26 (22.6)	15.08±6.49*	9.12±7.5	13±8.5	7.88±4.70
Extremely severe	25 (21.7)	14.84±9.7	7.32±6.2	13.12±9.97	8.12±4.88
p-value	-	0.016*	0.390	0.168	0.182
Feeling anxious about obtaining personal protective equipment					
Normal	9 (7.8)	11.11±6.76	8.67±6.2	11.44±6.2	6.33±7.47
Mild	27 (23.5)	8.93±6.64*	5.44±4.5	8.15±5.7	6.7±3.86
Moderate	24 (20.9)	12.71±5.92	6.38±4.68	11.21±7.06	6.29±3.47
Severe	29 (25.2)	12.45±6.40	6.93±6.98	10.55±7.55	6.66±4.09
Extremely severe	26 (22.6)	16.88±9.36*	8.46±6.48	15.19±10.43	8.08±5.98
p-value	-	0.021*	0.392	0.124	0.849
SP: Stress score, AP: Anxiety score, DP: Depression score, IP: Insomnia score, *Statistically significant difference, SD: Standard Deviation					

management during pandemic, being educated enough about working under pandemic conditions, achieving team-work with colleagues, job satisfaction had no significant effect on psychometric points of DHCWs (p>0.05). There was no significant difference between two groups of DHWCs (working on aerosol-generating treatments and the other ones) in all psychometric measures (p>0.05) (Table 4).

Discussion

The present study is conducted on DHCWs who worked actively at a specific university clinic during nationwide lockdown in Turkey. Most of the studies about the mental health of DHCWs during the COVID-19 pandemic have used internet platforms (23-28). To our knowledge, this is the first face-to face report on mental health outcomes and associated risk factors among DHCWs in Turkey during the COVID-19 pandemic. In the current investigation, Turkish DHCWs displayed overall high psychological impact of the COVID-19 outbreak in terms of stress, anxiety, depression, and insomnia as estimated by the DASS-42 and ISI survey systems. Among the remarkable findings of our study, the variation in working conditions and the state of being anxious about the supply of PPE were found to be related to the stress levels of the individuals. Dentists (endodontists and pedodontists) and their assisting dental staffs working on aerosol-generating treatments, did not differ in terms of psychological characteristics from the group that did not give aerosol-generating treatments. Since there was no study related to aerosol generating and non-aerosol generating procedures comparison in the dentistry, the results of this study could not be compared directly. However, the reason for the lack of difference between the two groups may be that nearly in all dental procedures, DHCWs have a fear that if they could have been exposed by aerosol with coughing or sneezing (etc.) even the procedure is a non-aerosol generating procedure like local anesthesia (29). Therefore, DHCWs had a long and stressful working life under heavy conditions before the pandemic due to their profession, and this situation facilitated their psychological adaptation to the pandemic conditions.

Our results revealed that both the high level of psychometric rates (40% vs 24% for anxiety, 54% vs 23% for depression, 40% vs 34% for insomnia) and the distribution of moderate-severe psychiatric symptoms

of DHCWs (30% vs 6.8% for anxiety, 35% vs 16% for depression) were significantly higher than the prevalence rates of HCWs reported in a recent meta-analysis study (30). In another study, severity distribution of depression, anxiety and insomnia symptoms of frontline HCWs treating patients with COVID-19 were very similar to the severity distribution of DHCWs in this study (31). Özarslan and Caliskan (25) reported that dentists taking part in the filiation service showed significantly higher stress levels than the dentists not involved in filiation. It is important to consider how these results that were collected during an infectious disease epidemic reflect the effects of psychological distress and burnout on dental staff. The results of the aforementioned studies reported important evidences about the negative psychological effects of the COVID-19 pandemic on healthcare workers.

