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The European Journal of Therapeutics will be published bimonthly, commencing in 2024 (six issues a year in February, April, June, August, October and December).

Formerly Gaziantep Medical Journal

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About the Journal

European Journal of Therapeutics (Eur J Ther) is the double-blind peer-reviewed, open access, international publication organ of the Gaziantep University School of Medicine. The journal is a quarterly publication, published on March, June, September, and December. The journal publishes content in English.

The European Journal of Therapeutics will be published bimonthly, commencing in 2024 (six issues a year in February, April, June, August, October and December).

European Journal of Therapeutics aims to contribute to the international literature by publishing Original Articles, Reviews, Case Reports, Special Editorial, Short Communication, Technical Notes, Letters to the Editor, Reply by Authors, Image, Withdrawn, Erratum, Retraction Notice, Editorial (EIC use only), In memory of (Invited by EIC only) and Book Review (Invited by EIC only) in the fields of medical sciences. The journal's target audience includes researchers, physicians, and healthcare professionals who are interested or working in in all medical disciplines.

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The editorial and publication processes of the journal are shaped in accordance with the guidelines of the International Committee of Medical Journal Editors (ICMJE), World Association of Medical Editors (WAME), Council of Science Editors (CSE), Committee on Publication Ethics (COPE), European Association of Science Editors (EASE), National Information Standards Organization (NISO). The journal is in conformity with the Principles of Transparency and Best Practice in Scholarly Publishing (doaj.org/bestpractice).

Processing and publication are free of charge with the journal. No fees are requested from the authors at any point throughout the evaluation and publication process. All manuscripts must be submitted via the online submission system, which is available at www.eurjther.com. The journal guidelines, technical information, and the required forms are available on the journal's web page.

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

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Instructions to Authors

European Journal of Therapeutics (Eur J Ther) is the double-blind peer-reviewed, open access, international publication organ of the Gaziantep University School of Medicine. The journal is a quarterly publication, published on March, June, September, and December and its publication language is English.

European Journal of Therapeutics aims to contribute to the international literature by publishing original clinical and experimental research articles, short communication, review articles, technical notes, and letters to the editor in the fields of medical sciences. The journal's target audience includes researchers, physicians and healthcare professionals who are interested or working in in all medical disciplines.

The editorial and publication processes of the journal are shaped in accordance with the guidelines of the International Council of Medical Journal Editors (ICMJE), the World Association of Medical Editors (WAME), the Council of Science Editors (CSE), the Committee on Publication Ethics (COPE), the European Association of Science Editors (EASE), and National Information Standards Organization (NISO). The journal conforms to the Principles of Transparency and Best Practice in Scholarly Publishing (doaj.org/bestpractice).

Originality, high scientific quality, and citation potential are the most important criteria for a manuscript to be accepted for publication. Manuscripts submitted for evaluation should not have been previously presented or already published in an electronic or printed medium. The journal should be informed of manuscripts that have been submitted to another journal for evaluation and rejected for publication. The submission of previous reviewer reports will expedite the evaluation process. 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.

Manuscripts submitted to European Journal of Therapeutics will go through a double-blind peer-review process. Each submission will be reviewed by at least two external, independent peer reviewers who are experts in their fields in order to ensure an unbiased evaluation process. The editorial board will invite an external and independent editor to manage the evaluation processes of manuscripts submitted by editors or by the editorial board members of the journal. The Editor in Chief is the final authority in the decision-making process for all submissions.

An approval of research protocols by the Ethics Committee in accordance with international agreements (World Medical Association Declaration of Helsinki "Ethical Principles for Medical Research Involving Human Subjects," amended in October 2013, www.wma.net) is required for experimental, clinical, and drug studies and for some case reports. If required, ethics committee reports or an equivalent official document will be requested from the authors. For manuscripts concerning experimental research on humans, a statement should be included that shows that written informed consent of patients and volunteers was obtained following a detailed explanation of the procedures that they may undergo. 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, the name of the ethics committee, and the ethics committee approval number should also be stated in the Materials and Methods section of the manuscript. It is the authors' responsibility to carefully protect the patients' anonymity. For photographs that may reveal the identity of the patients, signed releases of the patient or of their legal representative should be enclosed.

All submissions are screened by a similarity detection software (iThenticate by CrossCheck).

The similarity rate limit determined for our journal is 24%.

In the event of alleged or suspected research misconduct, e.g., plagiarism, citation manipulation, and data falsification/fabrication, the Editorial Board will follow and act in accordance with COPE guidelines.

Each individual listed as an author should fulfill the authorship criteria recommended by the International Committee of Medical Journal Editors **(ICMJE - www.icmje.org).** The ICMJE recommends that authorship be based on the following 4 criteria:

1. Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; AND
2. Drafting the work or revising it critically for important intellectual content; AND
3. Final approval of the version to be published; AND
4. Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

In addition to being accountable for the parts of the work he/she has done, an author should be able to identify which co-authors are responsible for specific other parts of the work. In addition, authors should have confidence in the integrity of the contributions of their co-authors.

All those designated as authors should meet all four criteria for authorship, and all who meet the four criteria should be identified as authors. Those who do not meet all four criteria should be acknowledged in the title page of the manuscript.

European Journal of Therapeutics requires corresponding authors to submit a signed and scanned version of the Copyright Agreement and Acknowledgement of Authorship Form (available for download through www.eurjther.com) during the initial submission process in order to act appropriately on authorship rights and to prevent ghost or honorary authorship. If the editorial board suspects a case of "gift authorship," the submission will be rejected without further review. As part of the submission of the manuscript, the corresponding author should also send a short statement declaring that he/she accepts to undertake all the responsibility for authorship during the submission and review stages of the manuscript.

European Journal of Therapeutics requires and encourages the authors and the individuals involved in the evaluation process of submitted manuscripts to disclose any existing or potential conflicts of interests,

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including financial, consultant, and institutional, that might lead to potential bias or a conflict of interest. Any financial grants or other support received for a submitted study from individuals or institutions should be disclosed to the Editorial Board. To disclose a potential conflict of interest, the ICMJE Potential Conflict of Interest Disclosure Form should be filled in and submitted by all contributing authors. Cases of a potential conflict of interest of the editors, authors, or reviewers are resolved by the journal's Editorial Board within the scope of COPE and ICMJE guidelines.

The Editorial Board of the journal handles all appeal and complaint cases within the scope of COPE guidelines. In such cases, authors should get in direct contact with the editorial office regarding their appeals and complaints. When needed, an ombudsperson may be assigned to resolve cases that cannot be resolved internally. The Editor in Chief is the final authority in the decision-making process for all appeals and complaints.

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Manuscript Types

Authors should determine the type of paper before submitting it and indicate the type of paper on the title page. This is because, depending on the type of paper, the rules to be followed, including formatting and word limits, change.The categorization system is at the discretion of the Editor-in-Chief. Authors may be asked to change the article type at the request of the editor at any stage of submission, including after acceptance.
The following types of papers will be considered for publication.

Original Articles: This is the most important type of article since it provides new information based on original research.
Abstracts should not exceed 500 words and should be structured with the following subheadings: Objective, Methods, Results, and Conclusion.
The main text should be structured with the following subheadings: Introduction, Material and Methods, Results, Discussion, Limitations, Conclusions, Acknowledgments, References, Main Points, and Figure Legends.
The main text should not exceed 3000 words, excluding the abstract, references, tables, and figure legends.
There should be a maximum of 50 references.
Statistical analysis to support conclusions is usually necessary. Statistical analyses must be conducted in accordance with international statistical reporting standards (Altman DG, Gore SM, Gardner MJ, Pocock SJ. Statistical guidelines for contributors to medical journals. Br Med J 1983: 7; 1489-93). Information on statistical analyses should be provided with a separate subheading under the Materials and Methods section and the statistical software that was used during the process must be specified. Units should be prepared in accordance with the International System of Units (SI).
Please check Table 1 for the limitations for Original Articles.

Review Articles: Reviews prepared by authors who have extensive knowledge on a particular field and whose scientific background has been translated into a high volume of publications with a high citation potential are welcomed. These authors may even be invited by the journal. Reviews should describe, discuss, and evaluate the current level of knowledge of a topic in clinical practice and should guide future studies. The main text should contain Introduction, Clinical and Research Consequences, and Conclusion sections. Please check Table 1 for the limitations for Review Articles.

Case Report: In the European Journal of Therapeutics, very interesting or rare cases can be published as Case Reports. However, due to the limited number of publications determined for this category, it is recommended that you submit such articles as Letter to the Editor. For the instructions for Letter to the Editor, please see below.

Technical Notes: This type of manuscripts should present a new experimental, computational method, test, procedure, or comparison of methods. The method described may either be completely new, or may offer a better version of an existing method. The technical note article must describe a demonstrable advance on what is currently available. Please check Table 1 for the limitations for Technical Notes.

Letter to the Editor: The European Journal of Therapeutics encourages authors to write letters to the editor on all topics covered by the journal. There is no abstract requirement for this type of manuscript. If authors prefer to include an abstract in the letter, they may include a short unstructured abstract of no more than 200 words.
It is recommended that a letter contains up to 10 references.
The letter recommends adding "Dear Editor" at the beginning of the main text and "Yours sincerely" at the end.

Table 1. Limitations for each manuscript type

Type of manuscript	Word limit	Abstract word limit	Reference limit	Table limit	Figure limit
Original Article	3000	500 (Structured)	50	6	7 or total of 15 images
Review Article	5000	250	50	6	10 or total of 20 images
Case Report	1500	200	20	5	1 or total of 5 images
Technical Note	1500	No abstract	15	No tables	10 or total of 20 images

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MANUSCRIPT PREPARATION

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 May 2023 - https://www.icmje.org/icmje-recommendations.pdf). Authors are required to prepare manuscripts in accordance with the 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 behavior.

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

Manuscripts submitted to the journal will first go through a technical evaluation process where the editorial office staff will ensure that the manuscript has been prepared and submitted in accordance with the journal's guidelines. Submissions that do not conform to the journal's guidelines will be returned to the submitting author with technical correction requests.

Authors are required to submit the following:
- Copyright Agreement and Acknowledgement of Authorship Form
- ICMJE Potential Conflict of Interest Disclosure Form (should be filled in by all contributing authors during the initial submission)

These forms are available for download at https://eurjther.com/index.php/home/forms.

Preparation of the Manuscript

Title page: A separate title page should be submitted with all submissions and this page should include:
- The full title of the manuscript as well as a short title (running head) of no more than 50 characters,
- Name(s), affiliations, and highest academic degree(s) of the author(s),
- Add the 16-digit ORCID of the author(s)
- Grant information and detailed information on the other sources of support,
- Name, address, telephone (including the mobile phone number) and fax numbers, and email address of the corresponding author,
- Acknowledgment of the individuals who contributed to the preparation of the manuscript but who do not fulfill the authorship criteria.

Abstract: An abstract should be submitted with all submissions except for Letters to the Editor. The abstract of Original Articles should be structured with subheadings (Objective, Methods, Results, and Conclusion). Please check Table 1 for word count specifications.

Keywords: Each submission must be accompanied by a minimum of three to a maximum of six keywords for subject indexing at the end of the abstract. The keywords should be listed in full without abbreviations. The keywords should be selected from the National Library of Medicine, Medical Subject Headings database (https://www.nlm.nih.gov/mesh/meshhome.html).

Main Points: All submissions except letters to the editor should be accompanied by 3 to 5 "main points" which should emphasize the most noteworthy results of the study and underline the principle message that is addressed to the reader. This section should be structured as itemized to give a general overview of the article. Since "Main Points" targeting the experts and specialists of the field, each item should be written as plain and straightforward as possible.

Main Text

Place the title, abstract, and keywords on the first page of the main text.
Organize the manuscript into four main headings: Introduction, Materials and Methods, Results, and Discussion.
Limitations, drawbacks, and shortcomings of original articles should be mentioned in the Discussion section before the conclusion paragraph.
Then place the references and figure legends in the main text, respectively.
All references, tables, and figures should be referred to within the main text, and they should be numbered consecutively in the order they are referred to within the main text.
Define abbreviations at first mention in the text and in each table and figure.
If a brand name is cited, supply the manufacturer's name and address (city and state/country).

DO NOT EMBED IMAGES or TABLES in the main text.

Text Formatting

Manuscripts should be submitted in Word.
Use a normal, plain font (e.g., 12-point Times Roman, 1.5 line spacing and justified) for text.
Do not use field functions.
Use the automatic page numbering function to number the pages.
Do not use field functions.
Do not indent at the beginning of a line.
Save your file in docx format (Word 2013 or higher).

Abbreviations

Abbreviations should be defined at first mention and used consistently thereafter.

Tables

All tables are to be numbered using Arabic numerals.
Tables should always be cited in the text in consecutive numerical order.
Each table must be uploaded as separate files (e.g. table 1, table 2, and table 3).

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Figures and Figure Legends

When there are figure subunits, the subunits should not be merged to form a single image. Each subunit should be submitted separately through the submission system. Images should not be labeled (a, b, c, etc.) to indicate figure subunits. Thick and thin arrows, arrowheads, stars, asterisks, and similar marks can be used on the images to support figure legends. Like the rest of the submission, the figures too should be blind. Any information within the images that may indicate an individual or institution should be blinded. To prevent delays in the evaluation process, all submitted figures should be clear in resolution and large in size (minimum dimensions: 100 × 100 mm).

Figures must be saved at a resolution of at least 600 dpi.
Figures, graphics, and photographs should be submitted as separate files (in TIFF or JPEG format) through the submission system (e.g. figure 1, figure 2 and figure 3).
The files should not be embedded in a Word document or the main document.
Figures should not be embedded in the manuscript text file.
Figure legends should be listed at the end of the main document.
All acronyms and abbreviations used in the manuscript should be defined at first use, both in the abstract and in the main text.
The abbreviation should be provided in parentheses following the definition.

Authors are responsible for the accuracy of references.

References

You can download the file named "EndNote style of the Eur J Ther" on the journal web page at the link.

In references, the names of all authors should be written. Usage of "et al" should not be preferred.
If available, please always include DOIs as full DOI links in your reference list.
(e.g. "https://doi.org/.....").

Use abbreviations for journal names. Journal titles should be abbreviated in accordance with the journal abbreviations in Index Medicus/MEDLINE/PubMed.

While citing publications, preference should be given to the latest, most up-to-date publications. Authors should avoid using references that are older than ten years. The limit for the old reference usage is 15% in the journal. If an ahead-of-print publication is cited, the DOI number should be provided. Journal titles should be abbreviated in accordance with the journal abbreviations in Index Medicus/MEDLINE/PubMed. In the main text of the manuscript, references should be cited using Arabic numbers in parentheses. The reference styles for different types of publications are presented in the following examples.

Journal Article
Yurci A, Gungor ND, Gurbuz T (2021) High Endometrial Thickness Does not Affect IVF/ICSI Outcomes. Eur J Ther. 27(1):94-98. https://doi.org/10.5152/eurjther.2021.20102

Example for Journal Article without English Titles
Aktan-İkiz A, Üçerler H, Orhan M (2007) Anatomic features of fossa navicularis at the skull base and its clinical importance [Kafa iskeletinde fossa navicularis'in anatomik özellikleri ve klinik önemi]. Sendrom 19:34–36 ([In Turkish])

Epub Ahead of Print Articles
Doruk M, Mustafaoglu R, Gül H (2023) The Impact of Using Technological Devices on Mental and Physical Health in Adolescents. Eur J Ther https://doi.org/10.58600/eurjther.20232902-592.y

Book
Anderson DM (2012) Dorland's illustrated medical dictionary, 32nd edn. Saunders Elsevier, Philadelphia

Book chapter
Gray H (1858) Anatomy Descriptive and Surgical 1st edn. In: John W, Parker and Son (eds), London, pp 150-155

Online Document
Bergman RA, Afifi AK, Miyauchi R (2007) Persistent congenital arterial anastomoses. Available from http://www.anatomyatlases.org/AnatomicVariants/Cardiovascular/Images0200/0232.shtml Accessed 22 Jan 2022

Reference citations in the text should be numbered in square brackets. Some examples:

Parent et al. [3] reported that .....
..... on medical radiation [21, 22].
..... sleep quality among adolescents [15, 18-21, 22, 25-30].
..... anxiety, depression, and a decrease in proprioception [5, 16-18].

Author-Suggested Reviewers

Authors are required to propose at least five reviewers when submitting their manuscripts.
It should be noted that there should be no conflict of interest between these proposed reviewers and the authors, and that these recommendations should comply with international ethical standards.
Recommended reviewers should have competence in the subject of the article.
The proposed reviewers must not have collaborated with the authors of the article in the last three years and must not be working in the same institution.

REVISIONS

When submitting a revised version of a paper, the author must submit a detailed "Response to the reviewers" that states point by point how each issue raised by the reviewers has been covered and where it can be found (each reviewer's comment, followed by the author's reply and line numbers where the changes have been made) as well as an annotated copy of the main document. Revised manuscripts must be submitted within 30 days from the date of the decision letter. If the revised version of the manuscript is not submitted within the allocated time, the revision option may be canceled. If the submitting author(s) believe that additional time

is required, they should request this extension before the initial 30-day period is over.

Accepted manuscripts are copy-edited for grammar, punctuation, and format. Once the publication process of a manuscript is completed, it is published online on the journal's webpage as an ahead-of-print publication before it is included in its scheduled issue. A PDF proof of the accepted manuscript is sent to the corresponding author and their publication approval is requested within 2 days of their receipt of the proof.

Corrections, Retractions, and Republications

European Journal of Therapeutics follows and implements the International Committee of Medical Journal Editors (ICMJE) recommendations on Corrections, Retractions, Republications and Version Control.

Corrections, Retractions, Republications and Version Control*

Honest errors are a part of science and publishing and require publication of a correction when they are detected. Corrections are needed for errors of fact. Matters of debate are best handled as letters to the editor, as print or electronic correspondence, or as posts in a journal-sponsored online forum. Updates of previous publications (e.g., an updated systematic review or clinical guideline) are considered a new publication rather than a version of a previously published article.

If a correction is needed, journals should follow these minimum standards:

- The journal should publish a correction notice as soon as possible detailing changes from and citing the original publication; the correction should be on an electronic or numbered print page that is included in an electronic or a print Table of Contents to ensure proper indexing.
- The journal also should post a new article version with details of the changes from the original version and the date(s) on which the changes were made.
- The journal should archive all prior versions of the article. This archive can be either directly accessible to readers or can be made available to the reader on request.
- Previous electronic versions should prominently note that there are more recent versions of the article.
- The citation should be to the most recent version.

Pervasive errors can result from a coding problem or a miscalculation and may result in extensive inaccuracies throughout an article. If such errors do not change the direction or significance of the results, interpretations, and conclusions of the article, a correction should be published that follows the minimum standards noted above.

Errors serious enough to invalidate a paper's results and conclusions may require retraction. However, retraction with republication (also referred to as "replacement") can be considered in cases where honest error (e.g., a misclassification or miscalculation) leads to a major change in the direction or significance of the results, interpretations, and conclusions. If the error is judged to be unintentional, the underlying science appears valid, and the changed version of the paper survives further review and editorial scrutiny, then retraction with republication of the changed paper, with an explanation, allows full correction of the scientific literature. In such cases, it is helpful to show the extent of the changes in supplementary material or in an appendix, for complete transparency.

* Corrections, Retractions, Republications and Version Control https://www.icmje.org/recommendations/browse/publishing-and-editorial-issues/corrections-and-version-control.html Date of Access: 05.10.2023

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Editorial

Welcome to the December 2023 Issue (Vol:29, No:4) and Current News of the European Journal of Therapeutics

Ayşe Balat[1, 2], Şevki Hakan Eren[3], Mehmet Sait Menzilcioğlu[4], İlhan Bahşi[5], İlkay Doğan[6], Davut Sinan Kaplan[7], Mehmet Karadağ[8], Ayşe Aysima Özçelik[9], Fatih Sarı[10], Hamit Yıldız[11]

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Dear Colleagues,

In this editorial, we would like to share with you important developments in the *European Journal of Therapeutics (Eur J Ther)*.

First of all, as the editorial team, we would like you to know that we hold frequent meetings to benefit our esteemed colleagues and continue to work with great devotion in line with our goal of taking the journal further. We have previously shared with you that we have applied to many indexes. It is with great pleasure that we would like to inform you that in the last few months, more of our index applications have been approved.

- Index Copernicus, as a result of this application, the ICV 2022 value of our journal was determined to be 100 (approved 2023-10-31) [1]
- BASE (Bielefeld Academic Search Engine) (approved 2023-11-30) [2]
- Sherpa Romeo (approved 2023-09-27) [3]
- MIAR (approved 2023-10-16) [4]

All indexes in our journal are currently included on the journal web page [5]

As the editorial team, we would like to inform you that we have determined a policy on this issue for our journal [6], taking into account the recommendations of important international ethics committees such as the Committee on Publication Ethics (COPE) [7] and the World Association of Medical Editors (WAME) [8], which have recently become a trendy topic of discussion about AI chatbots and academic studies prepared with the support of such tools.

As you know, when our journal was founded in 1990 [9], it was published in two yearly issues. It is an essential responsibility for us to carry our journal, which continued its publication life with three issues a year in 2009 [10] and four issues a year in 2014 [11]. With your valuable support, we would like to announce that we will increase our journal to 6 issues a year as of 2024 *(February, April, June, August, October* and *December)* with the rapidly growing progress of our journal.

Unfortunately, we cannot share the names of the referees who made significant contributions to our journal in 2023 due to the changes in the article submission interface during the year and the inaccessibility of some data in the previous interface. However, we would like to emphasize again that we are grateful to all of them for their valuable contributions. Moreover, to expand our journal's referee list, we would like to remind you that competent academics who volunteer in this regard can fill out the "*Become a Reviewer for the European Journal of Therapeutics*" form [12]. Finally, we would like to point out that we have strengthened our editorial team with an academician competent in dentistry, *Fatih Sari, DDS, PhD*.

Fatih Sari, DDS, PhD, is a new Editorial Board Member of the *Eur J Ther*. Dr. Sari is an Associate Professor in the Department of Prosthodontics at the Gaziantep University Faculty of Dentistry. He is a Vice Dean of the Faculty of Dentistry and Head of Clinical Departments. Dr. Sari is a prosthodontist and a member of the Turkish Dental Association. He has experience in implant-supported fixed prostheses, Cad/Cam applications and dental materials.

We look forward to being able to offer you a large number of high-quality and valuable articles over the coming year. In addition, we would like to thank the readers, authors and reviewers of the *Eur J Ther* for their continuous support.

Sincerely yours,

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Special Editorial

Zero Draft: A First Step in Research Writing

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The zero draft, often termed as the preliminary, unpolished version of a research paper, holds a pivotal role in the research process. Despite its initial status, this early draft offers myriad benefits to researchers. One crucial advantage is its ability to enhance clarity of thought. It compels authors to critically evaluate their research question, objectives, and methodologies, ensuring focused and purposeful work [1]. Additionally, it aids in identifying gaps in the research, shedding light on areas requiring further development or exploration.

Furthermore, crafting a zero draft promotes efficiency in time management, providing authors with a clear blueprint for judicious resource allocation. Overcoming writer's block is another advantage, as this initial draft breaks down the task into manageable segments, facilitating the writing process. Moreover, a well-crafted zero draft can serve as a robust foundation for potential publication in academic journals or conferences [1].

To create an effective zero draft, researchers should follow a methodical approach. This involves defining the research question, establishing a comprehensive outline, presenting data or findings succinctly, providing detailed descriptions of research methods, summarising pertinent literature, highlighting contributions to the field, offering a preliminary analysis of discoveries, proposing potential avenues for future research, and ensuring accurate citation of all sources while actively seeking feedback from peers [2].

In conclusion, the zero draft, though initially raw, plays a pivotal role in the research process. It fosters clarity of thought, aids in identifying research gaps, promotes collaboration, streamlines time management, assists in overcoming writer's block, and lays the groundwork for potential publication. By adhering to a systematic approach, researchers can fully harness the potential of their zero drafts to advance their research endeavors effectively.

Keywords: Academic Article writing, research, publication.

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Original Research

A New Vital Sign in Determining the Triage Category in Emergency Department Presentations: End-Tidal Carbon Dioxide

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ABSTRACT

Objective: To investigate whether patients' end-tidal carbon dioxide ($ETCO_2$) values measured at the time of their presentation to the emergency department can be used together with vital signs in determining their triage categories and predicting hospitalization.

Methods: This prospective, observational, cross-sectional study was conducted between May 1, 2023, and June 1, 2023, at the emergency department of a tertiary hospital. The study included patients aged >18 years who presented to the emergency department and were evaluated to have a triage category of level 2, 3, or 4 according to the five-step triage system. The patients' vital signs were measured at the time of their presentation to the emergency department and the $ETCO_2$ values measured through a nasal cannula were statistically evaluated in terms of their relationship with triage categories and hospitalization indications.

Results: A total of 1,100 patients were included in the study. According to the triage category of the patients, the mean $ETCO_2$ values for triage levels 2, 3, and 4 were 27.1±3.6, 30.6±3.1, and 35.4±3.5, respectively, indicating statistically significant differences ($p<0.001$). When the relationship of $ETCO_2$ and vital signs with hospitalization indications evaluated at the emergency department was examined, the area under the receiver operating characteristic curve for $ETCO_2$ was 0.733, which was statistically significant ($p<0.001$).

Conclusion: On completion of the study, it was concluded that the $ETCO_2$ values measured at the time of presentation to the emergency department can be a new vital sign that can be used to determine the triage categories of patients and identify those who require hospitalization.

Keywords: Emergency triage, end-tidal carbon dioxide, triage category, vital sign, hospitalization

INTRODUCTION

The increase in the number of patients seeking emergency care and the overcrowded nature of emergency departments make it difficult to provide effective care for critical patients [1]. Therefore, patients presenting to the emergency department are evaluated by grouping them according to the urgency of their conditions [2]. Various triage systems have been developed to perform this categorization, with the most commonly adopted being the Australasian Triage Scale, the Emergency Severity Index, the Manchester Triage Scale, and the Canadian Triage and Acuity Scale [3].

Many triage systems are based on patients' vital signs (VSs) measured at the time of their presentation to the emergency department. VSs are obtained by measuring physiological parameters, such as pulse, blood pressure, respiratory rate, oxygen saturation, and body temperature [4]. In many studies, VSs measured at the time of presentation to the emergency

department in all age groups have been associated with hospitalization requirements and in-hospital mortality [5-7]. VSs are very useful for emergency services since they are obtained using non-invasive techniques. However, the literature suggests that VSs alone may be insufficient for initial patient evaluation [8].

End-tidal carbon dioxide ($ETCO_2$) refers to the partial pressure of CO_2 available at the end of expiration, and the monitoring of this parameter provides information concerning the quality of ventilation and perfusion [9]. $ETCO_2$ has been the subject of numerous resuscitation studies. It has been shown that $ETCO_2$ is associated with the quality of resuscitation [10,11]. Due to its relationship with ventilation and perfusion, $ETCO_2$ has also been used in the triage of trauma patients and has been found to be successful [12,13].

Among patients presenting to the emergency department, it is essential to rapidly identify those who are likely to progress into mortality or morbidity, i.e., those who require hospitalization, and provide the appropriate treatment. For this purpose, VSs measurements are performed during triage. However, factors such as the noisy and stressful nature of the emergency department, measurement being dependent on the knowledge and experience of healthcare personnel, drugs used by patients, previous diseases, and age can affect VSs [14,15]. Therefore, there is a need for more objective methods to reliably demonstrate the clinical status of patients at the time of triage. In this study, we aimed to evaluate the relationship of $ETCO_2$, a ventilation and perfusion indicator, with triage categories and determine its ability to predict hospitalization indications in comparison with VSs.

Main Points;

- Although ETCO2 measurement is a parameter that is continuously measured during resuscitation, instantaneous ETCO2 measurements can provide information about the global perfusion status of patients.
- In this study, we found that ETCO2 values, which are an informative parameter for the determination of mortality and termination of resuscitation, can be used together with other vital signs to determine the triage category in emergency services.
- If this result can be strengthened by multicenter studies evaluating vital signs and ETCO2 together in emergency services, we believe that triage categories can be determined better.
- A single ETCO2 measurement was included in this study. Also, repeated ETCO2 measurements of patients will provide more reliable results.

MATERIALS AND METHODS

Study Design

This prospective, observational, cross-sectional study was conducted between May 3, 2023, and June 1, 2023, at the emergency department of a tertiary hospital. Local ethics committee approval was obtained for the study (ethics committee number: 3/14, date 05/02/2023). The study was carried out in accordance with the tenets of the Declaration of Helsinki. The total number of patient presentations to our emergency department is approximately 223,500/year; however, the number of applications per day varies. Therefore, in order to ensure that the patients to be included in our study were realistic, data collection was performed over one week on a full-time basis, using the number of patients on the same dates in the last two years as a reference.

Study Population and Patient Selection

At the time of presentation to the emergency department of our hospital, the triage categories of the patients are determined according to the five-step triage system. Very urgent patients who cannot be kept waiting in the emergency department (level 1 triage category according to the five-stage triage system) are provided care in the resuscitation room. Considering that the treatment of these patients is a priority, their $ETCO_2$ values were not measured to ensure that no time was wasted, and therefore these patients were excluded from the study. In addition, patients who do not have an emergency (level 5 triage category according to the five-stage triage category) are provided care in the green zone of our hospital's emergency department. These patients were also not included in the study since they did not require any urgent examination or treatment. All other patients are managed in yellow and red zones according to their VSs, previous diseases, and medical histories and categorized into triage levels 2, 3, and 4. This group constituted the population of our study. We also excluded patients who did not accept to participate in the study or who left the hospital without a doctor's approval while their examination and treatment were ongoing at the emergency department.

Patients aged >18 years who presented to the emergency department over the study period and were classified into triage category levels 2, 3, or 4 were included in the study. During the study period, a total of 1,864 patients were identified having presented to the emergency department and meeting the specified criteria. In five of these patients, the triage category was increased to level 1 because they developed a

cardiopulmonary arrest during their examination and treatment at the emergency department. Therefore, these patients were excluded from the study. A further 226 patients refused treatment or left the emergency department before the end of their examination or treatment. In addition, 148 of the patients did not want to participate in the study, and the triage category of 132 patients was not determined according to the five-triage system; therefore, these patients were also excluded from our study. Lastly, 253 patients whose VSs were not completely recorded, were excluded. As a result of the application of the inclusion and exclusion criteria, a total of 1,100 patients were included in the sample (Figure 1).

Data Collection and Recording

At the time of presentation to the emergency department, the reasons for patients' referrals were recorded in patient files by experienced triage nurses with at least three years of professional experience who had attended annual vocational training programs and received training on triage categories. Then, the patients' pulse, blood pressure, body temperature, and oxygen saturation values were recorded. The patients' $ETCO_2$ values (Capnostream-20, Medtronic, Israel) were measured using a nasal cannula and recorded in patient files at the time of presentation.

The triage categories of the patients were determined according to their VSs and complaints, according to the five-step triage system used in the Canadian triage system [16]. Level 1 patients who were very urgent and required simultaneous treatment were evaluated in the resuscitation room. In addition, level 5 patients whose complaints were not urgent and whose VSs were stable were separated to be evaluated in the green zone. The remaining patients were classified into levels 2, 3, or 4 and evaluated in relevant zones. The discharge or hospitalization status of these patients was recorded in their files. Age, gender, VSs, $ETCO_2$ values, triage categories, and outcomes (hospitalization or discharge) recorded in the files of the patients were transferred to the electronic environment.

Statistical Analysis

In this study, statistical analyses were performed using the IBM SPSS package program v. 25.0. The Kolmogorov-Smirnov test was used to evaluate the normality of the data distribution. Categorical variables were given as frequency and percentage, and continuous variables as mean and standard deviation. The chi-square test was conducted for the analysis of categorical variables. In the analysis of continuous variables, the Wilcoxon test was used for data showing a normal distribution, and the Kruskal-Wallis test for data that were not normally distributed. The Pearson correlation test was performed for the correlation analysis of the variables. The relationship of $ETCO_2$ and VSs with patient outcomes at the emergency department was investigated with a receiver operating characteristic (ROC) curve analysis, and the area under the curve values were calculated. In all analyses, $P < .05$ was considered statistically significant.

RESULTS

The mean age of the patients included in the study was 49.3 ± 18.8 years, and 645 (58.6%) of the patients were female. The mean $ETCO_2$ value was 28.1 ± 3.5. The triage category was level 2 in 148 (13.5%) patients and level 4 in 757 (68.8%) patients. The demographic characteristics of the patients and findings obtained at the time of their presentation to the emergency department are given in Table 1.

Table 1. Demographic characteristics of the patients and their findings at the time of admission to the emergency department

Variables	Median ± SD (min-max), n (%)
Age (years)	49.3 ± 18.8 (18-98)
Gender	
Male	455 (41.4%)
Female	645 (58.6%)
Systolic blood pressure (mmHg)	138.6 ± 26.1 (84-253)
Diastolic blood pressure (mmHg)	82.4 ± 15.8 (40-126)
Pulse (/minute)	90.7 ± 16.9 (50-175)
Body temperature (C°)	36.4 ± 0.5 (35.7-39.7)
Saturation (%)	92.7 ± 4.9 (50-100)
$ETCO_2$ (mmHg)	28.1 ± 3.5 (18-38)
Triage category	
Level 2	148 (13.5%)
Level 3	195 (17.7%)
Level 4	757 (68.8%)
Patient outcome	
Discharge	942 (85.6%)
Hospitalization	158 (14.4%)

SD: standard deviation; $ETCO_2$: end-tidal CO_2

Table 2 shows the comparison of the patients according to the triage category. Accordingly, the mean ages of level 2, 3, and 4 triage categories were 55.3 ± 16.8, 52.4 ± 18.4, and 47.4 ± 18.9 years, respectively, indicating statistically significant differences ($P < .001$). The mean $ETCO_2$ of the level 2, 3, and 4 triage

categories was 27.1 ± 3.6, 30.6 ± 3.1, and 35.4 ± 3.5, respectively, which also statistically significantly differed between the groups (P < .001).

The correlation of $ETCO_2$ with the triage category and other parameters is given in Table 3. Accordingly, $ETCO_2$ was positively correlated with the triage category (P < .001). and saturation at statistically significant levels (P < .001 for both).

Figure 2 presents the correlations of $ETCO_2$ and VSs with patient outcomes at the emergency department. Accordingly, the area under the ROC curve value of $ETCO_2$ was 0.733, which was statistically significant (P < .001). Other parameters, such as pulse, body temperature, and saturation, were also statistically significant in terms of patient outcomes (P > .05) (Figure 2, Table 4).

Table 2. Comparison of patients by triage category

Variables, median ± SD (min-max), n (%)	Level 2	Level 3	Level 4	p value
Age (years)	55.3 ± 16.8 (20-98)	52.4 ± 18.4 (18-90)	47.4 ± 18.9 (18-91)	**<.001**
Gender				
Male	66 (6%)	75 (6.8%)	314 (28.5%)	.460
Female	82 (7.5%)	120 (10.9%)	443 (40.3%)	
Systolic blood pressure (mmHg)	135.8 ± 24 (84-245)	144.5 ± 28.1 (85-225)	145.1 ± 30.8 (89-250)	**<.001**
Diastolic blood pressure (mmHg)	82.7 ± 17.5 (40-120)	85 ± 16.9 (44-126)	87.7 ± 15.1 (40-125)	**.040**
Pulse (/minute)	89 ± 17.8 (51-141)	89.7 ± 16.7 (56-156)	91.3 ± 16.8 (50-175)	.400
Body temperature (C°)	36.4 ± 0.4 (35.7-38.4)	36.4 ± 0.6 (35.8-39.7)	36.4 ± 0.4 (35.7-39.1)	.978
Saturation (%)	89.5 ± 9.2 (50-100)	92.9 ± 4.1 (62-99)	93.2 ± 3.6 (71-100)	**<.001**
$ETCO_2$ (mmHg)	27.1 ± 3.6 (19-38)	30.6 ± 3.1 (18-36)	35.4 ± 3.5 (19-38)	**<.001**
Patient outcome				
Hospitalization	41 (27.7%)	36 (18.5%)	81 (10.7%)	**<.001**
Discharge	107 (72.3%)	159 (81.5%)	676 (89.3%)	

SD: standard deviation; $ETCO_2$: end-tidal CO_2

Table 3. Correlation of $ETCO_2$ with the triage category and other parameters

$ETCO_2$	Age	Gender	Triage category	Saturation	Patient outcome
r	-0.108	-0.027	0.140	0.096	0.215
p	**<.001**	.363	**<.001**	**.001**	**<.001**

$ETCO_2$: end-tidal CO_2

Table 4. Results of ROC curve analysis of $ETCO_2$ and VSs in terms of patient outcomes at the emergency department.

Variables	AUC	SE	95% CI	P value
$ETCO_2$	0.733	0.023	0.687-0.779	**<.001**
Systolic blood pressure	0.509	0.030	0.450-0.569	.733
Diastolic blood pressure	0.551	0.028	0.496-0.605	.069
Pulse	0.426	0.030	0.367-0.485	.008
Body temperature	0.440	0.028	0.386-0.495	.032
Saturation	0.617	0.029	0.561-0.673	**<.001**

Note: ROC: receiver operating characteristic; $ETCO_2$: end-tidal carbon dioxide; VSs: vital signs; AUC: area under the curve; SE: standard error; CI: confidence interval

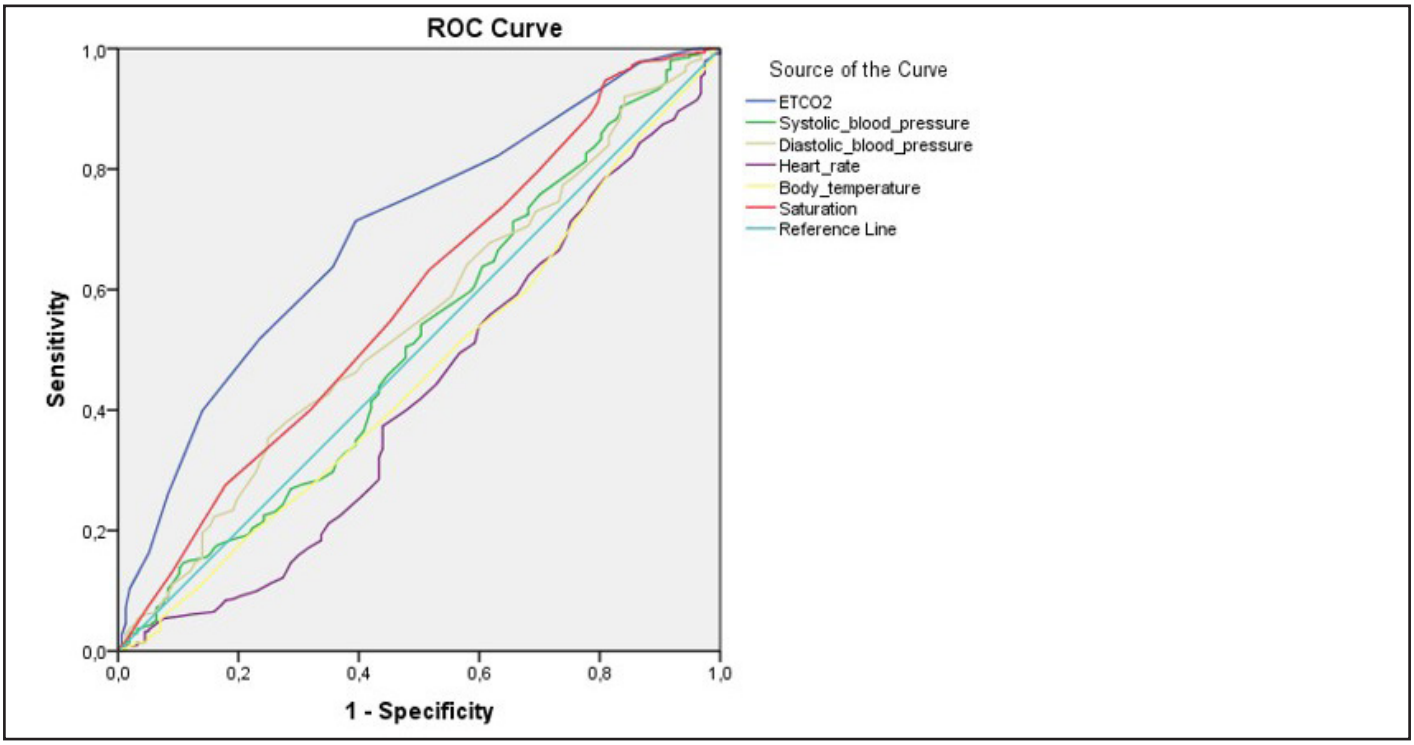

Figure 1. Flow chart of patient selection.

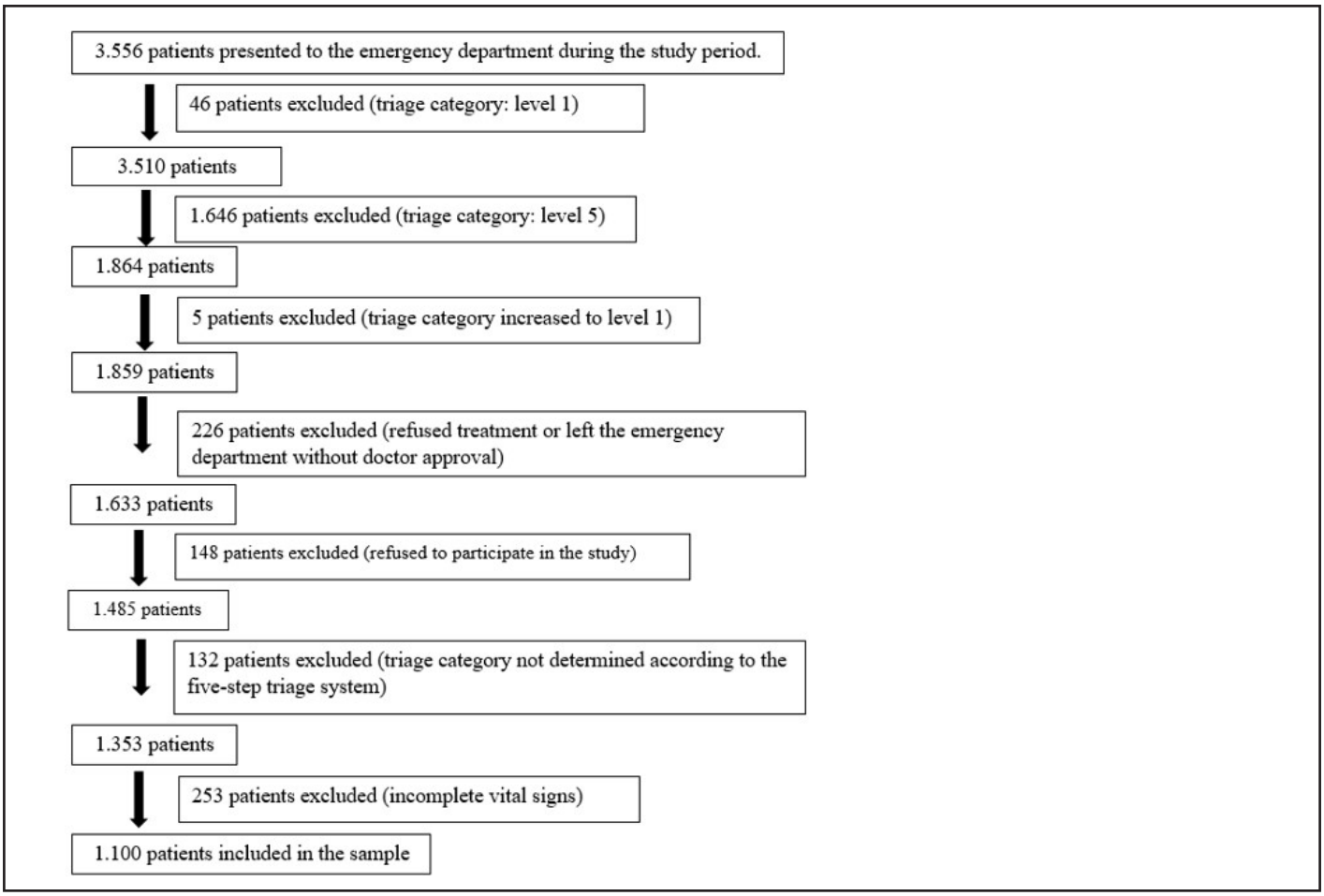

Figure 2. Correlation of $ETCO_2$ and VSs with patient outcomes at the emergency department

DISCUSSION

On completion of this study, it was concluded that the $ETCO_2$ values measured at the time of presentation to the emergency department can be new VSs that can be used to determine the triage categories of patients. In addition, it was determined that $ETCO_2$ values were more valuable than remaining VSs in identifying patients who required hospitalization.

$ETCO_2$ is an important part of the care provided for critically ill patients since it is considered very useful in assessing the quality of ventilation and perfusion of patients. It has been found to be particularly successful in predicting hemorrhagic shock, transfusion and surgical treatment requirements, and mortality in trauma patients [17]. Compared to standard VSs, $ETCO_2$ has been reported to be more successful in predicting mortality in trauma patients [18]. In addition, in a study evaluating patients with sepsis, $ETCO_2$ values were found to be valuable in the evaluation of mortality; however, in that study, $ETCO_2$ values did not have the discriminative ability to identify patients who required hospitalization [19]. It is known that $ETCO_2$ is also significant in the evaluation of the prognosis of patients with dyspnea and even in differentiating between cardiac and non-cardiac dyspnea [20]. Duyan et al. reported $ETCO_2$ to be valuable in differentiating non-cardiac chest pain from unstable angina [21]. Contrary to similar previous studies, in our study, we did not evaluate the relationship between $ETCO_{2,}$ which has an undisputable place in the literature, and hospital mortality. We evaluated the utility of the $ETCO_2$ values measured at the time of presentation to the emergency department in accurately identifying the triage category of patients. In our study, the $ETCO_2$ values of the patients in the level 2 group were lower than those of the patients in the remaining triage categories, since level 2 cases included those that had been exposed to multi-trauma and patients with sepsis, chest pain, and dyspnea. In addition, unlike other studies, when we used $ETCO_2$ to predict the hospitalization requirements of the patients, we determined that the selectivity of $ETCO_2$ was higher than that of standard VSs. This may be related to the low saturation values of the patients in this group.

VSs play an important role in determining the clinical risk of patients in emergency departments or inpatient clinics. However, deteriorations in VSs are often overlooked in the absence of clinical worsening or they are not detected until it is too late for treatment [6]. The main reason for this may be the incomplete recording of VSs, inappropriate response to abnormal values, and the insufficient flow of information on VSs among nurses and doctors [22]. The importance of VSs monitoring in clinical practice is indisputable; however, it remains unclear which VS is more associated with the clinical risk of patients, at what interval VSs should be evaluated, and the best way to monitor them [6]. There are many studies in the literature examining the relationship between VSs measured at the time of presentation to the emergency department and mortality [23-25]. However, only a few studies have explored the relationship between VSs alone and hospitalization indications. For example, in a study by Hong et al., pulse and blood pressure values were found to be unrelated to patients' mortality within the first 72 hours and 30 days or their risk of admission to the intensive care unit [23]. In the same study, it was also reported that the saturation value was valuable in predicting the clinical risks of patients [23]. In a study evaluating one-day mortality in the emergency department, Ljunggren et al. evaluated saturation, systolic blood pressure < 90 mmHg, and heart rate < 50/minute or >110/minute as determinant factors for mortality [24]. In another study, Tsai et al. observed that pulse, saturation, and systolic blood pressure were valuable in predicting in-hospital cardiac arrest among patients visiting the emergency department [25]. In addition, in the three studies mentioned above [23-25], increasing age was found to be associated with mortality. In the current study, we investigated the relationship between VSs and the triage category of patients presenting to the emergency department triage and evaluated the ability of VSs alone to predict hospitalization indications. We found statistically significant differences in the systolic blood pressure, diastolic blood pressure, and saturation values according to age. However, only the saturation value was clinically significant. When the VSs of the patients included in our study were compared in terms of their predictive ability for hospitalization, it was observed that saturation was more successful than the remaining parameters. This may be because the patients included in the study were from a heterogeneous group.

Erroneous measurements that may occur during the VSs evaluation may be due to reasons independent of the patient, such as the external environment and stress factors affecting the VSs, as well as the experience of the practitioner. In addition, VSs can be misleading for emergency services due to patients' chronic diseases, drug use, or older age that may affect these values [14,15]. Therefore, VSs may not provide the necessary assistance in accurately triaging of patients evaluated at the emergency department or rapidly identifying patients who

require hospitalization. Thus, there is still a need for new triage variables to be used for this purpose.

Limitations

The major limitation of our study is that VSs and $ETCO_2$ values were measured by eight different triage nurses. However, when measuring VSs and $ETCO_2$, great care was taken to record the measurements accurately and in a timely manner. Although necessary training was provided for each of the triage nurses, we did not have the opportunity to check the accuracy of the data recorded. In addition, our study was conducted with a very heterogeneous patient group. Therefore, we were not able to group patients according to their final diagnoses. Furthermore, we excluded level 1 and level 5 patients. Although we consider that the identification of level 1 patients was made accurately, level 5 patients may have been miscategorized by triage nurses. Therefore, these patients were excluded from the study. Additionally, our study was single-center and short research period of time. This may have affected the heterogeneity of errors included in the study. Another limitation is that since VSs and $ETCO_2$ are frequently evaluated as mortality markers in the emergency department, they were not evaluated in our study to avoid repetition of the literature.

CONCLUSION

We consider that $ETCO_2$ can be used as a new VS to allow the accurate determination of triage categories at the emergency department and prevent long waiting times in the emergency department for patients who require hospitalization. Thus, emergency physicians can make more objective decisions by eliminating external factors that may affect VSs.

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Original Research

Factors Affecting Treatment Compliance of Patients with COPD During the COVID-19 Pandemic

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ABSTRACT

Objective: This study aimed to investigate the effects of difficulties experienced in the follow-up and treatment during the Coronavirus 2019 pandemic (COVID-19p), which included COVID-19 phobia and depression, on treatment compliance in patients with Chronic Obstructive Pulmonary Disease (COPD).

Methods: This study included 123 patients with COPD. The COVID-19 Phobia Scale (C19P-S), Beck Depression Inventory (BDI), and Medication Adherence Report Scale (MARS) were used to assess the patients.

Results: The mean age of patients with COPD was 64.56 ± 9.31 years. It was determined that our patients did not maintain regular outpatient follow-up mostly because of the fear of COVID-19 transmission (75.9%). Overall, 24% of our patients showed treatment noncompliance. A statistically significant relationship was found between COPD stages B and D and treatment compliance (p=0.01). Patients with frequent emergency department admissions (p = 0.01) and those with high BDI (p = 0.01) and C19P-S (p = 0.02) scores during the pandemic were found to have reduced treatment compliance.

Conclusion: Patients with COPD with COVID-19 phobia, and depression had reduced treatment compliance. İt is necessary to be aware of these conditions and to plan appropriate interventions.

Keywords: Coronaphobia, Depression, Compliance, Treatment, Chronic obstructive pulmonary disease

INTRODUCTION

Chronic Obstructive Pulmonary Disease (COPD) is one of the leading causes of morbidity and mortality worldwide [1]. Regular monitoring of exposure to risk factors, symptoms, and treatment effectiveness in disease management is recommended. Although effective treatments are available for COPD management, treatment noncompliance among patients with COPD is a major issue [2].

The COVID-19 pandemic has adversely affected daily life activities. Strict measures, disruption in daily routines, fear of transmission and restrictions on health services cause widespread anxiety, fear, and depression among individuals in a society [3,4].