Since the SARS-CoV-2 pandemic, several studies have been performed investigating the impact of pandemic on dental professionals' mental health (23-28). When the literature is examined, the inclusion of DHCWs working from home and the collection of data with online surveys may create difficulties in interpreting the results of the previous studies. In our study, a negative relationship was found between sample age and the stress scores. The result of a recent study supported this finding, the stress levels of dentists with more than 20 years of professional experience were found to be lower (26). Consistent with these results, in two separate studies, younger endodontists had higher stress levels than older ones (27,28). Another study investigated depression, stress, and anxiety levels of physicians during the COVID-19 outbreak and reported that being male, older, and having more work experience were associated with lower depression, stress, and anxiety scores (32). Relatively higher prevalence rates of psychological symptoms might be related to the dominancy of young DHCW respondents in this study and longer practicing with acquired experience in time may have made it easier to cope with stress during the pandemic (33).

Feeling anxious about obtaining PPE during and after pandemic increased stress levels of DHCWs in the current study. Studies supporting this result have reported that high stress levels are correlated with a fear of contracting SARS-CoV-2 from a patient (23). In this study, despite a group of DHCWs treating fewer patients than the other group, they scored higher

Table 4. Analysis of psychological parameters of DCHWs with non-significant impacts						
Variables	n (%)	SS ± SD	AS ± SD	DS ± SD	IS ± SD	p-value
Gender						
Male	19 (16.5)	12.11±6.8	6.53±5.98	11.42±6.58	6.84±3.65	>0.05
Female	96 (83.5)	12.67±7.72	7.03±5.86	11.21±8.29	6.9±4.9	
Marital status						
Single	76 (66.1)	13.39±8	6.99±6.18	11.91±8.34	6.67±4.45	>0.05
Married	39 (33.9)	10.97±6.41	6.87±5.23	9.95±7.24	7.31±5.19	
Aerosol spreading work						
Yes	53 (46.1)	12.47±8.34	7.58±5.62	11.70±8.2	7.23±5.4	>0.05
No	62 (53.9)	12.66±6.88	6.40±6.04	10.85±7.8	6.60±4.02	
Isolation from family						
Yes	43 (37.4)	13.6±7.96	7.28±6.76	11.81±8.69	7.35±4.75	>0.05
No	72 (62.6)	12.22±7.34	6.75±5.28	10.90±7.62	6.61±4.69	
Working frequency						
Never	8 (7)	11.75±8.32	6.38±6.16	9.13±9.75	4.5±3.97	>0.05
Once in a month	4 (3.5)	9.75±3.10	3.5±3.0	5.25±2.5	5.25±2.06	
Twice in a month	75 (65.2)	12.89±7.9	6.99±5.58	11.59±8.24	7.03±4.87	
Once in a week	18 (15.7)	13.56±6.77	8.39±5.89	13.28±6.08	7.67±5.14	
Twice in a week/more	8 (7)	8.88±6.8	3.75±3.24	7.37±7.33	6.38±3.93	
Every day	2 (1.7)	15.5±9.19	14.5±19.1	16±12.73	9.5±2.12	
Psychiatric help before pandemic						
Yes	7 (6.1)	10.86±8.13	5.57±5.5	7±6.25	6.71±4.46	>0.05
No	108 (93.9)	12.69±7.55	7.04±5.89	11.52±8.05	6.90±4.74	
Psychiatric help during pandemic						
Yes	3 (2.6)	13±11.53	10.67±8.39	12±10.58	2.67±3.06	>0.05
No	112 (97.4)	12.56±7.50	6.85±5.79	11.22±7.99	7±4.70	
SARS-CoV-2 infection of relative						
Yes	6 (5.2)	16.17±8.77	9.33±6.02	14.83±9.22	8.33±5.82	>0.05
No	109 (94.8)	12.38±7.48	6.82±5.85	11.05±7.94	6.81±4.66	
SARS-CoV-2 related dead relative						
Yes	3 (2.6)	7.33±7.51	5.67±4.51	5.67±6.66	2.33±4.04	>0.05
No	112 (97.4)	12.71±7.54	6.98±5.90	11.39±8.01	7.01±4.68	
Negative reaction of society						
Yes	24 (20.8)	12.58±7.75	7.62±4.18	10.54±5.74	7.29±3.47	>0.05
No	91 (79.2)	12.57±7.5	6.77±6.23	11.43±8.52	6.78±4.99	
Stigmatization						
Yes	34 (29.6)	13.47±7.46	8.38±6.38	12.09±8.39	7.82±5	>0.05
No	81 (70.4)	12.20±7.62	6.35±5.55	10.89±7.87	6.49±4.55	
Hiding job from society						
Yes	7 (6.1)	13.71±10.40	8±6.19	10.57±11.10	5.57±3.16	>0.05
No	108 (93.9)	12.50±7.40	6.88±5.86	11.29±7.83	6.97±4.79	