COVID-19 anxiety, also known as “Corona phobia,” which is based on fear and anxiety refers to the state of constant and excessive fear of COVID-19 transmission, intolerance of

uncertainty, inappropriate behavior due to fear of transmission, emotional stress, and avoidance of people [3]. The fear of COVID-19 was more prevalent in Polish patients with taking anticoagulants, women and elderly [5]. However, the effect of COVID-19 phobia on treatment compliance of chronic diseases, especially how it affects compliance with COPD treatment, is not well known.

This study aimed to investigate the effects of depression, COVID-19 phobia, restrictions for pandemic control, transportation problems, and difficulty in accessing the health system during the pandemic on the treatment compliance of patients with COPD.

MATERIALS AND METHODS

This study is a single-center, cross-sectional study conducted between February 1, 2021 and March 30, 2022 in the Chest Diseases Clinic in a tertiary healthcare institution.

Patients who were diagnosed with COPD at least 3 months before the onset of the pandemic; aged >40 years; who had no communication, hearing, or speech problems; and who agreed to participate in the study were included in this study. The patients were informed with written consent form approved by the ethics committee of the institution, and patient consent was obtained. The sample size of the study was calculated by using power analysis. Minimum sample size was calculated as 123 (α=0.05), 1-ß=0.80). The patients included in the study were COPD patients whom we had previously diagnosed in this clinic in accordance with the GOLD guideline:

Stage A: Those who have no history of exacerbation in the last year or who have had one exacerbation that did not require hospitalization, who only have shortness of breath during heavy exercise or when walking fast on a flat road.
Stage B: Those who have no history of exacerbation in the last year or who have had one exacerbation that did not require hospitalization, who have to walk slower than their peers due to shortness of breath, or who have shortness of breath when walking 100 meters on a flat road, or who have shortness of breath at rest.

Stage C: Those who have had two or more exacerbations in the last year or have had one or more exacerbations requiring hospitalization and have shortness of breath only during heavy exercise or when walking fast on flat ground.

Stage D: Those who have had two or more exacerbations in the last year or have had one or more exacerbations requiring hospitalization and who have to walk slower than their peers due to shortness of breath or have shortness of breath when walking 100 meters on a flat road or have shortness of breath at rest [6].

Questionnaire form was prepared by the researchers by examining related literature [1-3]. This form had 19 questions regarding the sociodemographic and COPD disease-related characteristics of the participants.

The COVID-19 Phobia Scale (C19P-S): In order to evaluate the COVID-19 phobia status of patients, Arpacı et al. developed. It is a 20-item, 5-point Likert-type self-report scale developed to assess phobia in patients. The scale items are rated as follows: 1. I strongly disagree, 2. I disagree, 3. I agree, 4. Overall, I agree, and 5. I strongly agree. These scores ranged from 20 to 100 [7].

Beck Depression Inventory (BDI): was used to assess the depression status. The BDI scale consisted of 21 questions [8]. The Turkish validity and reliability test of the scale was conducted by Hisli et al. The questions were scored between 0–3 [9].

Medication Adherence Report Scale (MARS): The Medication Compliance Report Scale (MARS) was used to analyze treatment compliance [10]. The validity and reliability of the Turkish version were analyzed by Şen et al 2019 [11]. The scores for each item were added to determine the total score, which ranged between 5 and 25 High MARS scores indicated high levels of compliance.The patients were classified into two groups as ‘Compliant’ (MARS score $\geq$ 23) and ‘Noncompliant’ with treatment (MARS score < 23) [12]. The total scores obtained from each scale were recorded in the questionnaire form.

The questionnaire form and scales were filled by researchers using a face-to-face interview technique in the pulmonology department. Due to the few number of patients who applied to the outpatient clinic due to pandemic conditions, 30 patients were reached by phone and a questionnaire form and scales were applied.

Data were statistically analyzed using IBM SPSS Statistics (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.) on P5 to the Statistical Analysis.

Data Assessment

The patients were classified into two groups as 'Compliant' and 'Noncompliant' with treatment [12]. Treatment compliance was considered as the dependent variable. The association between categorical variables was analyzed using chi-square test. The association between numerical variables was tested using a correlation coefficient. For the comparison of scalar data between the subgroups of two categorical variables, Student's t-test was used for the normally distributed data, whereas the Mann–Whitney U test was used for the non-normally distributed data. The one-way analysis of variance or Kruskal–Wallis test was used to compare numerical variables in three independent groups. Binary logistic regression was applied to evaluate the COPD stage, frequency of emergency department admissions during the pandemic, BDI and C19P-S scores, which were associated with treatment compliance. P value of <0.05 was considered statistically significant.

RESULTS

The mean age of 123 patients with COPD included in this study was 64.5 ± 9.3 years, and 92.7% were male. The rates of comorbid diseases and smoking were 56.9% and 24.4%, respectively. Most patients were in COPD stages D (52.8%) and B (31.7%). The general characteristics and treatment compliance status of the patients are summarized in the Table 1. Overall, 75.6% (93) of the patients were compliant with the treatment. Overall, 24,4% (n = 30) of our patients were noncompliant with the treatment. A statistically significant relationship was found between COPD stages B and D and treatment compliance (p=0.01). Treatment compliance in stages B and D was less than in other stages.

Further, patients with frequent emergency department admissions (p = 0.01) and those with high BDI (p = 0.001) and C19P-S (p = 0.02) scores during the pandemic were found to have reduced treatment compliance. The binary logistic regression analysis of significant variables revealed that an increase in the BDI score by 1 unit increased the noncompliance with treatment by 1.15 units.

The frequency of admission to the emergency department due to COPD did not change in patients during the pandemic period, but outpatient follow-up decreased in 42.3% of them during the pandemic period (Table 1).

It was determined that the most common reason among patients for not visiting the outpatient clinic for regular follow-up was the fear of coronavirus transmission during the pandemic (33.3%). Patients who felt well, regardless of the pandemic restrictions, also missed their outpatient clinic visits (%4.9) (Table 2).

Variables	Number of patients (%. n)	Noncompliant with Treatment (%. n)	Compliant with Treatment (%. n)	p value
Age (Mean ± SD)	64.5±93.1	62.72±9.14	65.12±9.3	0.23
Sex				0.36
Male	92.7 (114)	23 (26)	77(77	
Female	7.3 (9)	33.3 (3)	66.7 (6)	
Education				0.73
Educated	109	24.8 (27)	75.2 (82)	
Uneducated	13	15.4 (2)	84.6 (11)	
Smoking				0.35
Not smoke	10.7 (13)	23.1 (3)	76.9 (10)	
Quit smoking	64.5 (78)	26.9 (21)	73.1 (57)	
Smoking	24.8 (30)	16.7 (5)	73.1 (57)	

Comorbid diseases				0.07
Yes	57.98 (69)	21 (30.4)	46 (696)	
No	42.02 (50)	8 (16)	42 (84)	
Number of drugs used for comorbid diseases	2 (2)	2.2 (2.5)	2.9 (1.9)	0.24
COPD stage				**0.01****
A	5.7 (7)	0 (0)	5.7 (7)	
B	30.9 (38)	10.5 (4)	31.1 (38)	
C	9.8 (12)	16.7 (2)	9.8 (12)	
D	35.4 (23)	64.6 (42)	53.3 (65)	
Number of inhalers used for COPD	2 (0.9)	2 (0.9)	1.8 (0.7)	0.37
Treatment regimen during the prepandemic period				0.08
Regular	95.8 (115)	21.7 (25)	78.3 (90)	
Irregular	4.2 (5)	60 (3)	40 (2)	
Outpatient follow-up during the prepandemic period				0.86
Regular	16.5 (20)	22.7 (20)	77.3 (68)	
Irregular	6.6 (8)	24.2 (8)	75.8 (25)	
Frequency of emergency service admission during the pandemic period				**0.01****
Increased	23.8 (28)	39.3 (11)	60.7 (17)	
Unchanged	50.4 (61)	13.1 (8)	86.9 (53)	
Decreased	27 (33)	30.3 (10)	69.7 (23)	
Frequency of hospitalization during the pandemic period				0.07
Increased	12.3 (15)	46.7 (7)	53.3 (8)	
Unchanged	66.4 (81)	19.8 (16)	80.2 (65)	
Decreased	21.3 (26)	23.1 (6)	76.9 (20)	
Outpatient follow-up during the pandemic period				0.12
Increased	13.1 (1)6	43.8 (7)	56.3 (9)	
Unchanged	44.3 (54)	22.2 (12)	77.8 (42)	
Decreased	42.6 (52)	19.2 (10)	80.8 (42)	
Beck Depression Inventory Scale score (mean ± SD)	11.9 (9.29)	20.2	9.15	**0.01***
COVID-19 Phobia Scale score (mean ± SD)	42.6 (12.5)	41.13	47.13	**0.02***

Note: SD: Standard deviation. *: Independent samples t-test. **: Chi-square test

Table 2. The reason for the decrease in outpatient control during the pandemic period

	Frequency	Percent
Fear of COVID-19 transmission	41	33.3
I could not get an appointment due to the restriction on outpatient clinic appointments.	1	0.8
I could't get out of house due to restriction	2	1.6
I could not provide transportation due to restrictions on public transportation	1	0,8
Due to restrictions imposed on elderly	2	1.6
No need for control because it feels good	6	4.9
Total	54	43.9

Table 3. Reasons for not using their medications regularly during the pandemic period

	Frequency	Percent
I couldn't get my medicines prescribed because I couldn't leave the house due to the fear of COVID 19 contamination.	5	4.1
I couldn't recipie drugs because I couldn't get an outpatient appointment due to COVID 19 restrictions.	1	0.8
I couldn't get drugs because I couldn't leave the house due to quarantine measures	1	0.8
Total	7	5.7

Our patients who received irregular treatment stated that they had difficulty for obtaining their medications because they could not go to the health institution because of the fear of COVID-19 transmission (%4.1) Restrictions on outpatient clinic appointments (%0.8) and quarantine measures (%0.8) prevented patients from obtaining their treatments (Table 3).

DISCUSSION

The appropriate use of adherence to daily controller medications is important for patients with respiratory illnesses during the coronavirus disease 2019 (COVID-19) pandemic [13]. In this study, 24,4 % of the patients were found to be noncompliant with treatment. Patients with higher COPD stage had less compliance to treatment. Patients with frequent emergency service visits and high BDI and C19P scores had higher treatment non-compliance. Studies conducted in the prepandemic period have shown that the rate of treatment noncompliance in patients with COPD were 40–60% [14,15]. There are few studies on treatment compliance rates in patients with COPD during the pandemic.

During the Covid 19 pandemic, Kaye et al. reported a noncompliance rate of 38.5–46.3% [13], whereas McAuley et al. reported a rate of 2.5% [16]. Different results may be due to the methods used for evaluating treatment compliance. Moreover, various studies in the literature have used electronic records and surveys completed by patients [17].

This may be because individuals with chronic diseases were informed and warned to continue their medications through media and social media during the pandemic. The high cost of treatment may a factor that reduces patients compliance with [15]. In Turkey COPD drug costs are covered by social insurance. All patients in this study had social security and could obtain their medications without paying any fee. In addition, the drugs prescribed for chronic diseases during the pandemic in Turkey were extended for one year without applying to health institutions. These situations may be the reason for the higher treatment compliance rates in this study.

The impact of the COVID-19 pandemic on adherence to chronic treatments has been discussed in chronic cardiovascular conditions and chronic autoimmune diseases and potential concerns have been raised about its impact on the short- and long-term outcomes of chronic diseases [18,19]. In these studies, treatment non-compliance was detected at rates of up to 24.4%.

We found that patients with advanced COPD stages were less likely to comply with treatment. Different results have been reported in the literature regarding the association between treatment compliance and COPD stage. Similarly, Park et al. reported that patients with advanced COPD had lower adherence to treatment [20]. This may be due to the complex treatment regimens and multiple drug administration in advanced stage patients. However, another study claim that patients with advanced COPD stage have better compliance as their symptoms are more severe [14].

In this study, we found that the most common causes of treatment non-adherence were COPD stage, COVID-19 phobia, depression level and frequency of emergency service admission during the pandemic period. However we did not find a statistically significant relationship between treatment non-adherence and age, gender, education level, number of comorbit diseases, number of drugs. Patients not compliant with treatment could not get their medicines due to pandemic conditions because they could not leave the house due to the fear of COVID-19 (5, 4.1%), quarantine measures (1, 0.8%), due to the fact that they cannot have their medications prescribed due to restrictions. Also, Zhang et al. reported that total of 27.3% of patients did not return to the hospital for a prescription for fear of the COVID-19 outbreak; 85.7% reported that they chose to visit the hospital whose condition worsened [21].

In this study, a higher number of emergency department admissions were reported in noncompliant patients (39,3%). Similarly, Chen et al. reported, noncompliance with treatment increases the frequency of admission to the emergency department (66 %) [22]. This situation suggested that the use of irregular and ineffective treatment may increase the number of attacks.

We found that patients with high COVID-19 phobia levels had lower compliance to treatment. Moreover, we found that patients with increased fear of COVID-19 had more frequent emergency admissions. This may be because these patients prefer applying to the emergency department owing to the restrictions imposed on outpatient clinic appointments during the pandemic.

This study we observed a significant association between the level of depression and non-compliance with treatment. Depression and anxiety were reported to be the most common psychological disorders in previous global epidemics [23]. A study conducted in the first two months of the pandemic revealed that the most important factor affecting treatment compliance was the level of depression [21].

Limitations

This study has several limitations; Data were collected from a single center and treatment compliance was evaluated based on patients self-reports. Another limitation of the study was that not all patients could be interviewed face to face due to pandemic conditions. A larger number of patients would be more beneficial for the study results.

CONCLUSION

One of the few studies reporting in the literature evaluating the relationship between fear of COVID-19 and compliance to treatment in COPD patients. We found that patients with COVID-19 phobia, depression, frequent emergency department admissions as well as those with advanced stage COPD had reduced compliance with treatment. For future epidemics or emergencies, it is necessary to be aware of these situations and plan appropriate interventions.

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Informed Consent: Participation in the study was based on voluntariness, and consent was obtained from all patients after being informed about the study by the researcher.

Author Contributions: Concept – SD; Design – SD, MT; Supervision – NB, SA; Materials – FF; Data Collection and/or Processing – SD, CS; Analysis and/or Interpretation –MT ; Literature Review –MU; Writing –SD; Critical Review –MU.

Ethics Committee Approval: Approval was obtained from Gaziantep University, Clinical Research Ethics Committee Committee to conduct the study (Dated 2021, Decision no 17). This study complied with the research and publication ethics. This study was carried out in accordance with the Helsinki Declaration.

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Original Research

Retrospective Analysis of Head and Maxillofacial Injuries: FIFA World Cup 2022 Report

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ABSTRACT

Objectives: The aim of this study is to analyze the incidence, causes and consequences of head and maxillofacial injuries in the 2022 FIFA World Cup.

Methods: This retrospective study, following the STROBE guideline, conducted an online investigation during the 2022 World Cup, with a specific focus on injuries resulting in player substitutions or absences of at least one match. To mitigate potential injury exaggeration, journalistic reports were prioritized over video analysis. The analysis of injuries involved the utilization of descriptive statistics, the Kolmogorov-Smirnov test, and the Pearson correlation test, with a significance threshold set at $p<0.05$. The analytical tools Microsoft Excel and RStudio were employed. Comparative insights were derived from previous FIFA World Cup data; however, injuries lacking sufficient recovery time were classified as preseason injuries, potentially introducing an element of bias to the analysis.

Results: During the FIFA World Cup 2022, a comprehensive analysis revealed a total of 123 injuries leading to player substitutions or subsequent game absences, of which seven were localized in the head or maxillofacial region. Statistical analysis indicated a departure from normal data distribution, and a robust correlation was observed between the number of players in national leagues and injury incidence. Noteworthy is the participation of 42 distinct national soccer leagues in contributing players to the tournament's national squads, with no specific league demonstrating a predisposition to higher injury rates. Within the dataset, three maxillofacial injuries were identified. It is of interest that five athletes opted for facemasks as protective measures for the maxillofacial region, and remarkably, only one of them experienced an injury during the World Cup but subsequently resumed play in subsequent matches, while the remaining four athletes had sustained injuries prior to the tournament.

Conclusion: The 2022 FIFA World Cup was associated with a low number of head and maxillofacial injuries, with collisions with other players being the most common cause. Despite their limited occurrence, these injuries can have severe implications. In response to the increase in concussions, FIFA implemented an improved safety protocol, which involves immediate game

halts for medical assessment and allows player substitutions without affecting the team's allotted substitutions. This change has been well-received by stakeholders. Furthermore, the use of protective equipment, such as custom-made shields, is growing in popularity and has the potential to reduce injury severity and shorten recovery time. Modern technology enables the creation of comfortable and effective protective gear, enhancing player safety. Overall, the study emphasizes the importance of injury prevention strategies in sports, calling for continued advancements in protective equipment design and increased transparency in injury reporting.

Keywords: Athletic Injuries; Facial bones; Maxillofacial Injuries; Sports Medicine; Soccer; Digital Planning; Virtual Planning; Protective Devices; Digital Technology

INTRODUCTION

Soccer is the most played sport in the world, as the Fédération Internationale de Football Association (FIFA) estimates that there are more than 200 million players worldwide [1,2]. Sports injuries constitute a recognized main cause of maxillofacial injuries. Due to the high number of players worldwide, soccer is among the most common sport associated with injuries worldwide [3,4]. Although incidences of trauma in other anatomical regions are more common, especially lower limbs, facial trauma is very common in soccer, and most frequently involves the nose [5] and middle third of the face [4,6]. There is an incidence of 12.5 head injuries per 1,000h played [7]. A remarkable increase in the number of sports-related injuries has been documented in the past 20 years [8–11].

FIFA World Cup is a soccer tournament that takes place every four years and is responsible for audiences that reach the entire globe [12]. The latest tournament, held in Qatar from November 20 to December 18, 2022, had 32 countries represented by about 830 different players. The increasing numbers show the connection between growing physical vitality and the occurrence of maxillofacial trauma. For this reason, an important question that arises is the need for quick rehabilitation in professional athletes [13].

The purpose of this paper is to discuss the frequency of maxillofacial injuries encountered during the championship, and future implications for the players.

Main Points;

- The article highlights that the frequency of head and maxillofacial injuries during the World Cup and its preseason is very low, with only 5.69% of injuries, mainly related to athlete substitution or absence.
- While the overall number of soccer-related injuries remains relatively stable, the article points out that the severity of these injuries is increasing over time, with concussions and facial fractures being observed more frequently in soccer.
- The article discusses how sports organizations like FIFA and the International Olympic Committee are refining their safety protocols, especially in cases of concussion injuries. They now require immediate medical attention, player substitution, and decisions made by medical staff rather than players themselves.
- The use of protective facial and head shields is on the rise in soccer, particularly among players with a history of previous injuries. Modern technology, such as computer-aided design and manufacture, is allowing for the creation of comfortable and effective protective gear, which can significantly reduce the severity of injuries and allow for shorter recovery times.

MATERIALS AND METHODS

This is a retrospective study and has followed the STROBE guideline [14]. Online research was conducted from the start of the World Cup on 20th November until December 20, 2022 (Table 1). The data is publicly available and does not require a separate ethical approval or written consent. Only injuries which resulted in player substitution during a match or absence for at least one game were considered. Literature review with the following strategies were used: 1. (FIFA World Cup) AND (maxillofacial OR face OR facial OR head) AND (injury OR fracture OR damage), 2. (soccer[title] maxillofacial injuries). Data were sorted in Microsoft Excel ® (Microsoft, Redmond, WA, USA). When available, data from previous FIFA World Cups were used as a comparison. The authors opted for journalistic reports rather than video analysis because some athletes might tend to dramatize and magnify the injury suffered. Any injury with not enough recovery time was considered in the preseason. This could impair the analysis of injuries [15].

Statistical Analysis

Injuries were calculated and summarized using descriptive statistics. For relevant data, the Kolmogorov-Smirnov test and the Pearson correlation test were used to comparison. Results with a $p<0.05$ were considered statistically significant. The analyses were performed with Microsoft Excel ® and RStudio ® (RStudio, GNU GPL).

RESULTS

A total of 123 injuries that resulted in player substitution or absence from a subsequent game were retrieved during the FIFA World Cup 2022. Seven of these injuries were in the head or maxillofacial region. Figure 1 shows the anatomical location of the injuries. Data were considered non-normally distributed ($d = 0.78193$, $p < 2.2e\text{-}16$). There was a strong correlation between the number of players in the national leagues and the number of injuries ($\rho=0.966037$, $p=0.001008$). Forty-two different national soccer leagues have contributed to the national squads participating in the tournament. None of these leagues have been identified as being particularly more dangerous to causing injuries. There were three maxillofacial injuries in 2022 FIFA World Cup (Table 2).

Five athletes used facemasks to protect the maxillofacial region during FIFA Soccer World CUP 2022 (Table 3). Only one of them was injured during the World Cup and returned to play in the following matches (Alireza Beiranvand, from Iran). The remaining four were injured previously to the World Cup.

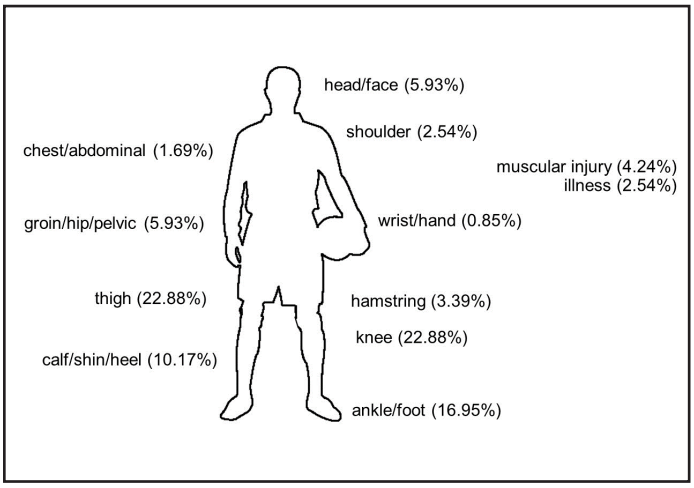

Figure 1. The anatomical location of the injuries.

Table 1. Websites used in online search.

Yahoo Sports	https://sports.yahoo.com/
International Olympics Committee	https://olympics.com
FIFA	https://fifa.com
Premier League Injuries	https://www.premierinjuries.com
Sporting News	https://www.sportingnews.com
NBC Sports	https://www.nbcsports.com

Table 2. Athletes with head or maxillofacial injuries during 2022 FIFA World Cup in order of occurrence.

Name	Country	Position	Type of collision	Against	Outcome
Alireza Beiranvand	Iran	goalkeeper	Head-to-head	Teammate	Concussion
Yasser Al-Shahrani	Saudi Arabia	defender	Knee-to-head	Teammate	Mandible, Midface fracture
Adrien Rabiot	France	midfielder	Head-to-head	Opposite team	Concussion

Table 3. Athletes played 2022 FIFA World Cup with protective face masks (alphabetic order).

Country	Name	Anatomic area injured
Iran	Alireza Beiranvand	Facial and nose injury
Tunisia	Ellyes Skhiri	Facial middle third
South Korea	Heung-Min Son	Orbit
Croatia	Josko Gvardiol	Nose
Belgium	Thomas Meunier	Facial middle third

DISCUSSION

The frequency of head and maxillofacial injuries during the World Cup and its preseason can be considered very low (5.69%) as stated before in the literature [1,12,15]. Approximately 9% to 18% [1,12,15] decreases to about 6%, but this can be explained since only injuries associated to athlete substitution or absence were included in the present report. Although few in number [1], these injuries may be severe and have significant implications for the players involved as well as the national squads and club teams [16]. Images showing maxillofacial injuries sustained by the Saudi Arabian player Mr. Yasser Al-Shahrani appeared quite remarkable and took the social media by storm. Although the overall number of soccer-related injuries remain relatively stable, the severity is increasing over time [5]. Concussions and facial fractures are not uncommon in contact sports and are observed more frequently in soccer [3]. Most of injuries involving the head and maxillofacial region tend to be associated with collision of heads involving a player of the opposite team [5]. This may be different to some other sports in which injuries may result from to falls to the ground or direct trauma from the ball as in cricket or hockey [3,4].

In the 2014 FIFA World Cup, an Argentinian player, Javier Mascherano suffered a concussion as did the Uruguayan player Alvaro Pereira in another match. These incidences prompted FIFA to refine their safety protocol for concussion injuries necessitating the game to be stopped immediately and seek medical attention [1]. If during the assessment, the concussion injury is considered a cause of concern, the involved player is substituted, and this substitution does not count towards the allocated quota of substitutions permitted for the team. This change has been viewed positively by the relevant stakeholders including player, coaching staff and the fans at large. The concussion injury suffered by the French national midfielder Adrien Rabiot was the first one to be dealt under this new protocol. The final decision regarding the fitness of the player is determined by the medical staff and not by the player. Not only FIFA, but other sports bodies such as the International Olympic Committee (IOC) are also amending their legislation to promote fair play, and improve player safety [1,17]. Other measures as harder penalties for the offending players and additional training of referees have been implemented and have demonstrated potential benefits in preventing injuries [12].

Sports are becoming increasingly more competitive, and players face higher expectations from their sports bodies and fans for a more consistent and predictable performance [3]. Such demands can potentially increase the risk of injuries in contact sports. In case of soccer, this is reflected in a greater number by players that used protective facial and head shields in 2022 FIFA World Cup. Similar trends are being observed in other football competitions especially amongst players who have experienced facial/head injuries [1]. The trend of using protective equipment with a history of previous injuries not only indicates a preventive approach but also an intention to protect further impact on previously injured sites. The use of protective equipment attenuates the impact force and load dispersion to reduce the occurrence and severity of facial fractures and brain damage [4].

Over the years, prevention of sports injuries has been recognized widely by sports bodies across the board with an increasing emphasis on use of protective gear. The use of custom-made protective shields is growing in popularity, as modern technology using computer aided design and manufacture allows manufacture of devices which are comfortable and do not interfere with player performance [13,18,19]. Regular use can significantly reduce the severity of injuries and thereby allow shorter recovery [4,18]. Although some improvised facial / head shields can be made using conventional techniques [4,20], digital planning is becoming more and more usual in sports face shield customization [21,22]. New materials allow the construction of protective shields which are not only lighter and more comfortable to use, but at the same time offer better mechanical protection [23,24]. Some qualities of digitally manufactured protective shields include comfort, better adaptation to individual head and face anatomy and improved fit [18] and minimal interference with the peripheral vision of the users [4].

A limitation of this study is that it did not use official data made available by FIFA or the national football bodies. However, such entities do not provide detailed information on the subject, which perhaps could be improved for better transparency.

CONCLUSION

Rules and protocols to minimize and manage sports injuries need close monitoring for strict implementation and updated regularly in the light of medical advice and research. The use of facial protective equipment has the potential to decrease the severity of maxillofacial injuries and reduce the athletes' time away from the game. Digital design and manufacture of protective equipment may make such equipment more comfortable for the players and encourage more frequent use.

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Original Research

Comparison of Topical Treatment Preferences of Physicians in Dermatological Diseases

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ABSTRACT

Objective: Regardless of their specialty, physicians frequently encounter dermatological conditions. We aimed to determine the topical medication choices of physicians for various dermatoses and to identify differences in preferences between dermatologists and non-dermatologist physicians.

Methods: Using an online survey, physicians were asked to select three preferred topical antibiotics/antiseptics, antifungals, and corticosteroids when treating a pyoderma, dermatophytosis, or a dermatosis necessitating topical corticosteroid therapy. Statistical analysis was performed using Statistical Package for the Social Sciences v.27.

Results: Among 358 physicians, 24.0% were dermatologists, and 76.0% were non-dermatologist physicians. The mean age was 38.40, and the average duration of medical practice was 14.04 years. The most frequently chosen topical antibacterials were fusidic acid (74.3%) and mupirocin (65.9%); topical antifungals were isoconazole nitrate + diflucortolone valerate (56.4%), tioconazole (27.7%), and naftifine (25.1%); and topical corticosteroids were clobetasol propionate (38.5%), methylprednisolone aceponate (36.6%), and mometasone furoate (34.6%). Dermatologists used nitrofurazone and izokonazol nitrate + diflucortolone valerate less frequently compared to non-dermatologists (0% vs. 27.6% and 8.1% vs. 71.7%, respectively; p-values <0.001). Family physicians/general practitioners constituted the largest group selecting clobetasol propionate (28.3%).

Conclusion: Physicians in our country predominantly choose fusidic acid and mupirocin as topical antibiotics, aligning with existing literature. However, nitrofurazone, causing contact dermatitis, and corticosteroid-containing antifungals with the potential for complications due to inappropriate use are frequently preferred by non-dermatologist physicians but not by dermatologists. The bold choice of clobetasol propionate, an ultrapotent topical corticosteroid, by family physicians/general practitioners is an important issue to address during medical education and post-graduation.

Keywords: Physicians, Antibiotics, Corticosteroids, Prescriptions

INTRODUCTION

Dermatology is a branch of medicine that includes diseases and conditions frequently encountered in daily practice. Regardless of their speciality, physicians face skin diseases of their patients or relatives and may have to recommend treatment for these conditions.

It is common for a family physician to treat a dermatophytosis or an eczema, for an orthopaedic surgeon to recommend an antibiotic ointment for the incision site after a surgical procedure, or for a paediatrician to prescribe a diaper dermatitis treatment for a patient. In addition, non-dermatologist physicians are encountering dermatological diseases with increasing frequency due to reasons such as patients having difficulty in reaching a dermatologist. A study conducted in the United States of America showed that only 41% of patients with dermatological diagnoses were examined by dermatologists in 1989 [1]. In another study, it was found that 39% of the referrals with dermatological diagnosis were made to family physicians [2]. However, in a previous study, it was found that family physicians had theoretical misconceptions about the etiopathogenesis and management of some skin diseases [3].

Sometimes, topical treatment preferences of physicians may differ due to differences in approaches between specialities. For example, it can be seen that a topical preparation containing nitrofurazone and polyethylene glycol, which is well known to cause allergic contact dermatitis by dermatologists, is widely preferred by orthopaedists, general surgeons, or plastic surgeons [4]. Although this issue is exemplified by case reports or case series demonstrating the complications caused by a single medication as in the above publication, there is no study in the literature showing which topical medication is preferred by physicians from different specialities in various dermatological conditions.

The aim of our study was to determine the medications chosen by physicians in the topical treatment of various skin diseases and to reveal the differences in preferences between dermatologists and non-dermatologists in this field.

Main Points;

- The most frequently preferred topical antibiotic among physicians is fusidic acid, topical antifungal is isoconazole nitrate + diflucortolone valerate, and topical corticosteroid is clobetasol propionate.
- The frequent preference of physicians for nitrofurazone, isoconazole nitrate + diflucortolone valerate, and clobetasol propionate, all of which can lead to various complications, is an issue that should be emphasized during medical education.

MATERIALS AND METHODS

The study was conducted by the dermatologists of our university and local ethics committee approval was obtained (Ethics committee approval code: 2023/0551). Based on the responses received, a questionnaire consisting of up to 12 questions was prepared in Google Forms format and sent online to both dermatologists and non-dermatologist physicians. The responses obtained from a total of 358 physicians were recorded.

Firstly, participants' age, gender, year of graduation, specialty, academic titles, type of institution they work at, whether they worked as general practitioners before their residency, the duration of their work as general practitioners if applicable, and the frequency of encountering dermatological conditions were inquired to gather general and professional characteristics. The physicians were then asked questions such as "which topical antibiotics/antiseptic creams or topical medications do you prefer more frequently when you encounter an infectious skin condition?", "when you diagnose a fungal infection on the skin, please tick the options you prefer most as topical antifungals" and "which one(s) do you prefer most as topical corticosteroid-containing medications?" and they were asked to select up to three topical antibacterial/antiseptic, topical antifungal and topical corticosteroid preparations, respectively.

Statistical Analysis

The statistical analysis of the data was conducted using IBM Statistical Package for the Social Sciences [SPSS] v.27. For the necessary variables, means and standard deviations were calculated for numerical data, and frequencies for categorical data. In comparing dermatologists and non-dermatologist physicians, the Pearson chi-square test was used for categorical data, and when necessary, the Fisher's test was employed (based on the ratio of the expected count to all cells and the minimal expected count). A p-value less than 0.05 was considered statistically significant.

RESULTS

Of the total of 358 physicians, 86 (24.0%) were dermatologists, while 272 were from other specialties or general practitioners. Among the participants, 54.7% were female, and 45.3% were male. The mean age of the physicians was 38.40 ± 10.03 years, and they had an average of 14.04 ± 10.28 years of experience in the medical field. When the physicians were classified based on the year they graduated from medical school, 11.5% graduated between 1978 and 1993, 13.4% between 1994 and

2003, 41.6% between 2004 and 2013, and 33.5% between 2014 and 2023. Among the participants, 48.6% were specialists, 25.7% were residents, 10.6% were associate professors, 2.5% were professors, and 12.6% were general practitioners. In terms of their medical specialties, following dermatology, the most common specialties among the participants were family medicine (10.9%), general practice (10.9%), and pediatrics (8.9%). Regarding their workplace, physicians were distributed as follows: training and research hospital (24.6%), state university (16.2%), city hospital (15.4%), state hospital (11.2%), family health center (10.6%), private hospital (9.5%), and private practice (7.3%). The proportion of physicians who encountered a dermatological issue at least once a day was 58.1% in total, including dermatologists (24.0%), those who encountered skin diseases many times a day (14.8%) and those who encountered them once or a few times a day (19.3%).

In response to the question "Which topical antibiotics/antiseptic creams or topical medications do you prefer more frequently when you encounter an infectious skin condition?" the preferred topical antibacterials were listed as follows: fusidic acid (74.3%), mupirocin (65.9%), bacitracin + neomycin (25.7%), nitrofurazone (20.9%) and oxytetracycline (16.2%), and the rate of those who answered this question as "I have not encountered such a situation" or "I refer to a dermatologist" was 1.7% (Table 1). In response to the question "when you diagnose a fungal infection on the skin, please tick the options you prefer most as topical antifungals" the most commonly chosen options were isoconazole nitrate + diflucortolone valerate combined preparations (56.4%), tioconazole (27.7%), naftifine (25.1%), sertaconazole (20.7%), and terbinafine (20.1%) (Table 2). Those who answered this question with "I have not encountered such a situation" or "I refer to a dermatologist" accounted for 9.2%. The question "which one(s) do you prefer most as topical corticosteroid-containing medications?" was answered with the following preferences: clobetasol propionate (38.5%), methylprednisolone aceponate (36.6%), mometasone furoate (34.6%), fusidic acid + betamethasone valerate combined preparations (31.8%), and hydrocortisone acetate (27.1%) (Table 3). Approximately 8.4% of the participants answered this question with "I have not encountered such a situation."

When the preferences for topical antibacterial drugs were compared between the two groups, dermatologists were significantly more likely to prefer fusidic acid (95.3% vs. 67.6%; p<0.001), mupirocin (79.1% vs. 61.8%; p=0.003), clindamycin solution (15.1% vs. 1.1%; p<0.001), and nadifloxacin (14.0% vs. 1.1%; p<0.001) compared to non-dermatologists. However, they never preferred nitrofurazone (0% vs. 27.6%; p<0.001) (Table 1). When the responses to the question "when you diagnose a fungal infection on the skin, please tick the options you prefer most as topical antifungals" were compared between the two groups, dermatologists were significantly more likely to prefer butenafine (25.6% vs. 5.1%; p<0.001), clotrimazole (15.1% vs. 6.3%; p=0.01), naftifine (73.1% vs. 9.9%; p<0.001), miconazole + aluminum hydroxychloride combined preparation (4.7% vs. 0.4%; p=0.013), terbinafine (33.7% vs. 15.8%; p<0.001), isoconazole (20.9% vs. 8.5%; p=0.002), and sertaconazole (52.3% vs. 10.7%; p<0.001), while they significantly less preferred isoconazole nitrate + diflucortolone valerate combination preparations (8.1% vs. 71.7%; p<0.001) (Table 2). When responses to the question "which one(s) do you prefer most as topical corticosteroid-containing medications?" were compared between the two groups, dermatologists were significantly more likely to prefer methylprednisolone aceponate (55.8% vs. 30.5%; p<0.001), betamethasone dipropionate (14.0% vs. 6.6%; p=0.032), prednicarbate (10.5% vs. 1.5%; p<0.001), clobetasol-17-butyrate (11.6% vs. 3.3%; p=0.005), hydrocortisone-17-butyrate (20.9% vs. 8.1%; p<0.001), mometasone furoate + fusidic acid combined preparation (8.1% vs. 2.6%; p=0.048), and mometasone furoate (73.3% vs. 22.4%; p<0.001). They were also significantly less likely to prefer hydrocortisone acetate (16.3% vs. 30.5%; p=0.01) and isoconazole nitrate + diflucortolone valerate combination (4.7% vs. 26.1%; p<0.001) (Table 3).

The evaluation also performed which specialty of physicians preferred a medication the most. Among the antibacterial drugs, 30.8% of those who chose fusidic acid, 28.8% of those who chose mupirocin, 81.3% of those who chose clindamycin solution and 80% of those who chose nadifloxacin were dermatologists, while 30.7% of those who chose nitrofurazone, 25% of those who chose povidone iodine, 26.1% of those who chose bacitracin + neomycin combination, 31% of those who chose oxytetracycline, 25% of those who chose rifamycin and 50% of those who chose tetracycline were family physicians (specialist/residents) or general practitioners (Table 4-5). Regarding antifungal drugs, 61.1% of butenafine users, 43.3% of clotrimazole users, 70% of naftifine users, 57.1% of miconazole + triamcinolone acetonide combination users, 27.9% of ketoconazole users, 80% of miconazole + aluminum hydroxide chloride combination users, 40.3% of terbinafine users, 43.9% of isoconazole users, and 60.8% of sertaconazole users were dermatologists. However, 27.7% of isoconazole nitrate + diflucortolone valerate

combination users, 39.4% of tioconazole users, 37% of ciclopirox users, and 33.3% of oxiconazole users were family physicians (specialists/residents) or general practitioners (Table 4-5). For topical corticosteroids, 44.4% of alclometasone users, 36.6% of methylprednisolone aceponate users, 34.8% of betamethasone valerate users, 69.2% of prednicarbate users, 52.6% of clobetasol-17-butyrate users, 28.1% of fusidic acid + betametazone valerate combination users, 45% of hydrocortisone-17-butyrate users, 53.8% of fusidic acid + mometasone furoate combination users, and 50% of mometasone furoate users were dermatologists. In contrast, 28.3% of clobetasol propionate users and 27.8% of hydrocortisone acetate users were family physicians (specialists/residents) or general practitioners (Table 4-5).

Table 1. Distribution of physicians' topical antibiotic/antiseptic preferences for all physicians, dermatologists and non-dermatologist physicians.

	All physicians	Dermatologists	Non-dermatologists	p
fusidic acid	%74.3	%95.3	%67.6	<0.001*
mupirocin	%65.9	%79.1	%61.8	<0.01*
bacitracin + neomycin	%25.7	%19.8	%27.6	0.15
nitrofurazone	%20.9	%0	%27.6	<0.001*
oxytetracycline	%16.2	%11.6	%17.6	0.19
povidone iodine	%12.3	%9.3	%13.2	0.33
rifamycin	%12.3	%9.3	%13.2	0.33
clindamycin	%4.5	%15.1	%1.1	<0.001*
nadifloxacin	%4.2	%14.0	%1.1	<0.001*
tetracycline	%1.1	%1.2	%1.1	1.00

Each ratio shows what percentage of the physician group in the relevant column prefers thedrug in the relevant row.

* p<0.05 for the difference between the preference rates of dermatologists and non-dermatologists

Table 2. Distribution of topical antifungal preferences of physicians for all physicians, dermatologists and non-dermatologist physicians.

	All physicians	Dermatologists	Non-dermatologists	p
isoconazole nitrate + diflucortolone valerate	%56.4	%8.1	%71.7	<0.001*
tioconazole	%27.7	%20.9	%29.8	0.11
naftifine	%25.1	%73.3	%9.9	<0.001*
sertaconazole	%20.7	%52.3	%10.7	<0.001*
terbinafine	%20.1	%33.7	%15.8	<0.001*
ketoconazole	%17.0	%19.8	%16.2	0.44
oxiconazole	%15.9	%19.8	%14.7	0.26
isoconazole	%11.5	%20.9	%8.5	<0.01*
butenafine	%10.1	%25.6	%5.1	<0.001*
clotrimazole	%8.4	%15.1	%6.3	0.01*
ciclopirox	%7.5	%5.8	%8.1	0.49
miconazole + triamcinoloneacetonide	%2.0	%4.7	%1.1	0.06
miconazole + aluminium, hydroxychloride	%1.4	%4.7	%0.4	0.01*

Each ratio shows what percentage of the physician group in the relevant column prefers the drug in the relevant row.

* p<0.05 for the difference between the preference rates of dermatologists and non-dermatologists.

Table 3. Distribution of physicians' topical corticosteroid preferences for all physicians, dermatologists and non-dermatologists.

	All physicians	Dermatologists	Non-dermatologists	p
clobetasol propionate	%38.5	%40.7	%37.9	0.64
methylprednisolone aceponate	%36.6	%55.8	%30.5	<0.001*
mometasone furoate	%34.6	%73.3	%22.4	<0.001*
fusidic acid + betamethasone valerate	%31.8	%37.2	%30.1	0.22
hydrocortisone acetate	%27.1	%16.3	%30.5	0.01*
isoconazole nitrate + diflucortolone valerate	%20.9	%4.7	%26.1	<0.001*
hydrocortisone-17-butyrate	%11.2	%20.9	%8.1	<0.001*
betamethasone dipropionate	%8.4	%14.0	%6.6	0.03*
betamethasone valerate	%6.4	%9.3	%5.5	0.21
clobetasone-17-butyrate	%5.3	%11.6	%3.3	<0.01*
fusidic acid + mometasone furoate	%3.9	%8.1	%2.6	<0.05*
prednicarbate	%3.6	%10.5	%1.5	<0.001*
prednisinolone	%3.1	%0	%4.0	0.07
alclomethasone dipropionate	%2.5	%4.7	%1.8	0.23

Each ratio shows what percentage of the physician group in the relevant column prefers thedrug in the relevant row.

* $p<0.05$ for the difference between the preference rates of dermatologists and non-dermatologists

Table 4. Drugs most frequently preferred by dermatologists and the proportion of dermatologists among physicians who prefer these drugs

Medication	Proportion of dermatologists among physicians who prefer this drug (%)
Antibacterial/antiseptic	
fusidic acid	%30.8
mupirocin	%28.8
clindamycin	%81.3
nadifloxacin	%80.0
Antifungal	
butenafine	%61.1
clotrimazole	%43.3
naftifine	%70
miconazole + triamcinolone acetonide	%57.1
ketoconazole	%27.9
miconazole + aluminium hydroxychloride	%80.0
terbinafine	%40.3
isoconazole	%43.9
sertaconazole	%60.8
Corticosteroids	
alclomethasone	%44.4
methylprednisolone aceponate	%36.6
betamethasone valerate	%34.8
prednicarbate	%69.2
clobetasone-17-butyrate	%52.6
fusidic acid + betamethasone valerate	%28.1
hydrocortisone-17-butyrate	%45.0
fusidic acid + mometasone furoate	%53.8
mometasone furoate	%50.0

Table 5. Drugs most frequently preferred by family medicine specialists/residents and practitioners and the proportion of family physicians/practitioners among physicians who prefer these drugs

Medication	Proportion of family physicians/ practitioners among physicians who prefer this drug (%)
Antibacterial/antiseptic	
nitrofurazone	%30.7
povidone iodine	%25.0
bacitracin + neomycin	%26.1
oxytetracycline	%31.0
rifamycin	%25.0
tetracycline	%50.0
Antifungal	
isoconazole nitrate + diflucortolone valerate	%27.7
tioconazole	%39.4
ciclopirox	%37.0
oxiconazole	%33.3
Corticosteroid	
clobetasol propionate	%28.3
hydrocortisone acetate	%27.8

Among physicians who graduated between 2004 and 2013, the proportion of dermatologists was significantly lower (10.1%) compared to those who graduated in other years (43.9% between 1978-1993, 29.2% between 1994-2003, 32.5% between 2014-2023; p<0.001). Those who graduated between 1978 and 1993 preferred klobetazon-17-butyrate (22.0%; p<0.001), betamethasone valerate (24.4%; p<0.001), tioconazole (61.0%; p<0.001), and oxiconazole (31.7%; p=0.018) more frequently but used fusidic acid + betamethasone valerate combination products less often (12.2%; p=0.007). Those who graduated between 1994 and 2003 preferred cyclopirox (20.8%; p<0.001) more often. Those who graduated between 2004 and 2013 preferred isoconazole nitrate + diflucortolone valerate combination more frequently (72.5%; p<0.001) but used mometasone furoate (25.5%; p=0.008), clindamycin solution (1.3%; p=0.018), naftifine (12.1%; p<0.001), and miconazole + triamcinolone acetonide combination (0%; p=0.015) less often. Finally, those who graduated between 2014 and 2023 preferred fusidic acid (84.2%; p=0.018) and ketoconazole (26.7%; p=0.005) more frequently.

When comparing the frequency of academic titles between dermatologists and non-dermatologist physicians, the proportions of associate professors and specialists were similar in both groups (10.5% vs. 10.7% and 48.8% vs. 48.5%, respectively). However, among dermatologists, there were significantly higher proportions of residents and professors (34.9% vs. 22.8% and 5.8% vs. 1.5%, respectively; p<0.001). The selection of certain drugs also varied according to academic title. General practitioners preferred isoconazole nitrate + diflucortolone valerate combination more frequently than residents and specialists (80% vs. 46.7% and 55.2%, respectively; p=0.006), oxiconazole more frequently than residents (31.1% vs. 7.6%; p=0.012), naftifine less frequently than residents and specialists (13.3% vs. 40.2% and 20.7%, respectively; p=0.003), sertaconazole and methylprednisolone aceponate less frequently than professors (13.3% vs. 55.6%; p<0.05 and 24.4% vs. 77.8%; p=0.037, respectively). Specialists preferred prednicarbate more frequently than professors (30.8% vs. 15.4%; p=0.033), while professors preferred prednacinolone more frequently than residents and specialists (22.2% vs. 2.2% and 2.9%, respectively; p=0.022). The selection of isoconazole nitrate + diflucortolone valerate combination products for corticosteroid use was also higher among general practitioners compared to residents, specialists, and associate professors (48.9% vs. 16.3%, 17.8%, and 15.8%, respectively; p<0.001).

DISCUSSION

In daily medical practice, all physicians generally face skin diseases and recommend topical medications to their patients or their close relatives. In our study, the medication choices of physicians in situations requiring the use of topical drugs in three commonly encountered scenarios, namely infected skin lesions, dermatophytosis, and the use of topical corticosteroid-containing drugs, were identified and compared between dermatologists and non-dermatologist physicians.

In our study, the responses to these three questions such as "I have not encountered such a situation" or "I refer to a dermatologist" remained at 1.7%, 9.2% and 8.4% for antibiotics, antifungals and corticosteroids, respectively. In addition, approximately 60% of the physicians stated that they encountered a dermatological problem at least once a day. Considering that the proportion of dermatologists among the participants was only 24%, it can be said that these findings confirm our suggestion that physicians, regardless of their specialty, frequently encounter skin diseases.

The first five topical antibiotics selected by all participating physicians and non-dermatologist physicians in our study were fusidic acid, mupirocin, bacitracin + neomycin, nitrofurazone, and oxytetracycline, respectively. Among dermatologists, the most frequently chosen antibiotics were fusidic acid, mupirocin, bacitracin + neomycin, clindamycin, and nadifloxacin, respectively. In our study, fusidic acid and mupirocin were the two most commonly preferred antibiotics by both dermatologists and non-dermatologist physicians. Furthermore, dermatologists significantly preferred these two antibacterials in a higher proportion compared to non-dermatologists. Our findings are in line with the literature, which suggests that these molecules are the most commonly preferred for superficial pyodermas and are equivalent in efficacy to oral antibiotics in impetigo treatment [5, 6]. The third most commonly preferred drug among both dermatologists and non-dermatologists being a preparation containing bacitracin + neomycin in nearly 20% of cases is surprising. Both bacitracin and neomycin are topical antibiotics associated with allergic contact dermatitis in the literature [7, 8]. The most significant difference between the two groups in terms of topical antibacterial preferences was observed in nitrofurazone. None of the dermatologists preferred this molecule, while it was the fourth most preferred antibiotic, chosen by 27.6% of non-dermatologists. Allergic contact dermatitis due to nitrofurazone is a well-known complication that dermatologists are familiar with it, having treated it many times throughout their careers, and it is well-described in the literature [4, 9]. However, our findings indicate that this experience and literature knowledge are not shared by non-dermatologist physicians. Among the specialities of physicians who chose nitrofurazone, the highest rate was family medicine and general practice with 30.7%. This data does not align with clinical experiences and the literature, which suggest that nitrofurazone is more commonly preferred by surgical specialties. However, this can be explained by the fact that family medicine and general practice specialists constituted the second-largest group after dermatologists among the participants of our study. Nevertheless, based on our findings, it can be said that nitrofurazone's complications should be emphasized, especially during dermatology rotations in medical education and in family medicine specialist training.

The top five topical antifungal choices in dermatophytoses for all participants were isoconazole nitrate + diflucortolone valerate, tioconazole, naftifine, sertaconazole and terbinafine, respectively. Dermatologists preferred naftifine, sertaconazole, terbinafine, butenafine, isoconazole and tioconazole, while non-dermatologists preferred isoconazole nitrate + diflucortolone valerate, tioconazole, ketoconazole, terbinafine and oxiconazole, respectively. The most pronounced differences in topical antifungal preferences between dermatologists and non-dermatologists were observed in the selections of isoconazole nitrate + diflucortolone valerate (8.1% vs. 71.7%), naftifine (73.3% vs. 9.9%), sertaconazole (52.3% vs. 10.7%), terbinafine (33.7% vs. 15.8%), and butenafine (25.6% vs. 5.1%) ($p<0.001$). Among these, the most striking one is that dermatologists rarely choose combinations containing isoconazole nitrate + diflucortolone valerate, whereas non-dermatologists prefer these preparations in over 70% of cases. Additionally, the majority of those who prefer these drugs (27.7%) are family physicians (specialists/residents) and general practitioners. Dermatologists are more likely to encounter problems caused by the choice of an antifungal combined with a steroid in a mycotic infection, such as discontinuation of treatment due to rapid improvement in symptoms or the development of complications such as atrophy, striae, hypertrichosis, iatrogenic tinea due to prolonged and irregular use [10]. Moreover, dermatologists are expected to be more conscious than other specialists about the side effects of the frequent use of these drugs, even iatrogenic Cushing's syndrome, especially in cases with a diagnosis of diaper dermatitis [11, 12]. Differences between dermatologists and non-dermatologists in the selection of antifungal monotherapies are generally related more to established habits, which active ingredients are used

more frequently in the relevant literature of their specialty, or the marketing and advertising strategies of these drugs, which can vary by specialty, rather than the efficacy or safety data of these drugs. It is not surprising that physicians who prefer drugs like thioconazole, cyclopirox, and oxiconazole, which have been well-known in the market in our country for many years, are mostly family physicians and general practitioners.