Table 4. Continued					
Support from colleagues					
None	6 (5.2)	7.67±5.82	2.83±3.76	6±5.10	5±4.65	
Some	15 (13)	14.40±9.6	7.4±8.6	11±9.43	7.73±3.43	>0.05
Medium-level	60 (52.2)	13.05±7.47	7.63±5.7	12.32±8.24	7.60±5.27	
A lot	22 (19.1)	11.95±6.5	6.14±4.88	9.18±6.13	5.27±2.86	
Quite a lot	12 (10.4)	11.50±7.63	6.5±4.76	12.58±8.45	6.17±5.32	
Feeling anxious during dental treatment					
Normal	5 (4.3)	7±6.40	3.60±3.65	8.60±5.77	4.60±3.36	
Mild	23 (20)	9.78±7.84	5.48±5.62	8.91± 8.18	6.35±4.01	>0.05
Moderate	39 (33.9)	12.46±6.30	6.31±5.64	10.38±6.65	6.03±3.07	
Severe	29 (25.2)	13.97±6.33	8.24±5.88	12.69±8.32	8.03±6.15	
Extremely severe	19 (16.59)	15.53±10.04	8.95±6.41	14.32±9.59	8.16±5.66	
Fear of infecting relatives					
Normal	4 (3.5)	8±8.49	6±5.35	8.75±6.34	5.75±5.91	
Mild	14 (12.2)	9.93±5.81	5.50±6.96	9.14±6.27	5.71±3.65	>0.05
Moderate	13 (11.3)	14.38±8.09	7.69±5.78	11.77±8.86	5.77±4.23	
Severe	41 (35.7)	11.12±6.89	6.78±5.94	10.76±7.85	6.59±4.08	
Extremely severe	43 (37.4)	14.70±7.98	7.44±5.62	12.47±8.60	8±5.50	
Management satisfaction					
Yes	58 (50.4)	12.24±7	6.78±5.88	10.69±7.83	6.38±5.05	>0.05
No	57 (49.6)	12.91±8.14	7.12±5.88	11.81±8.21	7.40±4.31	
Being educated enough about working under pandemic conditions					
None	25 (21.7)	14.44±9.47	7.80±7.36	12.68±10.69	7.76±5.24	
Some	33 (28.7)	12.67±6.35	7.45±5.25	11.67±5.69	6.06±5.05	>0.05
Medium-level	45 (39.1)	11.36±6.87	6.42±5.77	10.27±8.28	6.71±3.59	
A lot	10 (8.7)	13.80±9.15	6±4.57	11.30±6.52	9±6.20	
Quite a lot	2 (1.7)	9±7.07	4.50±3.54	8±2.83	3±4.24	
Achieving team-work with colleagues					
None	9 (7.8)	10.56±6.46	3.89±4.17	7.22±5.20	4.67±3.43	
Some	13 (11.3)	11.23±7.33	5.23±4.64	10±4.76	6.46±4.23	>0.05
Medium-level	36 (31.3)	13.14±8.48	7.53±6.28	11.97±9.38	7.06±4.70	
A lot	41 (35.7)	11.78±5.94	7.27±5.7	10.54±6.14	6.34±3.34	
Quite a lot	16 (13.9)	15.56±9.54	7.94±6.71	14.69±11.07	9.50±7.33	
Job satisfaction					
None	57 (49.6)	13.05±8.14	6.68±5.74	11.53±7.71	7.56±5.18	
Some	33 (28.7)	11.94±7.69	6.85±5.7	11.06±9.11	5.55±4.25	>0.05
Medium-level	22 (19.1)	12.45±5.8	7.05±5.15	10.50±7.17	7.32±4.05	
A lot	3 (2.6)	11.33±9.24	12.33±13.65	13.33±10.21	5.67±2.51	
SP: Stress score, AP: Anxiety score, DP: Depression score, IP: Insomnia score, SD: Standard Deviation, SARS-CoV-2: Severe acute respiratory syndrome coronovirus-2					

stress points than the latter. This finding is supported by a similar study which reported that DHCWs' perceived stress level was disproportionate with workload (23).

All psychological symptom scores were significantly and positively correlated with each other in line with literature (34). Feeling anxious and/or depressive before pandemic was correlated positively with all psychometric measurements during the pandemic in this study. Paulus and Stein (35) reported that negative feelings stemming from negative beliefs about self, others and future have key roles in developing anxiety and depressive disorders through dysfunctional interoceptive prediction schemas. Negative cognitive styles and stress-reactive rumination predicts greater number and longer durations of depressive episodes in a longitudinal study (36).

Pandemics are known to affect mental health of the general population and various at-risk groups like healthcare workers, students and people with chronic medical diseases (30). However, not much is known of the mental health of people with pre-existing mental illness during a pandemic. In our study, it was observed that a limited number of cases received psychiatric help before and during the pandemic, and it was determined that receiving psychiatric help before or during the pandemic did not have a significant effect on the psychometric points of DHCWs. Although it could not be generalized to the whole population due to the limited number of the cases, there was no relationship between receiving psychiatric help before and during the pandemic. A recent systematic review and meta-analysis of 15 studies investigates, whether people with pre-existing mental illness experience an increase in mental health symptoms and experience more hospitalizations during a pandemic (37). People with pre-existing mental illness have significantly higher psychiatric symptoms, anxiety symptoms and depressive symptoms compared to controls during a pandemic with pooled effect sizes (SMD) of 0.593 [95% confidence interval (CI) 0.46 to 0.72], 0.616 (95% CI 0.49 to 0.73) and 0.597 (95% CI 0.38 to 0.80) respectively. Major symptoms identified included increased anxiety, depression and insomnia. It is noteworthy that the aforementioned studies include mixed samples and general populations other than healthcare professionals.

The present study has several strengths and limitations. Strengths of this study are accuracy of data collection, homogeneous sample selection, acquiring data during the pandemic for preventing recall bias. One of the important limitation is its cross sectional nature and lack of the follow-up on a longitudinal level. Small sample size selected from a specific university clinic and female dominancy of DHCWs may also reduce generalizability of the results. We applied self-report questionnaire to assess psychological symptoms that do not rely on diagnostic evaluation. Adding a clinical mental health evaluation by psychiatric specialists would definitely contribute to the outcome of this survey. Regardless of the above limitations, conclusions of this study provide important information on the psychological impact of COVID-19 on DHCWs.

Conclusion

DHCWs had significantly high prevalence rates of depression, anxiety, stress and insomnia symptoms. Younger age, and feeling anxiety about acquiring PPE increases stress level. Feeling negative emotions before pandemic is positively correlated with depression, anxiety, stress and insomnia scores. Psychiatric screening before and during the pandemic, improving coping skills with negative emotions, encouraging and supporting vulnerable ones for psychological therapies like making regular aerobic exercise and scheduled psychiatric interviews, providing appropriate PPE and working conditions, regular screening for SARS-CoV-2 infection are essential interventions for alleviating psychological symptoms of DHCWs related to COVID-19 pandemic. Based on the nature of the dental profession, the stress on DHCWs will be increased with especially aerosol-generating procedures. Planning longitudinal studies about screening mental status of DHCWs and testing the effect of interventions for relieving the psychological impairments related to long-running traumatizing course of COVID-19 pandemic is strongly recommended.

Prof. Dr. Pelin Güneri for her support and valuable suggestions.

Ethics

Ethics Committee Approval: The present cohort study was designed as a survey and was approved by the Clinical Research Ethics Committee of Ege University Faculty of Dentistry (no: 65234543-050.06.04, date: 29.06.2020).