One of the most striking findings of our study was that clobetasol propionate, the most potent topical corticosteroid known, was the most preferred topical corticosteroid with a rate of 38.5% among all participating physicians. It is also interesting to note that clobetasol propionate was mostly preferred by family physicians and general practitioners. It can be said that the conditions in which clobetasol propionate should be preferred are skin diseases such as psoriasis, mycosis fungoides, lichen sclerosis, bullous pemphigoid which are mostly treated by dermatologists [13]. When used inappropriately, it can lead to many complications such as atrophy, striae, hirsutism, tinea incognito and iatrogenic Cushing's syndrome, as mentioned above for diflucortolone valerate [13, 14]. Considering that dermatologists treat skin diseases that cannot be effectively managed by other physicians, that they are well-informed about which drugs to choose, how much to apply, for how many days, and to which localization, and finally that they are experienced in recognizing and managing possible complications and educating their patients on this subject, it is understandable that an ultrapotent corticosteroid such as clobetasol propionate is chosen by dermatologists in a certain frequency. Nevertheless, the most frequently preferred topical corticosteroid by the dermatologists participating in our study was mometasone furoate, which has a medium-high potency. The fact that clobetasol propionate is preferred to such a high extent by non-dermatologist physicians -disregarding safety considerations- is an issue that should be emphasized during medical education and rotations. In our study, dermatologists preferred methylprednisolone aceponate, mometasone furoate, hydrocortisone- 17-butyrate and prednicarbate at higher rates than non-dermatologists. These corticosteroid drugs have various potencies and the fact that they are preferred more frequently by dermatologists can be explained by the habits acquired by physicians during their speciality training and the notion gained in the context of master-apprentice relationship rather than the efficacy or safety data of these drugs. Hydrocortisone acetate, a low potency topical corticosteroid, was preferred more frequently by non-dermatologists compared to dermatologists. This finding shows that physicians of other specialties may act boldly, as seen in the choice of clobetasol propionate, and sometimes they may prefer low potency corticosteroids by displaying a cautious and safety-first approach.

Limitations

There are numerous topical drugs available besides those listed as options in the study. Nevertheless, this limitation was addressed by providing participants with the opportunity to provide open-ended responses under the "other" option. Another limitation is that the proportion of participants in certain specialties may not necessarily align with the distribution of those specialists in our country, and therefore, potentially affecting the accurate determination of drug preferences among physicians in surgical branches.

CONCLUSION

Our study found that physicians in our country prefer two effective and safe topical antibiotics, fusidic acid and mupirocin, which are commonly used worldwide, as the top choices for superficial skin infections. However, it is noteworthy that nitrofurazone, a medication known to cause allergic contact dermatitis, was preferred at a significantly high rate by non-dermatologist physicians, despite none of the dermatologists selecting it. As a topical antifungal drug, the combination of isoconazole nitrate + diflucortolone valerate, which is rarely preferred by dermatologists, was found to be frequently preferred by non-dermatologists. Another striking finding of our study is that clobetasol propionate, considered the most potent topical corticosteroid, is the most frequently chosen topical corticosteroid among non-dermatologist physicians. Considering that this drug is mostly used in cases that should be managed by a dermatologist, this "brave" approach of a significant portion of our physicians is an issue that should be emphasised during medical education. During medical education and after graduation, it should be aimed that specialty-specific practices and traditions that guide the choice of topical medication should be compatible with the experience of dermatologists and be in accordance with the literature data.

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Original Research

Evaluation of Behavioral Characteristics After Hearing in Children with Cochlear Implants

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ABSTRACT

Objective: Cochlear implantation (CI) is an electronic device that converts mechanical sound energy into electrical signals and transmits it directly to the cochlea, allowing sound perception. These implants were applied to patients with severe sensorineural hearing loss who did not or had little benefit from the conventional hearing devices. This study aimed to investigate behavioral problems, find related factors, and determine the relationship between behavioral problems and parents' attitudes in children with CI.

Methods: The investigation involved the participation of fifty individuals, comprising 26 males and 24 females, between 4 and 18 years, with a mean age average of 4±1.56 without any neurological and developmental problems. Inclusion criteria required a minimum of one year post-CI follow-up and a corresponding minimum duration of one year utilizing CI. Achenbach's Child Behavior Checklist (CBCL) assessed behavioral aspects. Categories of Auditory Performance II (CAP) and The Speech Intelligibility Rating Scale (SIR) scales were employed to evaluate auditory performance and speech intelligibility. Parental attitudes were gauged using the Parent Attitude Research Instrument (PARI). The selection of fifty patients was accomplished through a simple random sampling technique, with no considerations for gender or social status differences during case selection.

Results: The patients who applied the CI bilaterally were more successful than the one-sided. The success rate of patients who had comorbidities was statistically significant. Aggressive behavior was less in patients operated on before age 4. There are no differences between the relations of friend circle, art, and sports-interested patients. For CAP II and SIR, there is a moderate statistical significance between the duration of use and CAP. CAP scores were analyzed high in patients who used the device for over six years. There is a moderate statistical significance between CAP and SIR correlation. Our research found a statistically significant decrease in all behavioral scales when comparing preoperative and postoperative scores. Although there was only a non-significant decrease in the delinquent behaviors score, a decrease was still observed. There were significant changes in males but no significant difference based on gender in our study.

Conclusion: The findings imply that implementing cochlear implants in younger children might yield even greater advantages. Our research adds to the expanding collection of evidence endorsing CI as a viable therapeutic choice for youngsters with hearing impairments, underscoring the necessity for continued investigations within this domain.

Keywords: Cochlear Implant, Sensorineural Hearing Loss, CBCL, CAP, SIR, PARI, Behavioral Disorder

INTRODUCTION

Hearing loss is an important public health problem affecting 32 million children worldwide and has a major impact on children's communication, social and educational development [1]. Hence, children facing severe to profound sensorineural hearing loss frequently encounter disruptions in their speech development or experience speech impairments, leading to adverse impacts on their communication skills and social interactions [2].

Important evidence has been obtained in young children indicating the effectiveness of CIs in speech perception, receptive and expressive language, and general communication skills [3]. Hearing, speaking, and language acquisition enable the child to develop independent thinking and self-control skills to maintain healthy relationships with others [4]. CI allows the child to develop the language and communication skills required to connect with peers and build effective social networks [5]. Deaf and hearing-impaired children have a higher risk of socially and emotionally negative development than their normal-hearing peers, leading to disruptive behavior problems [6].

Main Points;

- Children with severe to profound hearing loss face hurdles in speech development and social interactions, highlighting the importance of early interventions to foster proper speech and overall development.
- Cochlear implants have proven to be a transformative solution, significantly improving children's auditory perception and speech recognition, thereby aiding in robust language development and better communication abilities.
- The devices are central in enhancing a child's language acquisition, communication proficiency, and social skills, allowing them to engage more meaningfully with their environment and society.
- The study aims to explore the behavioral challenges faced by children with cochlear implants, seeking to identify the factors involved and develop insights to guide potential interventions, with the ultimate objective of enhancing these children's well-being and quality of life.
- Implementing early interventions like cochlear implants has wider public health and educational benefits, promoting inclusive education and healthier development for future generations, hence facilitating mainstreaming of children with hearing loss.

Our study aimed to investigate the behavioral problems of children with CIs aged 4-18 cross-sectionally and to find factors related to problematic behaviors.

MATERIAL AND METHODS

This study was designed at the Bozyaka Education and Research Hospital. The local ethics committee of the same hospital approved this study (approval No=03 and date 20/12/2016). Every participant's parent furnished written informed consent before engaging in the study.

Participants

Patients between 4-18 years of age who had CI surgery, followed up at our CI center, did not have any neurological and developmental problems, and were using verbal communication were included in the study.

The participants' parents filled out the demographic information form, and the educational background of the parents, professional status, and socioeconomic status were recorded five-point scale was employed to assess the extent of parental education (ranging from 1 for illiterate parents to 5 for those with postgraduate education) and professional status (ranging from 1 denoting unskilled workers to 5 representing professionals). The behavioral problems of the participants were compared with the normal hearing sample of the same age in the Turkish population. Patients and their relatives who were to participate in the study were informed about the research, and their consent was obtained.

Test materials

1. Behavioral problem scale: To assess the participants' outcomes, the study employed the Turkish adaptation of the Child Behavior Checklist (CBCL) for Ages 4-18. This questionnaire, known for its high reliability and user-friendly nature, facilitates the acquisition of psychometrically precise insights [7]. Akçakın,1985 [8] translated this questionnaire into Turkish and reliability study.

The scale comprises a set of thirteen items designed to capture behavioral and emotional issues prevalent in children and adolescents. This checklist has eight distinct subcategories: Anxious / Depressed, Withdrawn/Depressed, Somatic Complaints, Social Problems, Thought Problems, Attention Problems, Delinquent Rule-Breaking Behavior, and Aggressive Behavior. Each subcategory is scored on a 3-point scale (0 = never, 1 = sometimes, 2 = very often). The initial three subcategories contribute to the "Internalization Behavior Problem" scale, while the latter two subcategories contribute to the "Externalization Behavior Problem" scale. The cumulative score derived from these eight subcategories signifies the "Total General Behavior" level.

Raw scores transform T scores, which are adjusted based on a normative sample tailored for age and gender considerations. Elevated scores on the scales correlate with escalating behavioral challenges. The mean T score for each subcategory stands at 50 ± 10. Interpreting the T-scores, children's performances within each sub-dimension are categorized as falling within the normal range, bordering the threshold, or residing within the clinical range.

2. Evaluation of Speech Performance: The study employed the Categories of Auditory Performance (CAP) test and the Speech Intelligibility Rating (SIR) scales to gauge speech-related abilities.

The CAP test was utilized to assess the speech perception performance of children who had undergone CI. This evaluation focuses on supraliminal performance, providing a more realistic reflection of everyday auditory capabilities. The CAP test is structured as a hierarchical scale, encompassing various levels of auditory perceptual prowess. This spectrum ranges from a level of 0, representing "no awareness of environmental sounds," to a level of 7, indicative of the ability to "use the telephone with a familiar talker" [9]. (Refer to Appendix 1.)

Meanwhile, the SIR scales were employed to quantify children's speech intelligibility with CIs during spontaneous speech in everyday contexts. The SIR is a practical and efficient measure of speech intelligibility outcomes in real-life scenarios. It encompasses five distinct performance categories that span from "pre-recognizable words in the spoken language" to "connected speech intelligible to all listeners" [10]. (Refer to Appendix 2.) Notably, both scales were assessed by the same educational audiologist during the most recent follow-up visit for children who had undergone CI.

Appendix 1. Categories of the auditory performance score.

0	No awareness of environmental sound
1	Awareness of environmental sounds
2	Responds to speech sounds
3	Identifies environmental sounds
4	Discriminates speech sounds
5	Understands phrases without lip reading
6	Understands conversation without lip reading
7	Uses the telephone

Appendix 2. Speech intelligibility rate

Category 1	Pre-recognizable words in spoken language
Category 2	Connected speech is unintelligible but is developing for single words
Category 3	Connected speech is intelligible to a listener who concentrates and lip reads within a known context
Category 4	Connected speech is intelligible to a listener who has little experience of a deaf person's speech. The listener does not need to concentrate unduly
Category 5	Connected speech is intelligible to all listeners. The child is easily understood in everyday contexts

3. Assessment of Parental Attitudes: The study incorporated the Parent Attitude Research Instrument (PARI) to gauge parental perspectives regarding children with disabilities. This instrument comprises several subscales that delve into various dimensions of parental attitudes. The acceptance subscale gauges how parents embrace their child's disability and comprehend its influence on their lives. Meanwhile, the guilt subscale scrutinizes the presence of any guilt that parents might experience concerning their child's disability. The competence subscale delves into the parent's perception of their proficiency in effectively parenting a child with a disability. Lastly, the social isolation subscale evaluates the level of social isolation parents might perceive due to their child's disability [11].

Statistical Analysis

The information extracted from patient records was entered into a computer utilizing SPSS version 20.0 software from IBM Corp., located in Armonk, NY, USA. The test scores obtained from the CBCL for Ages 4-18 were subjected to statistical analysis using the Pearson Correlation coefficient and t-test. A significance level of $p < 0.05$ was adopted. The assessment involved comparing subscales of the CBCL scale, including internalization and externalization scales and total scores. The comparisons encompassed Anxious/Depressed, Withdrawn/Depressed, Somatic Complaints, Social Problems, Thought Problems, Attention Problems, Delinquent Rule-Breaking Behavior, and Aggressive Behavior subscales, alongside internalization and externalization scores and total scores. Moreover, ChatGBT artificial intelligence applications were enlisted to assist in grammar correction for writing purposes.

RESULTS

Demographic Data

A total of 50 patients, 26 males (52%) and 24 females (48%), between 4 and 18 years, with a mean age average of 4± 1.56, were included in the study. The demographic information of the patients is in Table 1. CIs were implanted in 35 patients (70%) at the age of 4 years or younger and 15 patients (30%) older than 4 years of age. The number of patients with 0-2 years of CI use was 4 (8%), the number of patients with 3-5 years of CI use was 11 (22%), and the number of patients with 6 years or more of CI use was 35 (70%). CIs were placed on the right side in 29 (58%) patients, on the left side in 16 (32%) patients, and bilateral in 5 (10%) patients. 7 (14%) patients had additional disabilities (disarticulation, spelling mistakes and word understanding). The socioeconomic status of the 18 (36%) parents was at the lower level, 27 (54%) of the parents were at the intermediate level, and 5 (10%) of the parents were at the upper level. The mean duration of CI use was 8.4 ±1.78 years, with a minimum of 1 and a maximum of 15 years. (Table 1.)

Table 1. Demographic Information

Demographic Information	Number of Patients	Percentage
Total Patients	50	100%
Male	26	52%
Female	24	48%
Age at Implantation		
≤4 years	35	70%
>4 years	15	30%
Duration of Cochlear Implant Use		
0-2 years	4	8%
3-5 years	11	22%
≥6 years	35	70%
Side of Implantation		
Right	29	58%
Left	16	32%
Bilateral	5	10%
Additional Disabilities	7	14%
Socioeconomic Status of Parents		
Lower	18	36%
Intermediate	27	54%
Upper	5	10%
Duration of Cochlear Implant Use (years)		
Mean	8.4	
Minimum	1	
Maximum	15	

CBCL, CAP, SIR, and PARI

The test scores obtained from the 4-18 Age CBCL were evaluated using the Pearson Correlation coefficient and t-test. The significance level was accepted as $p <0.05$. Subscales of the CBCL scale, internalization scale, externalization scale, and total scores were compared.

These comparisons were made among Anxious/Depressed, Withdrawn/Depressed, Somatic Complaints, Social Problems, Thought Problems, Attention Problems, Delinquent Rule-Breaking Behavior and Aggressive Behavior subscales and internalization scores, externalization scores, and total scores. The average time for receiving special education is 6.08 ±1.42 years, ranging from 0 to 13 years. No notable difference was observed between the duration of special education and involvement in sports, art, or friendship relations. However, a statistically significant positive correlation was discovered between special education and CAP duration. A strong statistically significant positive correlation was also observed between CAP and SIR. Furthermore, a significant difference was found between the duration of implant use.

Patients with bilateral implants were observed to have a significantly higher success rate than patients with unilateral implants. Success status was also significantly correlated with comorbidities. Regarding behavioral outcomes, aggressive behavior was significantly lower in patients who underwent surgery before the age of 4 years. Moreover, those who had a duration of implant use of 6 years or more had significantly higher CAP scores. (Table 2.A and B)

Table 2 presents the mean ranks and sum of ranks for various factors of two age groups, 0-5 years and > six years, and the total sample size. The mean rank for each factor is higher for the >6 years group than the 0-5 years group. The only exception is the CAP factor, which has a lower mean rank for the > 6 year group. The highest mean ranks are observed for Withdrawal and Aggression scores, respectively. The Wilcoxon test results indicated significant differences between the two groups for CAP (Z=-2.398, p=0.017). However, the two groups had no significant differences in other factors (p > 0.05). The results suggest that the two age groups did not significantly differ on most factors except for CAP. The test statistics in the table refer to the Mann-Whitney U and Wilcoxon W tests, which were conducted to compare the ranks of the different variables between the two age groups (0-5 years and >6 years). The Z score and Asymp. Sig. (2-tailed) values are also provided to indicate the significance of the differences observed.

Table 2. B shows all the test statistics for various factors between two age groups (0-5 years and >6 years). The factors included in the table are Factor 1, Factor 2, Factor 5, CAP, SIR, Total Score, Aggression Score, Anxiety Score, Socialization Score, and Attention Score. The table provides valuable information on the significance of differences between the two age groups for each factor, which can aid in further analysis and interpretation of the data. Based on the values in the "Asymp. Sig. (2-tailed)", there are some statistically significant differences between the groups for certain variables. Specifically, there is a significant difference between the 0-5 year and > 6-year groups for the "CAP" variable (p=0.017). There are no other significant differences between the groups for the other variables. The table presents the Mann-Whitney U and Wilcoxon W test results, the Z-score, and the asymptotic significance level (2-tailed) for each factor and score. The grouping variable is 0-5 years and >6 years.

Table 3 shows the CAP variable's central tendency and dispersion measures in two age groups: 0-5 years and >6 years. The median CAP score for the >6 years group (8.00) is higher than the median for the 0-5 years group (6.00). The standard deviation for the 0-5 years group is 2.031, and for the >6 years group is 1.991, indicating that the scores are more dispersed in the younger age group. Both groups' minimum and maximum scores are 1 and 9, respectively.

Table 2. PARI data corelation with age

A.

	0-5 years		**>6 years**		**Total**
Factor 1	N=15	Mean Rank=20.67	N=35	Mean Rank=27.57	N=50
Factor 2	N=15	Mean Rank=20.00	N=35	Mean Rank=27.86	N=50
Factor 5	N=15	Mean Rank=20.03	N=35	Mean Rank=27.84	N=50
CAP	N=15	Mean Rank=18.23	N=35	Mean Rank=28.61	N=50
SIR	N=15	Mean Rank=21.40	N=35	Mean Rank=27.26	N=50
Total Score	N=15	Mean Rank=25.77	N=35	Mean Rank=25.39	N=50
Aggression Score	N=15	Mean Rank=26.33	N=35	Mean Rank=25.14	N=50
Withdrawal Score	N=15	Mean Rank=26.97	N=35	Mean Rank=24.87	N=50
Anxiety Score	N=15	Mean Rank=21.00	N=35	Mean Rank=27.43	N=50
Socialization Score	N=15	Mean Rank=24.37	N=35	Mean Rank=25.99	N=50
Attention Score	N=15	Mean Rank=23.87	N=35	Mean Rank=26.20	N=50

B.

Test Statistics	Factor 1	Factor 2	Factor 5	CAP	SIR	Total Score	Aggression Score	Withdrawal Score	Anxiety Score	Socialization Score	Attention Score
Mann-Whitney U	190,000	180,000	180,500	153,500	201,000	258,500	250,000	240,500	195,000	245,500	238,000
Wilcoxon W	310,000	300,000	300,500	273,500	321,000	888,500	880,000	870,500	315,000	365,500	358,000
Z	-1.537	-1.764	-1.740	-2.398	-1.388	-0.085	-0.266	-0.472	-1.437	-0.369	-0.523
Asymp. Sig. (2-tailed)	0.124	0.078	0.082	0.017	0.165	0.932	0.791	0.637	0.151	0.712	0.601

Table 3. CAP

	0-5 years	>6 years
N	15	35
Median	6.00	8.00
Std. Dev	2.031	1.991
Minimum	1	1
Maximum	9	9
25%	5.00	7.00
50%	6.00	8.00
75%	7.00	9.00

DISCUSSION

The hearing status of the hearing-impaired children and the resulting verbal language delays are thought to be associated with behavioral problems due to difficulty in perceiving the environment and events and the inability to express themselves adequately. The majority of the literature has reported higher rates of internalization problems (e.g., anxiety and depression) and externalization problems (e.g., hyperactivity and behavioral problems) in children with hearing loss compared to normal hearing children [12,13]. One of the important effects of behavioral problems in children is that they can limit the audiological benefit they receive from their implants. Behavioral problems can make it difficult for children to adapt to implant use and speech education. Children presenting with behavioral problems benefit less from the CI. It can be thought that the audiological benefits of CI can also lead to greatly improved cognitive abilities [14-16]. It is completed with findings a strong statistically significant positive correlation was observed between CAP and SIR after the CI surgery.

In addition to studies showing that they exhibit behavioral problems even after successful hearing aid use or CI interventions, studies indicate a significant decrease in behavioral, emotional, and social problems after CI implants in deaf and hard-of-hearing children [15,16]. Preschool children with CIs have been reported to perform similarly to their hearing peers in CBCL measurements after one year of implant use [17]. It has also been reported that deaf children successfully cope with social and school-life demands, regardless of their speech and language success after CI [17-20]. Our study shows a significant difference between the CAP variable's 0-5 year and > 6-year groups (p=0.017).

It was observed that they actively participate in school and sports activities, as in the normal-hearing student group [18]. Language deficiencies can lead to communication difficulties and consequently trigger social problems and aggressive behavior. SIR of auditory performance test scores (i.e., CAP) can be considered determinants of social and aggressive

behavior problems. The study by Wei-Chieh Chao et al. showed that the CAP scale scores of CI patients were correlated with all CBCL test contents except "Somatic Complaints" and "Thought Problems." Children with more problematic behaviors had lower CAP / SIR scores [20-25]. It has the same correlation in our study.

Early initiation of hearing rehabilitation with hearing aids or CIs improves language development and social-emotional adaptation [20]. It has been stated that early implantation in children with hearing loss has an important effect on the development of hearing due to the development of early neuronal plasticity, allowing the development of speech perception and verbal language acquisition [21]. Prolonged CI usage time, better performance in early-age implantation, and auditory speech perception affect the performance of expressive and receptive verbal language [26-28]. In our study, the number of patients with 0-2 years of CI use was 4 (8%), the number of patients with 3-5 years of CI use was 11 (22%), and the number of patients with six years or more of CI use was 35 (70%). The mean duration of CI use was 8.4 years, with a minimum of 1 and a maximum of 15 years. There were significant changes between the CI using period and behavioral status.

Compared with the normative language acquisition process, it shows that these children develop expressive and receptive oral language skills and have linguistic skills patterns under their chronological age. The language skills of children who were implanted after one year old were shown to decrease [23]. In addition, the implantation age of CI users, children's hyperactivity and attention deficit disorder, and behavioral problems negatively correlate with [24]. In recent years, a significant increase in the number of children undergoing CI with significant disabilities, in addition to their deafness, was seen. Additional problems are known to decrease these children's language and auditory performance levels compared to deaf children with CIs without additional problems. This situation is much more important in children with CIs with more than one additional disability. However, CI helps them improve their communication skills [25].

High family income has been associated with better language performance before CI and accelerated improvement in language understanding after CI [26]. In early communication interactions, maternal sensitivity has been shown to affect language outcomes positively [29, 30]. In our study, the socioeconomic status of 18 (36%) parents was at the lower level, 27 (54%) of the parents were at the intermediate level, and 5 (10%) of the parents were at the upper level. Patients with bilateral implants were observed to have a significantly higher success rate than patients with unilateral implants. Success status was also significantly correlated with comorbidities. Regarding behavioral outcomes, aggressive behavior was significantly lower in patients who underwent surgery before the age of 4 years, and it is associated with other literature regarding the ''parent and children'' relation in different situations and diseases [31, 32]. Moreover, those who had a duration of implant use of 6 years or more had significantly higher CAP scores.

Our study suggests that socioeconomic factors, including the 36% of parents from lower socioeconomic backgrounds, potentially affected children's access to quality rehabilitation services and specialized education, influencing behavioral outcomes measured by the CBCL scale. The education and professional level of the parents, assessed on a five-point scale, might have significantly dictated the parental involvement in the children's recovery process. This is exemplified by the finding of reduced aggressive behavior in children who underwent surgery before the age of four, indicating possible better access to early intervention resources. Thus, socioeconomic disparities appear to have a notable impact on post-surgery outcomes.

There are several positive aspects of this study. The study provides valuable insights into the behavioral outcomes of children with CIs, which can help inform clinical decision-making and improve the quality of care for this patient population. Using standardized measures, such as the CBCL, CAP II, and Speech Intelligibility Rating Scale, enhances the validity and reliability of the findings. The study provides evidence for the effectiveness of CI in improving speech perception and language skills in children with hearing loss, which can lead to improved communication and social interactions. Additionally, the study highlights the importance of early detection and treatment of hearing loss in reducing the risk of behavioral problems in children with hearing loss. Finally, the study emphasizes the need for a multidisciplinary approach to caring for children with CIs, including specialized education and support services for the child and their family.

Limitations

There are several limitations to this study. The sample size was relatively small, which may limit the generalizability of the results to larger populations. Additionally, the study design was cross-sectional, which limits the ability to establish causal relationships between CI and behavioral outcomes. Longitudinal studies are needed to examine the long-term effects of CI on behavioral outcomes over time. The study only included patients who were using verbal communication, which may exclude patients who are nonverbal or use sign language as their primary mode of communication. The study did not include a control group of children with hearing loss who did not receive CIs. This makes it difficult to determine whether the observed improvements in behavioral outcomes are specifically related to CI or other factors, such as access to specialized education and support services. Future research should consider these factors to understand better the complex interplay between hearing loss, CI, and behavioral outcomes in diverse populations

CONCLUSIONS

Our study underscores the value of early CIs in enhancing speech and reducing aggressive behavior in children with hearing loss. The key to success is early surgery, ideally before age 4, and a multidisciplinary approach encompassing specialized education and family support. This work adds to the mounting evidence that CIs are a viable treatment option, spotlighting the necessity for further research.

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Ethical Approval: This study was designed at the University of Healt Sciences, Izmir Bozyaka Education and Research Hospital. The ethics committee of the approved this study (approval number: 03 and date 20/12/2016).

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Original Research

The Relationship Between the Resected Colon Length and the Number of Lymph Nodes in Colorectal Cancer: A Retrospective Cohort Study

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ABSTRACT

Objective: The prognostic factors that are important for colorectal cancer are the pathological grade of the tumor and the existence of lymph node involvement. Currently, the curative treatment option is resection of the tumor with adequate length and margin along with complete dissection of lymph nodes draining the site of resection. Our study investigated into retrieving adequate lymph nodes for accurate staging as well as the relationship between lymph nodes and colon and rectum specimen length.