Informed Consent: They were informed about the study in written form and only the individuals who signed the consent form were included.

Peer-review: Internally and externally peer-reviewed.

Authorship Contributions

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

References

1. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med 2020; 382: 727-33.

2. Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet 2020; 395: 565-74.

3. Peiris JS, Guan Y, Yuen KY. Severe acute respiratory syndrome. Nat Med 2004; 10(12 Suppl): S88-97.

4. Zaki AM, van Boheemen S, Bestebroer TM, Osterhaus AD, Fouchier RA. Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. N Engl J Med 2012; 367: 1814-20.

5. Sabino-Silva R, Jardim ACG, Siqueira WL. Coronavirus COVID-19 impacts to dentistry and potential salivary diagnosis. Clin Oral Investig 2020; 24: 1619-21.

6. He F, Deng Y, Li W. Coronavirus disease 2019: What we know? J Med Virol 2020; 92: 719-25.

7. Ong SWX, Tan YK, Chia PY, Lee TH, Ng OT, Wong MSY, et al. Air, Surface Environmental, and Personal Protective Equipment Contamination by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) From a Symptomatic Patient. JAMA 2020; 323: 1610-2.

8. Cheng AC, Williamson DA. An outbreak of COVID-19 caused by a new coronavirus: what we know so far. Med J Aust 2020; 212: 393-4.e1.

9. He X, Lau EHY, Wu P, Deng X, Wang J, Hao X, et al. Temporal dynamics in viral shedding and transmissibility of COVID-19. Nat Med 2020; 26: 672-5.

10. Lavezzo E, Franchin E, Ciavarella C, Cuomo-Dannenburg G, Barzon L, Del Vecchio C, et al. Suppression of a SARS-CoV-2 outbreak in the Italian municipality of Vo'. Nature 2020; 584: 425-9.

11. Gamio L. The workers who face the greatest coronavirus risk. The New York Times (New York) 2020 March 15. Available from URL: https://www.nytimes.com/interactive/2020/03/15/business/economy/coronavirus-worker-risk.html. Last Accessed: 15 Mar, 2020.

12. Kampf G, Todt D, Pfaender S, Steinmann E. Persistence of coronaviruses on inanimate surfaces and their inactivation with biocidal agents. J Hosp Infect 2020; 104: 246-51.

13. Xu J, Li Y, Gan F, Du Y, Yao Y. Salivary Glands: Potential Reservoirs for COVID-19 Asymptomatic Infection. J Dent Res 2020; 99: 989.

14. To KK, Tsang OT, Yip CC, Chan KH, Wu TC, Chan JM, et al. Consistent Detection of 2019 Novel Coronavirus in Saliva. Clin Infect Dis 2020; 71: 841-3.

15. The American Dental Association. ADA Recommending Dentists Postpone Elective Procedures. Available online: https://tinyurl.com/wpp647r (accessed on 16 March 2020).

16. Centers for Disease Control and Prevention. CDC Recommendation: Postpone Non- Urgent Dental Procedures, Surgeries, and Visits. Available online: https://www.cdc.gov/oralhealth/infectioncontrol/statement-COVID.html (accessed on 27 March 2020).

17. Xiang YT, Yang Y, Li W, Zhang L, Zhang Q, Cheung T, et al. Timely mental health care for the 2019 novel coronavirus outbreak is urgently needed. Lancet Psychiatry 2020; 7: 228-9.

18. Liu S, Yang L, Zhang C, Xiang YT, Liu Z, Hu S, et al. Online mental health services in China during the COVID-19 outbreak. Lancet Psychiatry 2020; 7: e17-8.

19. Lovibond PF, Lovibond SH. The structure of negative emotional states: comparison of the Depression Anxiety Stress Scales (DASS) with the Beck Depression and Anxiety Inventories. Behav Res Ther 1995; 33: 335-43.

20. Hekimoglu L, Altun ZO, Kaya EZ, Bayram N, Bilgel N. Psychometric properties of the Turkish version of the 42 item Depression Anxiety Stress Scale (DASS-42) in a clinical sample. Int J Psychiatry Med 2012; 44: 183-98.