Methods: This retrospective cross-sectional study examined the correlation between resected colon length and lymph node count in patients with colorectal cancer diagnosed between January 2010 and June 2018. We defined a cutoff value for the segment length to be resected to allow adequate staging of the tumor. Furthermore, we examined the relationship between the resected segment lengths and survival.

Results: Of the patients who were included in this study, 211 were men and 169 were women. The mean resected colon length was 26.47±17.09 cm and the mean dissected lymph node count was 29.05±20.84. There was a positive correlation between specimen length and total lymph node count as well as specimen length and the existence of reactive lymph nodes that were statistically significant (r=0.319, p=0.001; r=0.312, p=0.001, respectively). In our study, tumor localization was described in three regions: the right colon, left colon and rectum. The mean right colon region specimen length was 28.8±15.5 cm, while it was 22.0±11.0 cm for the left colon region, and 21.7±10.6 cm for the rectum region. The 5-year overall survival rate was 53.2%, whereas the 5-year disease-specific survival rate was 58.2%.

Conclusion: The mean specimen length to achieve the cutoff value for adequate lymph node retrieval (least 12 lymph nodes) was 16 cm in our study (ROC curve, AUC=0.689±0.05, p=0.001). The resected colon length and the number of retrieved lymph nodes were positively correlated in our study. Based on this confirmation, a cutoff value of 16 cm was calculated to achieve an adequate segment length to be resected.

Keywords: Colorectal cancer, lymph node, prognosis, colon-rectum length, stage

INTRODUCTION

Colorectal cancer is the third most common tumor of the gastrointestinal system [1,2]. It is ranked second among cancer-related deaths [1,2]. The prognostic factors that are important for colorectal cancer are the pathological grade of the tumor and the existence of lymph node involvement. Determining the prognostic factors that affect survival, accurate staging and the treatment of choice is important for colorectal cancer [3]. Currently, the curative treatment option is resection of the tumor with adequate length and margin along with complete dissection of lymph nodes draining the site of resection [3,4]. While a variety of classifications have been used for staging, the TNM staging system is currently used. This staging is determined based on spread to lymph nodes, invasion through the colon wall and metastasis to distant organs. T indicates the degree of invasion, N is related to the metastatic locoregional lymph node count and M signifies whether there is metastasis or not [5]. Following accurate staging, postoperative adjuvant therapy options are determined for patients [6,7].

For accurate staging in patients who have undergone colorectal cancer resection, many institutions, including the American Joint Commission on Cancer (AJCC) guidelines, the National Cancer Institute (NCI), and the American Society of Clinical Oncology (ASCO), state that a minimum of 12 lymph nodes should be dissected [8]. For this reason, an adequate colon length should be resected during surgery. In our research, we aimed to determine the relationship between sufficient lymph node dissection and adequate colon length resection in patients with colorectal cancer.

Main Points;

- The most important two prognostic factors in nonmetastatic resectable colorectal cancer are the grade of the tumor and the involvement of lymph nodes.
- The curative treatment option is the resection of the tumor by resecting the colon at an adequate length to obtain tumor-free margins and the complete dissection of lymph nodes draining the site of resection.
- For accurate staging, a minimum of 12 lymph nodes should be retrieved.
- The retrieval of more than 12 lymph nodes was associated with longer survival compared to the dissection of 11 or fewer lymph nodes.
- Our study has shown the positive and statistically significant correlation between the length of the resected colon specimen and the number of retrieved lymph nodes.
- We found a cut-off value of 16 cm for the specimen length to retrieve an adequate number of lymph nodes.

MATERIALS AND METHODS

This study investigated the relationship between resected colon length and lymph node count retrospectively in patients diagnosed with colorectal cancer who underwent surgery between 01 January 2010 and 20 June 2018 at Gaziantep University Hospital, Department of General Surgery. The study also examined other parameters that affect the lymph node count.

The study included 380 patients who were diagnosed with colorectal cancer. Age and sex information was used to evaluate their demographic and clinical features. Specimens retrieved from patients who had undergone surgery were evaluated by the department of pathology. The histopathological examination determined colon and rectum specimen length, total lymph node count, existence of reactive lymph nodes, existence of malignant lymph nodes, T staging, N staging, metastasis, overall stage, lymphovascular invasion, and perineural invasion. Tumor localization and the surgical procedures that were performed were determined through operative records. Parameters of age, sex, colon specimen length and lymph node count were compared. For patients diagnosed with malignant disease, overall survival and disease-specific survival analyses were performed. Parameters affecting either type of survival were evaluated separately. Thirty-three patients who died within the early postoperative period due to comorbidities were excluded from disease-specific survival analyses. In patients with malignant disease, differences in sex for specimen length, total lymph node count, existence of reactive lymph nodes and malignant lymph nodes were questioned. Survival analyses were performed with respect to staging. Specimen length and lymph node count were evaluated in relation to the type of surgical procedure performed and tumor localization.

This study was approved by the ethics committee of Gaziantep University on July 4, 2018 with approval number 2018/166.

Statistical Analysis

The distribution of collected data was assessed using the Shapiro–Wilk test. Spearman's rank correlation was used to determine the correlation between variables with a nonnormal

Table 1. Summary of the tested variables; n:number of patients

		n	%
Status	Survivors	270	71.1
	Non-survivors	110	28.9
Gender	Male	211	55.5
	Female	169	44.5
T1234	1	15	3.9
	2	51	13.4
	3	210	55.3
	4	87	22.9
	T *in situ*	17	4.5
N0123	0	219	57.6
	1	94	24.7
	2	67	17.6
M01	0	281	73.9
	1	99	26.1
Stage	0	17	4.5
	1	44	11.6
	2A	105	27.6
	2B	13	3.4
	3A	14	3.7
	3B	88	23.2
	4	99	26
Surgical Procedure	Anterior resection	35	9.2
	Abdominoperineal resection (APR)	38	10.0
	Low anterior resection	101	26.6
	Right hemicolectomy	113	29.7
	Segmental resection	17	4.5
	Sigmoidectomy	20	5.3
	Left hemicolectomy	39	10.3
	Total colectomy	17	4.5
Tumor Region	Right	130	34.2
	Left	92	24.2
	Rectum	142	37.4
	Total	16	4.2
Lymphovascular Invasion	Present	89	23.4
	Absent	291	76.6
Perineural Invasion	Present	47	12.4
	Absent	333	87.6
Type of Surgery	Emergency	48	12.6
	Elective	332	87.4

distribution. For the comparison of features with a nonnormal distribution in the two groups, the Mann–Whitney U test was used. Survival rates were estimated using the Kaplan–Meier method. To determine the factors that impact survival, Cox regression analysis was used. As descriptive statistics, the mean±standard deviation for quantitative variables and the number and percentage for categorical variables were used. Statistical analyses were performed using SPSS for Windows (v 24.0), and $p<0.05$ was accepted as statistically significant.

RESULTS

Of the patients included in this study, 211 were men and 169 were women. The overall mean age with standard deviation was 57.86±14.48. The mean age in men was 56.37±14.59, while it was 59.69±14.15 in women. The mean resected colon length was 26.47±17.09 cm, and the mean dissected lymph node count was 29.05±20.84. In men, the mean dissected lymph node count was 30.51±23.83; while in women, it was 27.22±27.22. In our study, tumor localization was described in three regions: the right colon, left colon and rectum. The rectosigmoid region was included in the rectum region. There were 130 right colon region patients, 92 left colon region patients and 142 rectum region patients. The mean right colon region specimen length was 28.8±15.5 cm, while it was 22.0±11 cm for the left colon region, and 21.7±10.6 cm for the rectum region. Among the majority of the patients with transverse colon tumors, 113 patients underwent right hemicolectomy, 17 patients with central transverse colon tumors underwent segmental resection, 39 patients underwent right hemicolectomy, 20 patients underwent sigmoidectomy, 38 patients underwent abdominoperineal resection (APR), 101 patients underwent low anterior resection, 35 patients underwent anterior resection and 17 patients underwent total colectomy. The mean specimen length for right hemicolectomy was 30±15.6 cm. The segmental colon resection mean specimen length was 29.2±16.8 cm. The mean specimen length for sigmoidectomy was 21.8±11.8 cm. The left hemicolectomy mean specimen length was 27.3±13 cm. For anterior resection, low anterior resection and APR, the mean specimen lengths were 20±7.7 cm, 20±9.6 cm and 25.3±10.6 cm, respectively (Table 1).

For 291 patients, lymphovascular invasion was reported to be negative, while it was positive for 89 patients. For 333 patients, perineural invasion was reported to be negative, while it was positive for 47 patients. According to staging parameters, 17 patients were stage 0, 44 patients were stage 1, 118 patients were stage 2, 102 patients were stage 3, and 99 patients were stage 4. Ninety-nine patients had metastasis. The number of patients with malignant lymph nodes was 161. Of the 380 patients, 332 underwent elective surgery, while 48 underwent emergency surgery. Among patients who had an adequate number of lymph nodes dissected (12 or more), the mean specimen lengths showed a significant difference between elective surgery patients who had a mean specimen length of 35±20.6 cm and emergency surgery patients who had a mean specimen length of 26.7±17.1 cm (Table 1).

According to ACJJ, the minimum adequate dissected lymph node count, was 12. From 329 of our patients, 12 or more lymph nodes were retrieved and the mean specimen length of these patients was 27.61±17.65 cm. From 51 patients, an inadequate number of lymph nodes were retrieved (11 or less). The mean specimen length for these patients was 19.1±10.29 cm.

To calculate a cutoff value for colon specimen length, ROC curve analysis was conducted. In ROC curve analysis, the discrimination threshold is determined based on the AUC value. AUC>0.8 is considered to have excellent discriminating ability. In our study, the AUC was calculated as AUC=0.689±0.05, p=0.001 (Table 2).

This indicates that a resected colon specimen of longer than 16 cm will be associated with an 82% likelihood of having retrieved an adequate number of lymph nodes. However, a specimen length of shorter than 16 cm will be associated with a 53% likelihood of ending in the retrieval of an inadequate number of lymph nodes (Figure 1).

Patient age and specimen length were found to have a weak, negative correlation, which was significant (r=-0.130, p=0.011). Patient age and the number of total retrieved lymph nodes were found to have a weak, negative correlation, which was significant (r=-0.156, p=0.002). Patient age and the existence of reactive lymph nodes were found to have a weak, negative correlation, which was significant (r=-0.149, p=0.004). There was no statistically significant correlation between patient age and other parameters (T, N, and M stage).

There was a positive correlation between specimen length and total lymph node count as well as specimen length and the existence of reactive lymph nodes, which were significant (r=0.319, p=0.001; r=0.312, p=0.001, respectively). There was no significant correlation between specimen length and other

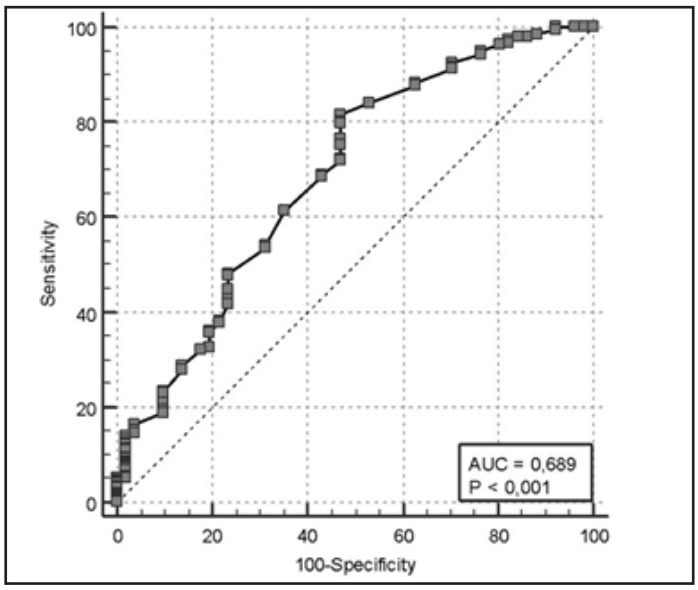

Figure 1. AUC curve for the specificity and the sensivity analysis of a specimen length of >16cm to achieve an adequate number of retrieved lymph nodes.

parameters (the existence of malignant lymph node, T, N, and M stage). There was a positive correlation between total lymph node count and reactive lymph nodes, which was significant (r=0.957, p=0.001). There was no significant correlation between total lymph node count and other parameters (the existence of malignant lymph node, T, N, and M stage). The existence of malignant and reactive nodes was found to have a weak, negative correlation, which was significant (r=-0.144, p=0.005) (Table 3).

N stage and the existence of reactive lymph nodes were found to have a weak, negative correlation, which was significant (r=-0.146, p=0.004). N stage and the existence of malignant lymph nodes were found to have a strong, positive correlation, which was significant (r=0.938, p<0.001). N stage and T stage were found to have a strong, positive correlation, which was significant (r=0.261, p<0.001).

Table 2. ROC analysis findings for a specimen length of >16 cm.

Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	-LR
>16 cm	81.76	77.2 – 85.8	52.94	38.5 – 67.1	1.74	0.34

Table 3. Correlations between variables

	Correlation	Specimen Length	Total Number of Lymph Nodes	Number of Reactive Lymph Nodes	Number of Malignant Lymph Node	T Staging	N Staging	M Staging	Overall Stage of the Tumor
Age	r	-0.130*	-0.156**	-0.149**	-0.030	-0.048	-0.012	-0.029	0.043
	p	0.011	0.002	0.004	0.554	0.360	0.821	0.568	0.403
Specimen Length	r	1.000	0.319**	0.312**	-0.027	-0.025	-0.049	-0.054	0.054
	p		0.001	0.001	0.601	0.631	0.346	0.294	0.297
Total Number of Lymph Nodes	r		1.000	0.957**	0.055	-0.035	0.032	-0.070	-0.085
	p			0.001	0.287	0.505	0.528	0.171	0.099
Number of Reactive Lymph Nodes	r			1.000	-0.144**	-0.109	-0.146	-0.138	-0.079
	p				0.005	0.038	0.004	0.007	0.124
Number of Malignant Lymph Nodes	r				1.000	0.251	0.938	0.254	0.048
	p					0.000	<0.001	0.001	0.346
T-Staging	r					1.000	0.261	0.382	-0.048
	p						<0.001	0.001	0.364
N-Staging	r						1.000	0.256	0.061
	p							<0.001	0.233
M Staging	r							1.000	0.012
	p								0.811

r:Spearman rank correlation coefficient, *significant for p<0.05; **significant for p<0.01

Colon-rectum specimen length, total lymph count, and the existence of reactive lymph nodes showed significant differences with varying regions (Table 4). According to Dunnett's C multiple comparison test, conducted to determine which groups caused differences: for specimen length, right-left, right-rectum, total-right, and total-left; for total lymph node count, right-left, right-rectum, total-right, total-left, and total-rectum pairs, had significant correlations (Table 5).

Specimen length, total lymph node count, the existence of reactive and malignant lymph nodes, T stage, N stage, M stage, and stage with respect to sex showed no significant difference (p>0.001) (Table 6).

Survival by Tumor Stage and Lymph Node

The numbers of patients with 12 or more lymph nodes retrieved in stages 0, 1, 2, 3, and 4 were 15 (88.2%), 39 (88.6.4%), 105 (88.2%), 86 (85.1%), and 84 (84.8%), respectively. The number of patients with an inadequate number of lymph nodes retrieved in stages 0, 1, 2, 3, and 4 was 2 (11.8%), 5 (11.4%), 14 (11.8%), 15 (14.9%), and 15 (15.2%), respectively. An increase of one node in the number of malignant lymph nodes increased the hazard ratio by a factor of 1.04. An increase in the reactive lymph node number by each unit decreased the hazard ratio by approximately 3%. The M stage positivity increased the hazard ratio by a factor of 2.44. There was no statistically significant difference between sexes in terms of survival rates (Table 6).

Table 4. Colon specimen length by tumor stages

T Stage	Colon Specimen Length (cm) Mean±SD
0	23.23±17.11
1	26.33±15.82
2	25.49±14.85
3	25.79±16.31
4	28.96±20.61

Table 5. Mean number of retrieved lymph nodes and mean specimen length by tumor location

Tumor Location	Mean Number of Total Lymph Nodes	Specimen Length
Right	33.1±18.9	28.8±15.5
Left	23.7±17.4	22.0±11.0
Rectum	25.6±15.8	21.7±10.6
Total	58.1±49.0	75.6±23.6
P	<0.001	<0.001

Table 6. Cox regression analysis. Evaluation of the effects of the specimen length, the total number of retrieved lymph nodes, the presence of reactive and malignant lymph nodes, the T-stage, N-stage, and M-stage of the tumor, and the effect of gender on overall survival.

Variable	HR(95% CI)	P
Specimen Length	1.01 (0.99 -1.02)	0.092
Total Number of Lymph Nodes	0.98 (0.97 -1)	0.010*
Number of Reactive Lymph Nodes	0.97 (0.95 -0.98)	<0.001*
Number of Malignant Lymph Nodes	1.04 (1-1.08)	0.036*
T-Stage	1.01 (0.75 -1.35)	0.943
N-Stage	0.36 (1.03 -1.80)	0.029*
M-Stage	2.44 (1.61 -3.68)	<0.001*
Gender	1.35 (0.92 -1.98)	0.123

HR: Hazard Ratio, CI: Confidence Interval, * significant for p<0.05.

Patients with 12 or more lymph nodes dissected showed better long-term survival than patients with 11 or fewer lymph nodes dissected (log-rank test p=0.03) (Figure 2).

The survival rates for patients were as follows: 94.1% for stage 0, 84.1% for stage 1, 85.6% for stage 2, 65.7% for stage 3, and 49.5% for stage 4. The 1-year survival rates, 2-year survival rates and 5-year survival rates were 89.2%, 82%, and 75%, respectively for stage 0. However, these rates were 87.2%, 80%, and 68% for stage 1; 89%, 84%, and 72% for stage 2; 89.2%, 84%, and 72% for stage 3; and 81%, 73.5%, and 65% for stage 4, respectively.

Our results indicated a decrease in survival rates with advanced disease (p=0.001) (Table 7) (Figure 3). Overall survival rates were not different with respect to the location of the tumor (on the right side, left side, or in the rectum) (log-rank test. p=0.759).

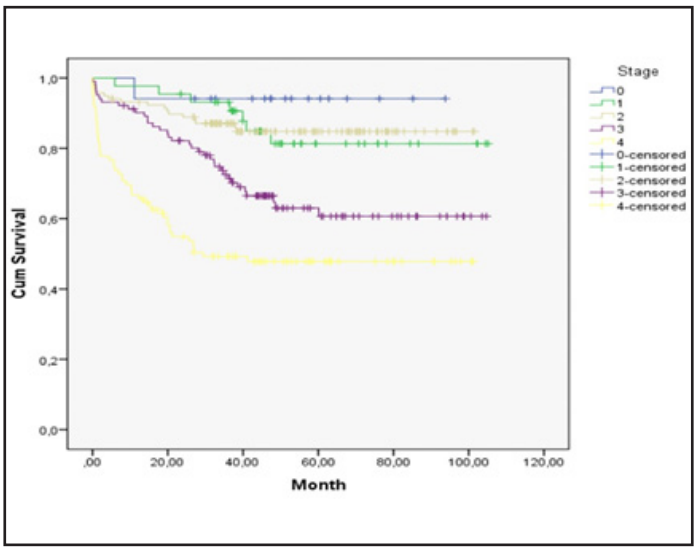

Figure 3. Kaplan-Meier survival depending on stages log-rank test P:0.001

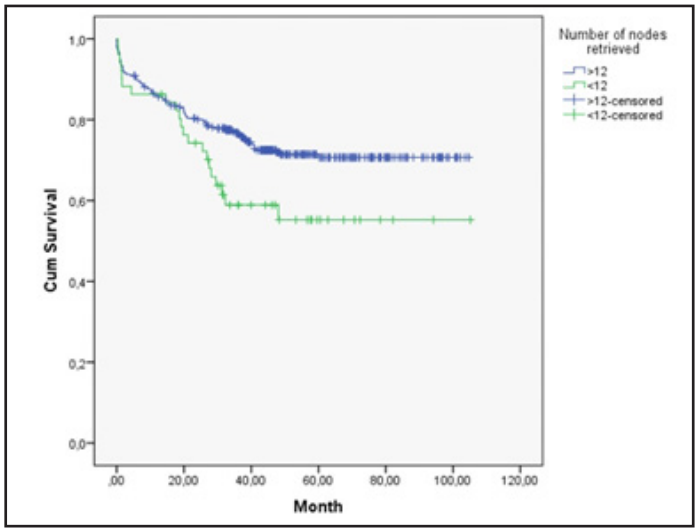

Figure 2. Kaplan-Meier survival analysis with respect to the number of dissected lymph nodes. Patients with 12 or more lymph nodes dissected appeared to have better long-term survival than patients with 11 or fewer lymph nodes dissected (Log-rank test, p:0.03).

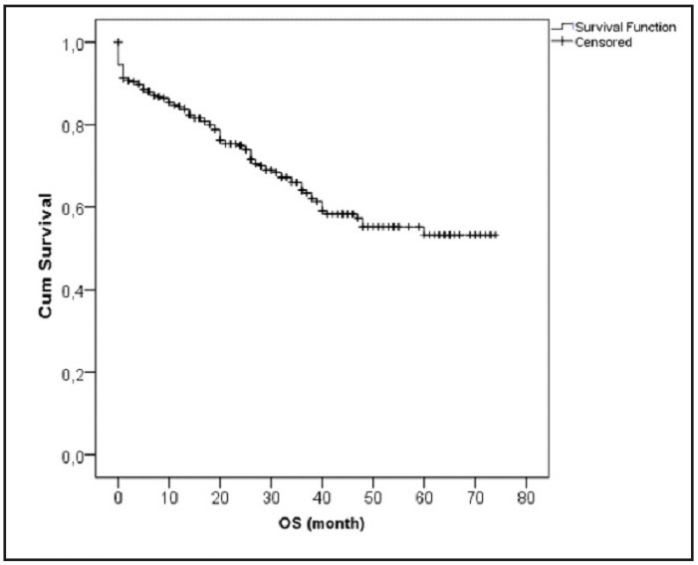

Figure 4. Overall survival rates

Table 7. Correlation between tumor stages and survival

Tumor Stage	Mean[a]			
	Estimated Survival Rate	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
0	88.967	4.719	79.718	98.217
1	91.555	4.691	82.361	100.748
2	88.370	2.948	82.591	94.149
3	73.634	4.240	65.324	81.944
4	53.655	4.720	44.405	62.905
Overall	77.748	2.205	73.425	82.070

Log-Rank test. P=0.001

Overall Survival

Of the 380 patients with malignant disease, 71.1% were survivors and 28.9% were nonsurvivors at the time of the analysis. For these patients, the mean duration of survival was 49.87±1.82 months, the one-year survival rate was estimated to be 84.4%, and the 5-year survival rate was estimated to be 53.2% (Figure 4).

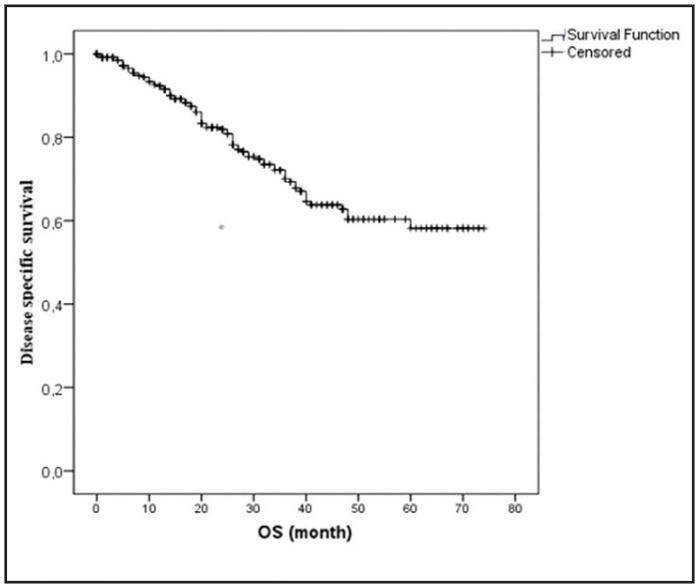

Figure 5. Disease spesific survival

Disease-Specific Survival

Of the 347 patients with malignant disease, 77.8% were survivors and 22.2% were nonsurvivors at the time of the analysis. For the survivors by the time of the analysis, the mean duration of survival was 56.91±1.33 months, the one-year survival was estimated to be 92.3%, and the 5-year survival was estimated to be 58.2% (Figure 5).

DISCUSSION

Accurate staging is crucial for colorectal cancer. For accurate staging, a minimum of 12 lymph nodes must be retrieved. Other studies in the literature have shown that patients with more than 12 lymph nodes dissected had longer survival than patients who had 11 or fewer lymph nodes dissected [9,10,11]. In our study, an adequate number (12 or more) of lymph nodes were retrieved from 329 patients. Patients with an adequate number (12 or more) of lymph nodes dissected had longer survival than patients with an inadequate number (11 or less) of lymph nodes dissected (Kaplan–Meier survival analysis, log-rank test p=0.03).

Pages et al. have shown that the number of malignant lymph nodes does not increase or the number of reactive lymph nodes increases as the length of the resected colon increases, despite having performed colorectal surgeries on different patients using the same techniques, due to biological differences in the lymph nodes of the operated portion of the colon mesentery and differences in the immunological response of the body [12,13]. In our study, specimen length showed a positive correlation with total and reactive lymph node counts (r=0.319, p=0.001; r=0.312, p=0.001, respectively). Specimen length showed no correlation of statistical significance with other parameters.

In the present study, lymph node count was correlated with age. This correlation between total lymph node count and age was an inverse correlation (r=-0.156, p=0.002). The lymph node count decreases with increasing age [14-16]. Our study has revealed similar results. Reasons for this may be weaker immunological response and the unwillingness of surgeons to perform wide resections on older patients.

While some studies in the literature have reported an increased number of lymph nodes in women [17-19], in our study, more lymph nodes were retrieved from men. However, this difference in our study was not significant.

Important prognostic factors for colorectal cancer include stage and the existence of metastatic lymph nodes [20]. In our study, with more advanced stages, survival rates decreased through Kaplan–Meier survival analysis with the log-rank test (p=0.001).

Many factors are known to affect overall survival, such as tumor localization, stage, tumor differentiation and lymph node involvement [21]. In our study, while metastatic lymph nodes affected survival, varying tumor localization showed no significant difference (log-rank test p=0.759).

There are various published studies on the relationship of specimen length and lymph nodes for colorectal cancer. F. Stracci et al. reported inadequate lymph node dissection in the case of specimen lengths less than 20 cm [22]. In our study, contrary to the literature, the cutoff value of mean specimen length for the retrieval of an adequate number (12 according to ACJJ) of lymph nodes was calculated as 16 cm (ROC curve, AUC=0.689±0.05, p=0.001). In 79.73% (n=303) of patients, as a result of operations where correct surgical techniques were applied, a minimum specimen length of 16 cm was achieved. This length and at least 12 lymph nodes are recommended for

all oncological colon resections, regardless of the location of the tumor.

Limitations

Although our study was a single center and retrospective study with important limitations, there is a positive correlation between resected colon specimen length and the number of lymph nodes retrieved.

CONCLUSIONS

The mean specimen length to achieve the cutoff value for adequate lymph node retrieval (the retrieval of at least 12 lymph nodes for an adequate pathological examination as defined by AJCC) was 16 cm in our study (ROC curve, AUC=0.689±0.05, p=0.001). Furthermore, the length of the resected colon specimen and the number of retrieved lymph nodes were positively correlated. Based on this confirmation, a cutoff value of 16 cm achieved an adequate segment length to be resected.

There was a positive correlation between the number of lymph nodes evaluated after surgical resection and the survival of patients with colon cancer. The results indicate that the number of lymph nodes analyzed should be considered as an indicator of the level of quality in colon cancer management. Our findings indicate that the removal of the colon can have a significant impact when following oncological surgical standards. In this study, we concluded that colorectal tumor surgery would be effective when performed by expert surgeons by applying correct techniques.

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Original Research

Baseline Sodium-Glucose Cotransporter-2 Inhibitor Use Strongly Attenuates the Uric Acid-Elevating Effect of Thiazide Exposure

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ABSTRACT

Objective: Thiazide diuretics are among the major anti-hypertensive medications. However, their hyperuricemic effect restricts their use in patients with gout. Sodium glucose co-transporter 2 inhibitor (SGLT-2i) initiation lowers serum uric acid (SUA) levels. It is not known whether existing SGLT-2i use affects the SUA increasing effect of thiazides.

Methods: Post-hoc data analysis of our published study was conducted. Hypertensive patients who were initiated on thiazide diuretics or whose dose escalated were included (thiazide exposure). Demographic, clinical, and laboratory data were acquired via an electronic database. Patients were grouped according to SGLT-2i presence at the time of thiazide exposure. Since the number of SGLT-2i users was low, bootstrapping via simple random sampling was performed.

Results: 144 patients were included in the study, of whom 13 were on SGLT-2i. Initial sample analysis revealed that while baseline SUA levels were similar between groups, SUA change was significantly lower after thiazide exposure among patients receiving SGLT-2i (0.6 vs. 0.2, p = 0.039). Similarly, baseline SUA levels were similar, but SUA change after thiazide exposure was significantly lower among patients receiving SGLT-2 on bootstrapped data (0.13 [-0.25 - 0.57, 95%CI], vs. 0.61 [0.45 - 0.78, 95%CI], mean difference = 0.48, [0.04 - 0.91, 95%CI], p = 0.029).

Conclusion: This study revealed that thiazide diuretics may be a safe anti-hypertensive medication in terms of hyperuricemia among patients using SGLT-2i. Further studies with similar outcomes may result in the elimination of restrictive recommendations for the use of thiazides in patients with hyperuricemia or gout, provided patients are on SGLT-2i.

Keywords: Sodium glucose cotransporter 2 inhibitors, hypertension, uric acid, thiazides

INTRODUCTION

Hypertension is one of the major cardiovascular risk factors and affects almost 1 in 2 adults in the United States [1]. Hypertension is also a component of the metabolic syndrome, a syndrome characterized by abdominal adiposity, insulin resistance characterized by high fasting glucose, hypertension, and dyslipidemia [2]. Metabolic syndrome itself is also a cardiovascular disease risk factor [3]. Although not a component of metabolic syndrome, increased serum uric acid (SUA) levels and gout disease are associated with metabolic syndrome [4].

Hypertension and metabolic syndrome are encountered in 74% and 63% of gout patients, respectively [5, 6]. Besides the association, SUA levels are correlated with blood pressure, as a 1 mg/dl increase in SUA level is associated with a 10 mm Hg increase in systolic blood pressure [7].

Hyperuricemia and gout are not only associated with hypertension and metabolic syndrome, but they are also associated with other cardiometabolic diseases such as chronic kidney disease and atherosclerotic heart disease [8]. These close associations result in many patients receiving drug therapies that target multiple components of these diseases. While plenty of these effects are beneficial, some are deleterious. For example, sodium glucose co-transporter 2 inhibitors (SGLT-2i) emerged as anti-diabetic medications due to their glycosuric effects [9]. However, it was also discovered that they improve cardiovascular and kidney outcomes, resulting in their use in heart failure and chronic kidney disease patients, regardless of diabetes [10]. Recent studies also illustrated that SGLT-2i, compared to other oral anti-diabetics, decreases SUA levels [11].

However, not all co-effects are beneficial. Thiazide diuretics, either hydrochlorothiazide or thiazide-like, elevate SUA levels to some extent [12]. It is known that thiazide diuretics are associated with higher rates of gout flares [13]. Although it is not absolutely contraindicated, some guidelines assert either a relative contraindication warning or remark cautious use [14-16]. These recommendations cause only renin-angiotensin-aldosterone system inhibitors (RAASi) and calcium channel blockers (CCB) to be used as first-line treatment options among hypertensive patients with gout. However, many patients require more than two anti-hypertensive medications in order to achieve target blood pressures [16]. At this point, clinicians are left with only spironolactone and doxazosin, which both have low tolerability due to their hormonal and vascular side effects, respectively [17, 18].

Main Points;

- SGLT-2i drugs have uric acid-lowering effects when initiated. This study demonstrated that baseline SGLT-2i presence attenuates the uric acid-elevating effects of thiazide diuretics as well. This finding indicates that thiazide use among patients with high baseline uric acid levels or patients with gout may be safe, provided that patients are on SGLT-2i.

In this study, we wanted to investigate whether baseline SGLT-2i use at the time of thiazide initiation had any effect on thiazide diuretics' uric acid-increasing effect compared to the absence of SGLT-2i at the baseline.

MATERIALS AND METHODS

Design, Settings, and the Study Population

This study was designed as a post-hoc analysis of our recently published retrospective cohort study on hypertensive patients [19]. We analyzed our patients' data via electronic medical records (EMR). Four clinics, consisting of secondary and tertiary-level general internal medicine and cardiology clinics, contributed to the study.

The inclusion criteria were as follows:

- Having a hypertension diagnosis
- Having a thiazide diuretic initiated or its dose increased if already on thiazide (collectively named "thiazide exposure")
- Having data regarding SGLT-2i use
- Having a control visit for renal function and an electrolyte check within 4 weeks
- Having the relevant EMR data for the study
- Being over 18 years old

In daily practice, renal functions and electrolytes are not routinely checked within 4 weeks when only thiazides are initiated or their doses increased (i.e., thiazide exposure). However, since our published study's aim was to evaluate the effect of RAASi dose change on renal parameters, we have the relevant data for this study as well. This also means that the data acquired via EMR represents both thiazide and RAASi exposure. However, because RAASi other than losartan—which was used only in a few patients in the study cohort—is known not to affect SUA levels, we did not consider which RAASi patients were exposed to due to their lack of effect on SUA levels.

Clinical Data

The data acquired (and calculated) for each patient were as follows:

- Demographics: age and sex
- Relevant comorbidities: diabetes mellitus, coronary artery disease, heart failure, chronic kidney disease, and airway diseases
- Medications other than thiazides: RAASi, CCB, beta blockers, loop diuretics, beta-2 agonists
- Initial and Control Laboratory Values: Urea (mg/dL),

creatinine (mg/dL), estimated glomerular filtration rate (mL/min/1.73 m2), uric acid (mg/dL), sodium (mEq/L), potassium (mEq/L)

- Change in values: Initial renal function and electrolyte values (values at T0) were subtracted from control values (values at T1) to calculate the changes in values (values at T1 and T0).

Statistics and Bootstrapping

For descriptive statistics, continuous variables were presented as "mean (±standard deviation)" or "median (interquartile range)" according to their distribution pattern. Categorical variables were presented as "numbers (percentages)". For comparison of continuous variables' between-group differences, the student's t-test or Mann-Whitney U test was used according to the variables' distribution patterns. Pearson's chi-squared test ($\chi 2$ test) (or Fisher's exact test when needed) was used for comparison of categorical variables' between-group differences. Since the number of patients was small in the SGLT-2i receiving group and did not distribute normally, bootstrapping was performed for resampling, using 1000 samples. Simple random sampling was chosen as the bootstrap sampling methodology. Since bootstrapped samples were distributed normally, between-group differences were analyzed using a t-test. Confidence intervals were calculated for 95%, and lower-upper bound values were presented. Two-sided significance testing was performed to calculate p-values, and p-values less than 0.05 were considered significant. All analyses were conducted using IBM SPSS Software version 23.0 (SPSS Inc., Chicago, IL), licensed to the institution where the study was carried out.

Each patient in the study was assigned an anonymous identification number to protect confidentiality. The processing of the data did not require informed consent, and written informed consent was not obtained due to the study's retrospective design. The study complies with the principles outlined in the Declaration of Helsinki, and this study was approved by the Hacettepe University Institutional Review Board (Project number GO22/734).

RESULTS

Patient Characteristics

A total of one hundred and forty-four patients were found to be eligible and included in the study. Of whom, 13 were using SGLT-2i and 131 were not. Age and gender were similar between the two groups. All patients who were receiving SGLT-2i had diabetes mellitus, and none were receiving SGLT-2i solely for heart failure or chronic kidney disease progression slowing. Coronary artery disease was more frequent among SGLT-2i users. On the other hand, heart failure, chronic kidney disease, and airway disease rates were similar between SGLT-2i users and non-users. Considering initial anti-hypertensive medications, thiazides, calcium channel blockers, beta blockers, and loop diuretic use were similar between SGLT-2i users and non-users. However, RAASi use was slightly higher among SGLT-2i users (76.9 vs. 40.5%, $p < 0.01$). Table 1 illustrates patient characteristics in detail among SGLT-2 groups.

Renal Functions and Electrolytes of the Sample

Uric acid, urea, creatinine, estimated glomerular filtration rate, sodium, and potassium levels were similar between SGLT-2i users and non-users both during thiazide exposure (T0) and during the control visit (T1). Although T0 uric acid levels seemed lower among SGLT-2i non-users, they did not reach statistical significance (5.1 vs. 5.6, $p = 0.35$). Changes (Δ) in renal functions and electrolytes were also similar for urea, creatinine, estimated glomerular filtration rate, sodium, and potassium. However, Δ uric acid was significantly lower among SGLT-2i users compared to non-users (0.2 vs. 0.6, $p = 0.039$). Table 2 illustrates the renal functions and electrolytes of the sample before thiazide exposure, during the control visit, and the change between them in detail among the SGLT-2i groups.

Renal Functions and Electrolytes of the Bootstrapped Sample

Although Δ uric acid was significantly lower among SGLT-2i users, there were only 13 patients in the SGLT-2i user group. Because they did not meet parametric assumptions, this analysis was calculated via the Mann-Whitney-U test. We performed bootstrapping for uric acid at T0, T1, and Δ in order to compare newly generated data using a parametric test. Parametric testing of bootstrapped data showed uric acid levels at T0 and T1 were similar between SGLT-2i users and non-users. However, Δ uric acid was significantly lower among SGLT-2i users compared to non-users after thiazide exposure (0.13, -0.25 – 0.57, 95%CI vs. 0.61, 0.45 – 0.78, 95%CI, mean difference = 0.48, 0.04 – 0.91, 95%CI, $p = 0.029$). Table 3 illustrates uric acid values and changes in bootstrapped data according to patients SGLT-2i use and the results of the T-test in detail.

Table 1. Patient characteristics according to baseline SGLT-2i use

	Total (N = 144)	**SGLT-2i absent** (N = 131)	**SGLT-2i present** (N = 13)	**p***
Demographics				
Age	61 (14)	61 (13)	58 (16)	0.78
Female sex	95 (66%)	85 (64.9%)	10 (76.9%)	0.54
Comorbidities				
DM	81 (56.3%)	50 (38.2%)	13 (100%)	**< 0.001**
CAD	29 (20.1%)	21 (16%)	8 (61.5%)	**0.001**
HF	4 (2.8%)	4 (3.1%)	0	1
CKD	7 (4.9%)	6 (4.6%)	1 (7.7%)	0.49
Airway diseases	15 (10.4%)	14 (10.7%)	1 (7.7%)	1
Initial Medications				
RAASi	63 (43.8%)	53 (40.5%)	10 (76.9%)	**0.01**
Thiazides	19 (13.2%)	16 (12.2%)	3 (23.1%)	0.38
CCBs	39 (27.1%)	36 (27.5%)	3 (23.1%)	1
Beta blockers	46 (31.9%)	39 (29.8%)	7 (53.8)	0.11
Loop diuretics	3 (2.1%)	3 (2.3%)	0	1

CAD: Coronary Artery Disease, CCB: Calcium Channel Blockers, CKD: Chronic Kidney Disease, DM: Diabetes Mellitus, HF: Heart Failure, RAASi: Renin-Angiotensin-Aldosterone System Inhibitors, SGLT-2i: Sodium-glucose cotransporter-2 inhibitors

* p values less than 0.05 are shown in bold

Table 2. Renal functions and electrolytes of patients during initiation (or dose escalation) of thiazides, at control visit, and the difference between, according to baseline SGLT-2i use

	T_0			**T_1**			**Δ**		
Parameter	**SGLT-2i absent**	**SGLT-2 present**	**p***	**SGLT-2i absent**	**SGLT-2 present**	**p***	**SGLT-2i absent**	**SGLT-2 present**	**p***
Uric acid	5.1 (1.4)	5.6 (1.8)	0.35	5.7 (1.7)	5.7 (0.8)	0.80	0.6 (1.0)	0.2 (0.9)	**0.039**
Urea	28 (11)	33.5 (5)	0.20	34 (13)	39 (18)	0.08	3.8 (10)	5.5 (5.4)	0.43
Creatinine	0.75 (0.24)	0.74 (0.26)	0.57	0.76 (0.27)	0.71 (0.32)	0.44	0.02 (0.11)	0.01 (0.07)	0.95
eGFR	94 (21)	97(18)	0.84	93 (25)	97 (24)	0.50	-2 (10)	-2 (3.5)	0.49
Sodium	139 (3)	140 (2.5)	0.69	139 (3)	139 (2)	0.85	-1.08 (3.8)	-1.18 (2.1)	0.76
Potassium	4.3 (0.4)	4.3 (0.4)	0.82	4.2 (0.5)	4.1 (0.8)	0.43	-0.1 (0.6)	-0.2 (0.4)	0.42

eGFR: Estimated Glomerular filtration rate, SGLT-2i: Sodium-glucose cotransporter-2 inhibitors

T_0: Value at the initiation (or dose increase) of thiazides, T_1: Value at control visit, Δ: Calculated as (value at T_1) minus (Value at T_0)

* p values less than 0.05 are shown in bold

Table 3. Uric acid values and changes of bootstrapped data according to baseline SGLT-2i use and results of the T-test

	SGLT-2i absent		SGLT-2i present		Bootstrapped T-Test		
Parameter	**Mean**	**95% CI Lower - Upper**	**Mean**	**95% CI Lower - Upper**	**Mean Difference**	**95% CI Lower - Upper**	**p***
T_0 Uric Acid	5.19	4.96 – 5.41	5.50	4.91 – 6.06	-0.30	-0.91 – 0.30	0.30
T_1 Uric Acid	5.81	5.56 – 6.06	5.63	4.96 – 6.22	0.18	-0.46 – 0.85	0.58
Δ Uric Acid	0.61	0.45 – 0.78	0.13	-0.25 – 0.57	0.48	0.04 – 0.91	**0.029**

CI: Confidence Interval, SGLT-2i: Sodium-glucose cotransporter-2 inhibitors

T_0: Uric acid at the initiation (or dose increase) of thiazides, T_1: Uric acid at control visit, Δ: Calculated as (Uric acid at T_1) minus (Uric acid at T_0)

* p values less than 0.05 are shown in bold

DISCUSSION

This study illustrated that the uric acid increase due to thiazide exposure is significantly lower among patients who receive SGLT-2i compared to non-users. While it has been recently shown that "SGLT-2i initiation" has uric acid-lowering effects, to the best of our knowledge, this is the first study to reveal the uric acid-attenuating effects of "existing SGLT-2i use" among thiazide-exposed patients.

The link between uric acid and hypertension has been a subject of debate. Hyperuricemia is common among patients with hypertension as well as those with metabolic syndrome and diabetes mellitus, but hypertension is also a strong predictor of these comorbidities [11]. Clinical trials of uric acid-lowering medications for blood pressure reduction have resulted in inconsistent and conflicting results [20]; thus, although higher uric acid levels are associated with higher blood pressures, lowering uric acid levels via drugs is not part of the anti-hypertensive treatment strategy. Thiazide diuretics are among the major anti-hypertensive medication classes, with a uric acid-increasing effect. While thiazides' hyperuricemic effect does not alter their anti-hypertensive properties, it causes cautious use among patients with high uric acid levels [16]. Moreover, some guidelines [14, 15] recommend against the use of thiazides in gout patients due to the increased risk of gout flares [21] mediated via uric acid elevations. SGLT-2i initiation is shown to attenuate uric acid levels in type 2 diabetic patients when compared with other oral anti-diabetic medications [11, 22]. The mechanism responsible for the uric acid-lowering effect of SGLT-2i is the expression of glucose transporter 9 isoform 2 in the kidney tubules, which causes the excretion of D-glucose and uric acid in urine [22]. In vitro studies indicate that lower uric acid levels attained via SGLT-2i play a role in the anti-inflammatory effects of SGLT-2i [23].

Although not statistically significant, uric acid levels before thiazide exposure were higher among SGLT-2i users compared to non-users (5.1 vs. 5.6) in the study. This non-significant trend may be explained by the fact that all patients in the SGLT-2i user group were diabetic, which is associated with higher uric acid levels, but only 38.2% of the patients in the non-user group were diabetic. A meta-analysis illustrated that the uricosuric effect of SGLT-2i ranges between 0.6 and 0.7 mg/dl [24]. However, this meta-analysis shows the effect of SGLT-2i "initiation" on uric acid levels. Our study differs from the existing literature because we did not calculate the SGLT-2i initiation's uric acid-lowering effect but calculated whether baseline SGLT-2i presence could alter the uric acid-increasing effect of thiazide diuretics. We have shown that SGLT-2i presence during thiazide exposure was responsible for a 0.48 (0.04–0.91, 95%CI) mg/dL mean difference at uric acid levels compared to SGLT-2i absence. Moreover, uric acid levels only increased by 0.13 (-0.25–0.57, 95%CI) mg/dL in SGLT-2i users. Compared with the 0.61 (0.45–0.78, 95%CI) mg/dl at SGLT-2i non-users, this increase could be described as "negligible".

This finding is of particular importance since many hyperuricemic patients or patients with gout are deprived of the robust anti-hypertensive effects of thiazides due to their worrisome hyperuricemic effects. By adding SGLT-2i to eligible patients' treatment regimens, thiazides might find a role for hypertension treatment in hyperuricemic patients. This

strategy may also be the key to improving blood pressure target achievement rates.

Limitation

We acknowledge limitations of our study. The major limitation of the study was the fact that this was a post-hoc analysis of a retrospective study, thus prone to the limitations of retrospective analysis. Secondly, although SGLT-2i is used among non-diabetic heart failure and chronic kidney disease patients, patients in our study were receiving SGLT-2i for diabetes treatment; therefore, our results may not be generalized to non-diabetic patients. Thirdly, although we have performed bootstrapping in order to meet parametric assumptions, the number of patients in the SGLT-2i user group was low compared to non-users. And finally, we analyzed SGLT-2i medications as a group but did not analyze whether both empagliflozin and dapagliflozin, two SGLT-2i available in the area where the study was carried out, had the same effect on uric acid after thiazide exposure.

CONCLUSIONS

In conclusion, this study showed for the first time that not only the initiation but also the presence of SGLT-2i have profound effects on uric acid levels after thiazide exposure. The findings of our study should be tested in future pragmatic randomized trials. Results of this study as well as further trials may affect the hypertension guidelines' restrictive recommendations of thiazides, ease clinicians' hypertension management among patients with gout, and help patients achieve their target blood pressure.

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Original Research

Analytical Comparison of Maxillary Sinus Segmentation Performance in Panoramic Radiographs Utilizing Various YOLO Versions

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ABSTRACT

Objective: In this study, we aimed to evaluate the success of the last three versions of YOLO algorithms, YOLOv5, YOLOv7 and YOLOv8, with segmentation feature in the segmentation of the maxillary sinus in panoramic radiography.

Methods: In this study, a total of 376 participants aged 18 years and above, who had undergone panoramic radiography as part of routine examination at Gaziantep University Faculty of Dentistry, Department of Oral and Maxillofacial Radiology, were included. Polygonal labeling was performed on the obtained images using Roboflow software. The obtained panoramic radiography images were randomly divided into three groups training group (70%), validation group (15%) and test group (15%).

Results: In the evaluation of the test data for maxillary sinus segmentation, sensitivity, precision, and F1 scores are 0.92, 1.0, 0.96 for YOLOv5, 1.0, 1.0, 1.0 for YOLOv7 and 1.0, 1.0, 1.0 for YOLOv8, respectively.

Conclusion: These models have exhibited significant success rates in maxillary sinus segmentation, with YOLOv7 and YOLOv8, the latest iterations, displaying particularly commendable outcomes. This study emphasizes the immense potential and influence of artificial intelligence in medical practices to improve the diagnosis and treatment processes of patients.

Keywords: Maxillary sinus; Segmentation; Artificial intelligence; Deep learning models

INTRODUCTION

There are four pairs of paranasal sinuses in the maxillofacial region and cranium; maxillary, frontal, ethmoid and sphenoid sinuses. These sinuses are air-filled cavities lined with mucosa and connected to the nasal cavity. The nose and paranasal sinuses constitute both a functional unit and an integral component of the respiratory system [1]. Specialized epithelial tissue within these structures filters, warms and humidifies the air we breathe, thereby optimizing its suitability for the exchange of oxygen and carbon dioxide within the lungs [2].

The maxillary sinus, pyramid-shaped and the largest among paranasal sinuses, has its frontal wall formed by the facial surface of the maxilla and indented internally by the canalis sinuosus. The posterior wall is formed by the infratemporal surface, the superior wall is composed of the delicate, triangular orbit floor with the infraorbital groove, and the medial wall separates the sinus from the nasal cavity [3]. In the realm of dentistry, the maxillary sinus assumes particular significance among these structures due to its adjacency to dentoalveolar structures, the prevalence of pathologies, and the potential for the symptoms of these pathologies to be confused with symptoms of dental diseases.

Inflammatory paranasal sinus disease is the most prevalent condition affecting the maxillary sinuses. [4]. Dentists frequently encounter the task of distinguishing between dental diseases and other conditions when the maxillary sinus is implicated. Around 10-12% of instances of inflammatory maxillary sinus disease originate from dental sources. Sinus retention cysts usually occur at the floor of the maxillary sinus and are often detected incidentally on dental radiographs and cross-sectional imaging [5]. Mucoceles are formed by the accumulation of mucus when sinus drainage is obstructed and can occupy the sinus entirely. Additionally, mucoceles may induce bone expansion due to pressure effects [6]. Paranasal sinus osteomas are rare, yet they represent the prevalent benign bone growths within the paranasal region. These growths frequently exhibit no symptoms until they attain a particular size, typically being discovered coincidentally during medical examinations [7]. While malignancies arising from the paranasal sinuses are relatively uncommon, approximately 80% of such malignancies occur in the maxillary sinus [8]. Malignant paranasal sinus diseases typically become apparent in advanced stages when the tumor attains a size that triggers symptoms. The paranasal sinus mucosa, unlike oral mucosa, is not as readily accessible for routine examination, making early mucosal abnormalities harder to detect. Dentists can contribute to the diagnosis of maxillary sinus malignancies. The convergence of patient symptoms and clinical indicators should elicit concerns regarding the potential presence of maxillary sinus malignancy, prompting the need for timely referral to a specialized medical practitioner [9].

Panoramic radiography, employed as a standard imaging modality in dentistry, mostly includes the maxillary sinuses in the field of view. Therefore, dentists play a crucial role in diagnosing these diseases by examining the maxillary sinuses in panoramic radiographs where all these pathologies can be seen.

Main Points;

- The effectiveness of YOLOv5, YOLOv7, and YOLOv8 algorithms in segmenting the maxillary sinus on panoramic radiographic images was evaluated in the present study.
- The latest YOLO versions, YOLOv7 and YOLOv8, attained notably high success rates, whereas the success rate of YOLOv5 was comparatively lower than these versions.
- The realm of oral and maxillofacial radiology has also greatly benefited from the progress of artificial intelligence, offering robust assistance in the identification of anatomical structures and pathological conditions.