21. Bastien CH, Vallières A, Morin CM. Validation of the Insomnia Severity Index as an outcome measure for insomnia research. Sleep Med 2001; 2: 297-307.

22. Boysan M, Gülec M, Besiroglu L, Kalafat T. Psychometric properties of the Insomnia Severity Index in Turkish sample. Anadolu Psikiyatri Derg 2010; 11: 248-52.

23. Shacham M, Hamama-Raz Y, Kolerman R, Mijiritsky O, Ben-Ezra M, Mijiritsky E. COVID-19 Factors and Psychological Factors Associated with Elevated Psychological Distress among Dentists and Dental Hygienists in Israel. Int J Environ Res Public Health 2020; 17: 2900.

24. Uçan Yarkaç F, Tekin Atay Ü, Dinçer NN, Öncü E. Evaluation of dentists' stress and anxiety levels in the COVID-19 pandemic. Int Dent Res 2021;11(Suppl 1): 259-65.

25. Özarslan M, Caliskan S. Attitudes and predictive factors of psychological distress and occupational burnout among dentists during COVID-19 pandemic in Turkey. Curr Psychol 2021; 40: 3113-24.

26. Sarialioglu Gungor A, Donmez N, Uslu YS. Knowledge, stress levels, and clinical practice modifications of Turkish dentists due to COVID-19: a survey study. Braz Oral Res 2021; 35: e048.

27. Kumar Anil RN, Karumaran SC, Kattula D, Thavaeajah R, Anusa AM. Perceived stress and psychological (dis) stress among Indian endodontists during COVID-19 pandemic lock down. MedRxiv 2020. Doi: doi.org/10.1101/2020.05.06.20092601

28. Yu J, Hua F, Shen Y, Haapasalo M, Qin D, Zhao D, et al. Resumption of Endodontic Practices in COVID-19 Hardest-Hit Area of China: A Web-based Survey. J Endod 2020; 46: 1577-83.e2.

29. Dziedzic A, Tanasiewicz M, Tysiąc-Miśta M. Dental Care Provision during Coronavirus Disease 2019 (COVID-19) Pandemic: The Importance of Continuous Support for Vulnerable Patients. Medicina (Kaunas) 2020; 56: 294.

30. Pappa S, Ntella V, Giannakas T, Giannakoulis VG, Papoutsi E, Katsaounou P. Prevalence of depression, anxiety, and insomnia among healthcare workers during the COVID-19 pandemic: A systematic review and meta-analysis. Brain Behav Immun 2020; 88: 901-7.

31. Lai J, Ma S, Wang Y, Cai Z, Hu J, Wei N, et al. Factors Associated With Mental Health Outcomes Among Health Care Workers Exposed to Coronavirus Disease 2019. JAMA Netw Open 2020; 3: e203976.

32. Elbay RY, Kurtulmuş A, Arpacıoğlu S, Karadere E. Depression, anxiety, stress levels of physicians and associated factors in Covid-19 pandemics. Psychiatry Res 2020; 290: 113130.

33. Fleisher LA, Sweeney RE, Clapp JT, Barsade SG. Managing anxiety in anesthesiology and intensive care providers during the Covid-19 pandemic: an analysis of the psychosocial response of a front-line department. Nejm Catalyst Innovations in Care Delivery 2020; 8.

34. Wang W, Tang J, Wei F. Updated understanding of the outbreak of 2019 novel coronavirus (2019-nCoV) in Wuhan, China. J Med Virol 2020; 92: 441-7.

35. Paulus MP, Stein MB. Interoception in anxiety and depression. Brain Struct Funct 2010; 214: 451-63.

36. Robinson MS, Alloy LB. Negative cognitive styles and stress-reactive rumination interact to predict depression: A prospective study. Cognit Ther Res 2003; 27: 275-91.

37. Neelam K, Duddu V, Anyim N, Neelam J, Lewis S. Pandemics and pre-existing mental illness: A systematic review and meta-analysis. Brain Behav Immun Health 2021; 10: 100177.