In 1956, John McCarthy of Dartmouth College introduced the term "artificial intelligence," which now serves as a general descriptor for machines emulating human intelligence's capabilities and functions [10]. Artificial intelligence has made dramatic advances in many fields in recent years, and medicine is one of the fields where artificial intelligence is currently making great progress [11].

Artificial intelligence is developing in the field of dentistry, as in other fields. Artificial intelligence can perform some tasks in dentistry with more precision, reduced need for personnel and minimized errors; It has proven successful in various tasks, ranging from organizing appointments to assisting in clinical diagnoses and treatment planning [12].

You Only Look Once (YOLO) is a popular and widely used artificial intelligence algorithm [13]. This model is a convolutional neural network based real-time object detection algorithm that is fast and high performing compared to its competitors. Redmon et al. introduced the first version of YOLO in 2015 [14]. Since then, new versions of YOLO have been developed and various features have been added to the algorithm.

In this study, we examined the success of the last three versions of YOLO with segmentation feature in segmenting the maxillary sinus in panoramic radiography. We believe that this study will both help dentists in determining the boundaries of the sinus in panoramic images and shed light on the success rates of YOLOv5, YOLOv7 and YOLOv8 algorithms.

MATERIALS AND METHODS

Patient Selection

For our research, a total of 376 participants, 188 males and 188 females, between the ages of 18 and 50, who had undergone panoramic radiography as part of routine examination at Gaziantep University Faculty of Dentistry, Department of Oral and Maxillofacial Radiology, were included. This study received approval from the Gaziantep University Clinical Studies Ethics Committee (Decision No : 2023/310).

Panoramic Radiography Protocol

Uniform digital panoramic images were captured using a consistent machine (Planmeca Proline XC, Helsinki, Finland) with the subsequent exposure parameters: 64 to 66 kVp; 6 to 9 mA; and an exposure time of 10 seconds. The patients were positioned within the dental panoramic machine, aligning the

machine's vertical line with the patient's midsagittal plane, while ensuring that the horizontal line (Frankfurt plane) remained parallel to the floor.

Image Labelling and Model Training

Polygonal labelling was performed on the obtained images using Roboflow software (Roboflow, Inc., Des Moines, Iowa, USA) (Figure 1).

The obtained panoramic radiography images were randomly divided into three groups training group (70%), validation group (15%) and test group (15%).

The obtained panoramic radiography images were resized from 1429x697 pixel size to 640x640 pixel size for the algorithm to work at the best performance. Resizing is a key preprocessing step, ensuring uniformity in image size (640x640 pixels) during dataset integration. This uniformity runs algorithms more quickly during training.

Also, the training data;

- Auto Orientation,
- Horizontal Flipping,
- Vertical Flipping,
- Rotation: Ranging from -15° to +15°,
- Grayscale: Applied to 25% of images,
- Blur: Up to 2.5 pixels was applied and it was aimed to increase the learning success of the model by increasing the number of images.

After these changes were applied to the training data, the number of images in the training, validation and test groups were as follows.

- Education group:786 image
- Verification group: 57 images
- Test group: 57 images

The methodology employed up to this point is outlined in Figure 2 as a summarized template.

Deep Learning Procedure

This study utilized the deep learning PyTorch Library and the open-source Python programming language (version 3.6.1; Python Software Foundation, Wilmington, DE, USA), along with the transfer learning approach. The architectures of YOLOv5, YOLOv7, and YOLOv8 were employed for the purpose of maxillary sinus segmentation.

Model Development

The open-source version of Python programming language (v.3.6.1) and PyTorch library were preferred for the model development process. Model training was performed on a computer with 16 GB RAM and equipped with an NVIDIA Tesla V100 graphics card. All model training was done in 10 epochs (training rounds). Google's COLAB platform was used for training and validation, which provides a virtual Linux computer.

Segmentation

In this study, the success of segmentation of maxillary sinuses in panoramic radiography images with YOLOv5, YOLOv7 and YOLOv8 deep learning models was investigated. Figure 3 illustrates the segmented maxillary sinuses achieved through YOLOv5, YOLOv7, and YOLOv8.

Statistical Analysis

True positive (TP): The maxillary sinus segmented by the model is actually the maxillary sinus.

False negative (FN): This is when the model does not segment the maxillary sinus, but it is actually the maxillary sinus.

False positive (FP): This is when the model segments the maxillary sinus, but it is not actually the maxillary sinus.

Sensitivity, accuracy and F1 score are calculated with these values;

- Sensitivity: Refers to the ability not to miss true positives. That is, it shows how few missed positive cases there are.
- Precision: It expresses the rate at which the values predicted as positive by the model are actually positive. That is, it shows how little the model makes false positives.
- F1 Score: A metric that balances precision and sensitivity. By considering the balance between sensitivity and precision, it helps to better evaluate the performance of the model.
- The Receiver Operating Characteristic (ROC) is a common metric used to evaluate the performance of a classification model. It graphs the true positive rate (TPR) of the model on the y-axis and the false positive rate (FPR) on the x-axis.
- The Area Under the Curve (AUC) represents the area beneath the ROC curve. AUC is a measure used to assess the overall classification performance of the model. The AUC value takes a value between 0 and 1:

If AUC = 0.5, the performance of the model is the same as random forecasting.

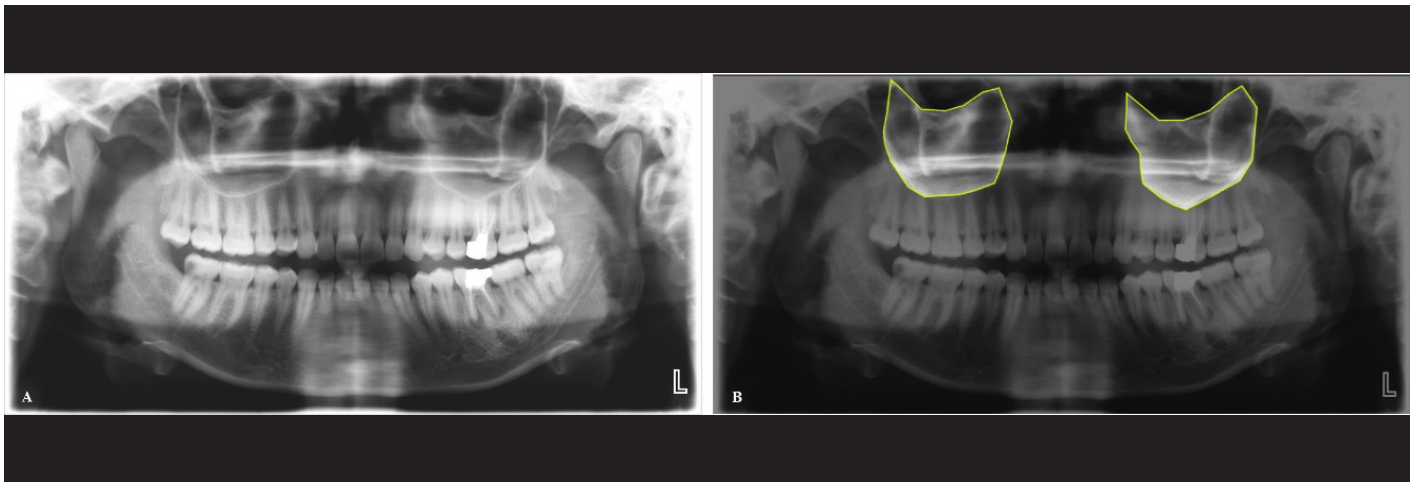

Figure 1. A: Original panoramic image B: Polygonal labelling of images using Roboflow software

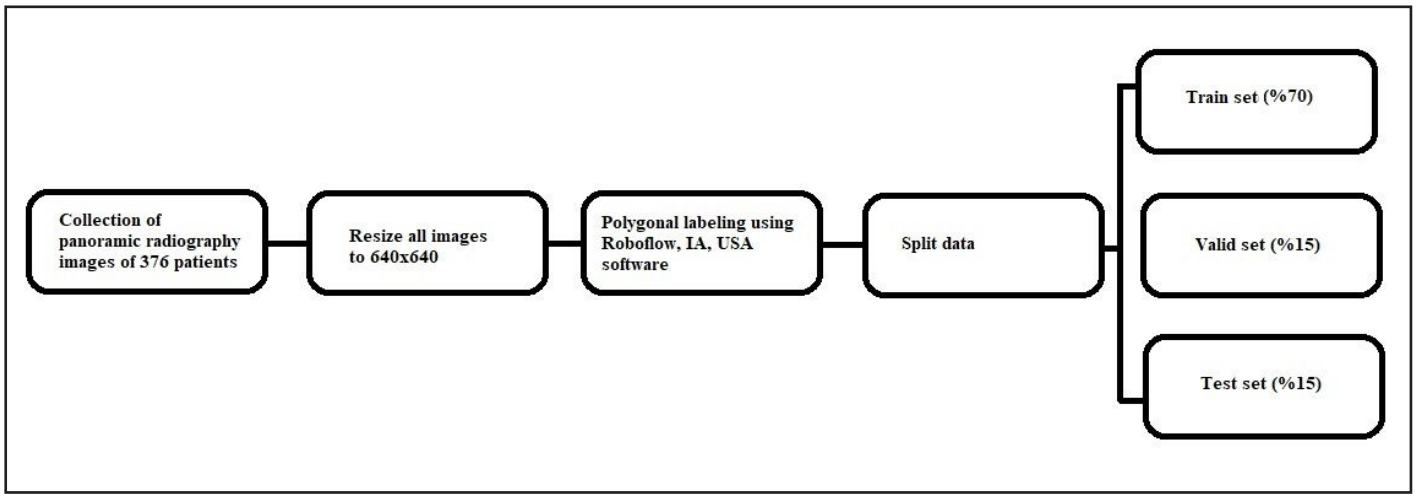

Figure 2. Summarized template of the methodology

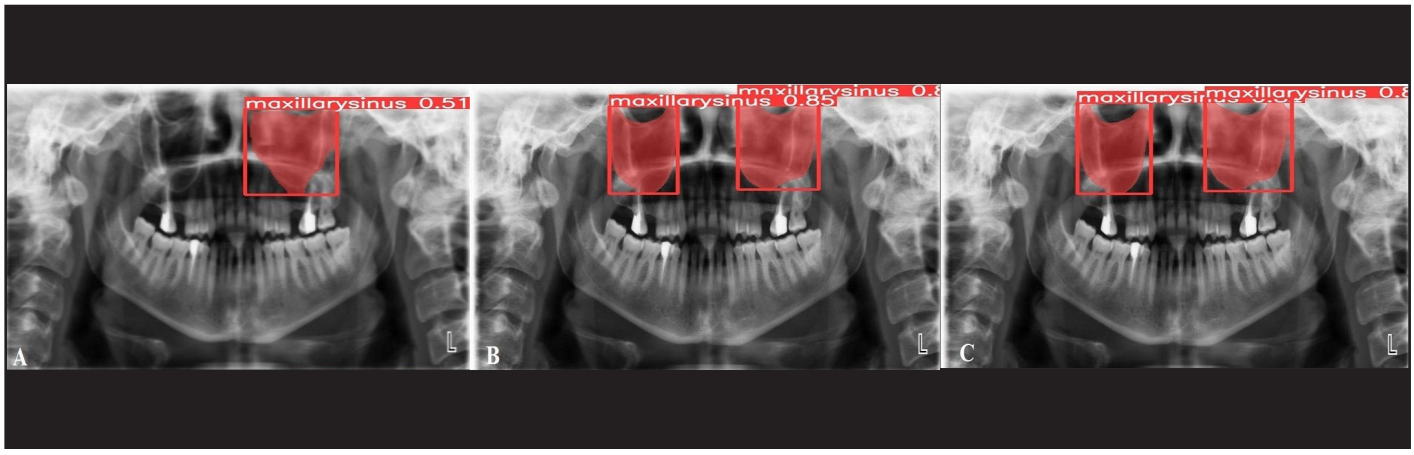

Figure 3. A: Panoramic image segmented utilizing YOLOv5 algorithm B: Panoramic image segmented utilizing YOLOv7 algorithm C: Panoramic image segmented utilizing YOLOv8 algorithm

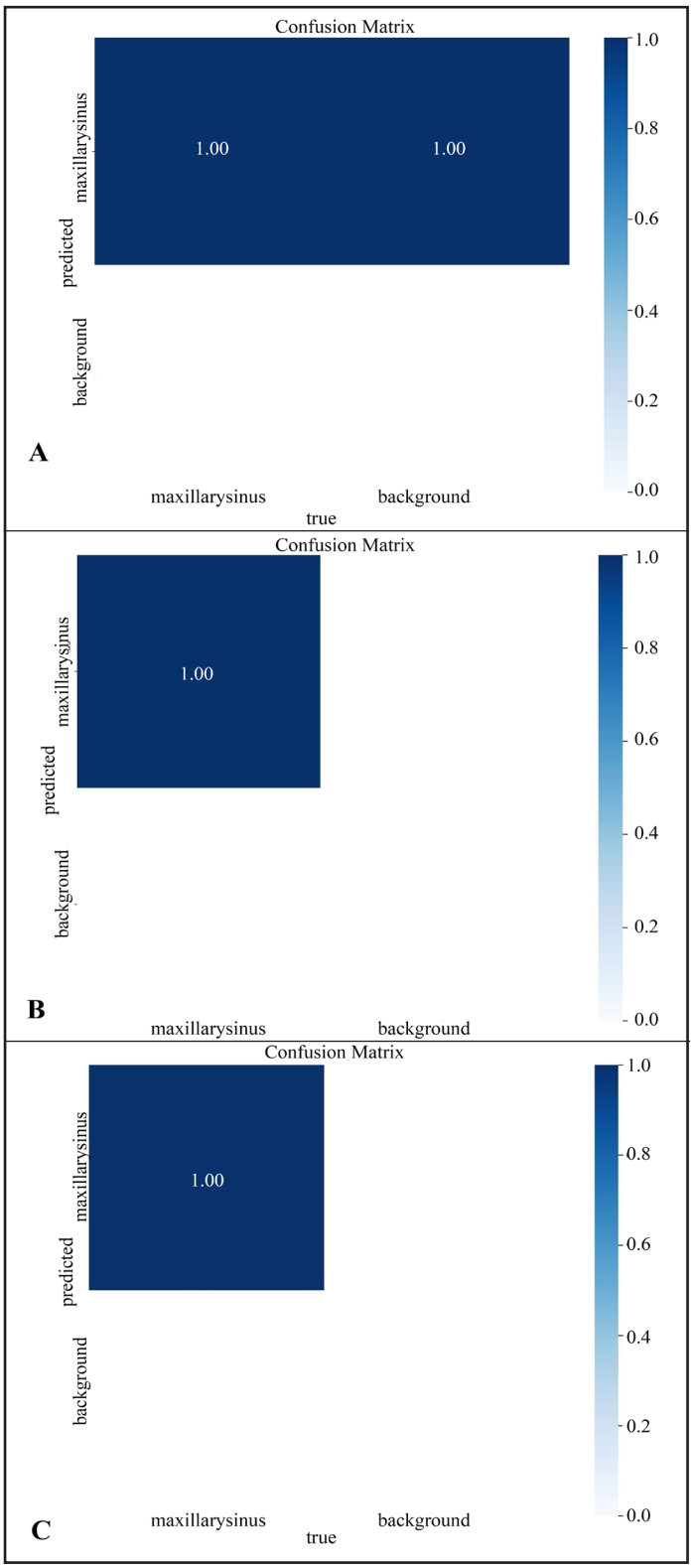

Figure 4. A: Confusion matrix plot for YOLOv5 algorithm B: Confusion matrix plot for YOLOv7 algorithm C: Confusion matrix plot for YOLOv8 algorithm

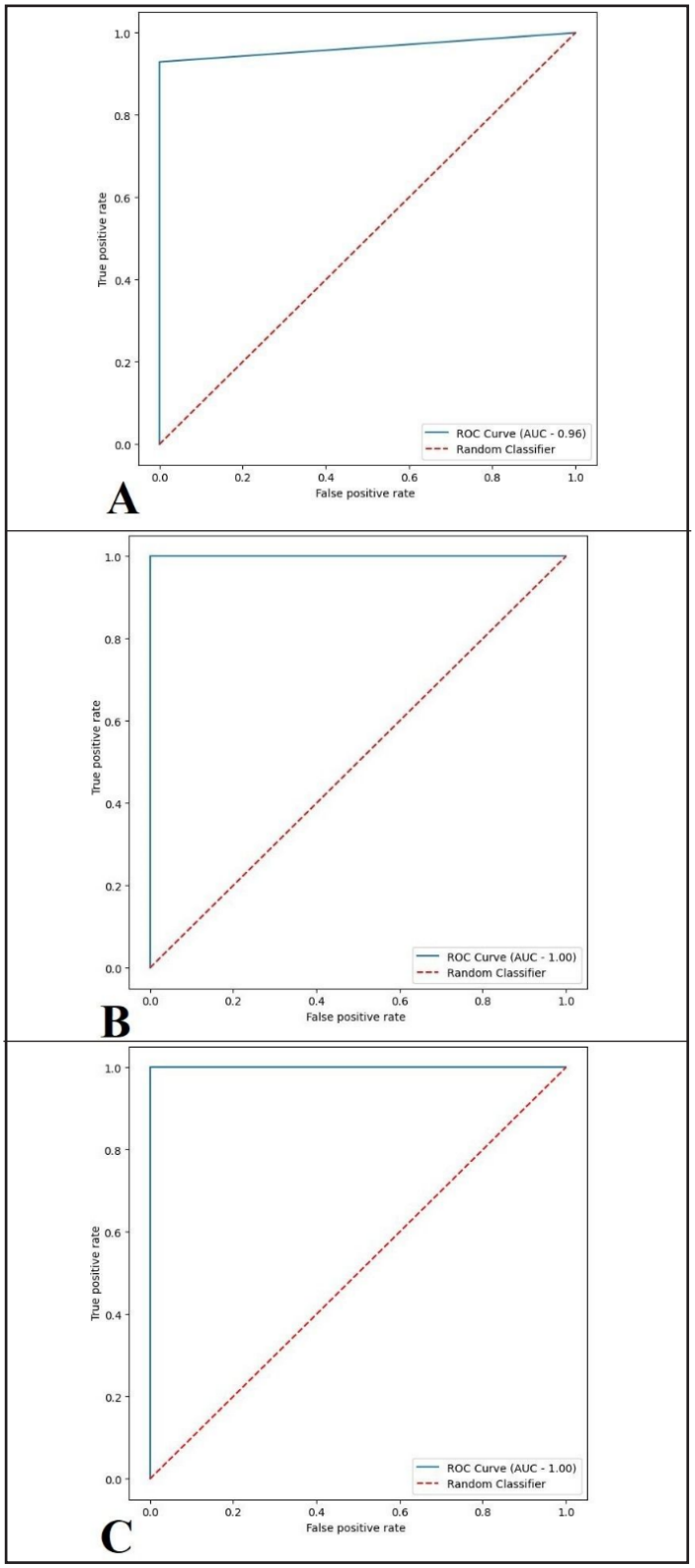

Figure 5. A: Receiver operating characteristic (ROC) curve and area under the curve (AUC) plot for YOLOv5 algorithm B: Receiver operating characteristic (ROC) curve and area under the curve (AUC) plot for YOLOv7 algorithm C: Receiver operating characteristic (ROC) curve and area under the curve (AUC) plot for YOLOv8 algorithm

If AUC > 0.5, the performance of the model is good and indicates that it makes better predictions. As the AUC value increases, the performance of the model improves further.

If AUC < 0.5, the performance of the model is worse than random forecasting. As the AUC value decreases, the performance of the model gets worse.

Higher AUC values indicate that the model has a better discrimination power and better distinguishes between true positive and negative classes.

RESULTS

The confusion matrix plots of YOLOv5, YOLOv7 and YOLOv8 deep learning models for maxillary sinus segmentation are shown in Figure 4.

Figure 5 displays the ROC curves and AUC values for the YOLOv5, YOLOv7, and YOLOv8 deep learning models employed in the segmentation of maxillary sinuses.

In the evaluation of the test data for maxillary sinus segmentation, TP, FP and FN values are 106, 0, 8 for YOLOv5, 114, 0, 0, 0 for YOLOv7 and 114, 0,0 for YOLOv8, respectively. The calculated sensitivity, precision, and F1 scores based on these values are presented in Table 1.

Table 1. F1, Sensitivity and Precision values in the evaluation of test data for maxillary sinus segmentation

	YOLOv5	YOLOv7	YOLOv8
F1	0.96	1.0	1.0
Sensitivity	0.92	1.0	1.0
Precision	1.0	1.0	1.0

DISCUSSION

Artificial intelligence has developed in numerous scientific disciplines in recent years and has become an indispensable part of daily life. The utilization of artificial intelligence in the fields of medicine has aspired not only to aid physicians in diagnosis and treatment within areas such as pharmacology [15], ophthalmology [16], pathology [17], cardiology [18], psychiatry [19], and radiology [20] but also to achieve time and cost efficiencies.

Dentistry is one of the medical fields experiencing rapid and dynamic advancements in new technologies [21]. Within restorative dentistry, artificial intelligence can accurately identify tooth decay or pre-existing restorations and facilitate the selection of the optimal approach for caries removal [22-24]. Artificial intelligence within the field of endodontics can provide valuable assistance in the identification of periapical lesions and root fractures, evaluation of root canal systems, estimation of pulp root cell viability, determination of working length measurements, and prediction of the efficacy of retreatment procedures [25-29]. They can ease diagnosis and treatment planning in orthodontics, identify cephalometric landmarks, conduct anatomical analyses, evaluate growth and development, and assess the outcomes of treatment [30-34]. In oral surgery, artificial neural networks can offer assistance in orthognathic surgical and implant treatment planning, as well as predicting post-extraction complications and detecting bone lesions [35-40]. In the domain of periodontology, these networks have been employed to evaluate both periodontal bone deterioration and the loss of bone around dental implants [41, 42].

The use of artificial intelligence in oral and maxillofacial radiology is becoming increasingly widespread. Studies in this field are frequently used in cone beam computed tomography (CBCT) [28], panoramic radiography [43], intraoral radiography [44], cephalometric radiography [45] and ultrasonography [46] images for the purpose of segmentation [47], detection [48], classification [24]. Thereby, it aims to help physicians identify anatomical structures and diagnose pathologies within this region through its applications.

In the literature, there are many studies on the examination of maxillary sinuses employing artificial intelligence. Ki-Jung et al. [48] aimed to segment the maxillary sinus into distinct components such as maxillary bone, air, and lesions. They subsequently conducted an analysis by juxtaposing these outcomes with evaluations conducted by experts. The study demonstrated that the integration of a deep learning framework can effectively reduce the time required for instructive labeling on limited CBCT datasets. Murata et al. [49] utilized a deep learning system for the diagnosis of maxillary sinusitis. In their study, where they evaluated 800 panoramic images, they indicated that the deep learning system exhibited a notably elevated diagnostic performance for maxillary sinusitis in panoramic radiographs. Morgan et al. [50] developed a CNN model with a 3D U-Net architecture for the automated segmentation of the maxillary sinus in CBCT images. They reported that the CNN model offers a time-efficient, precise, and consistent automated segmentation, which enables the generation of an accurate 3D model for diagnosis and treatment

planning purposes. Choi et al. [51] developed a segmentation model for maxillary sinuses employing the U-Net architecture. They determined that the deep learning model exhibited strong performance in effectively segmenting both clear and hazy maxillary sinuses. Kabak et al. [52] conducted a study to assess and compare manual, semi-automatic, and automatic approaches for evaluating maxillary sinus volume using CBCT. They reached the conclusion that the data obtained through the application of artificial intelligence exhibited a strong correlation with the results of sinus morphometry achieved through manual and semi-automatic techniques. Kuwana et al. [53] aimed to determine the performance of deep learning object detection techniques in detecting maxillary sinuses and classifying maxillary sinus pathologies in panoramic radiography. They stated that deep learning can consistently recognize maxillary sinuses and accurately distinguish cysts within the maxillary sinus region and cases of maxillary sinusitis. Youngjune et al. [54] utilized a deep learning algorithm to label maxillary sinuses depicted in Waters radiographs as either sinusitis or normal sinus. They affirmed that the deep learning algorithm has the potential to diagnose maxillary sinusitis in Waters radiographs. Serindere et al. [55] utilized a convolutional neural network (CNN) for the evaluation of maxillary sinusitis in both panoramic radiographs and CBCT images. They concluded that the diagnostic performance of CNN for maxillary sinusitis in panoramic radiographs was moderate, but significantly higher in CBCT images. In their study, Hung et al. [56] employed a CNN algorithm for the automatic detection and segmentation of mucosal thickening and mucous retention cysts in the maxillary sinus using CBCT. They stated that this approach holds the potential to accurately detect and segment these pathological conditions.

The current study examined the success of maxillary sinus segmentation in panoramic radiographs using different versions of the YOLO algorithm. The study concluded that the latest YOLO versions, YOLOv7 and YOLOv8, attained notably high success rates, whereas the success rate of YOLOv5 was comparatively lower than these versions. YOLOv7 was unveiled on ArXiv in July 2022 by the creators of YOLOv4. Similar to YOLOv4, it was trained solely on the MS COCO dataset without the use of pre-trained backbones. YOLOv7 introduced various architectural modifications that enhance accuracy while specifically targeting training time, without impacting the inference rate. These architectural changes likely account for the improved success of YOLOv7 compared to its predecessors.

All of these examples from the literature demonstrate how widespread and effective the utilization of artificial intelligence is as a tool in the examination of maxillary sinuses. Artificial intelligence assumes a pivotal role in identifying, segmenting, diagnosing, and strategizing treatment for maxillary sinuses, primarily due to its capableness in image processing and analysis. Deep learning algorithms exhibit high performance, especially in maxillary sinus segmentation and diagnosis and follow-up of sinus diseases. Many studies emphasize the advantages of technology in the field of medicine and the potential of artificial intelligence in clinical applications.

Limitations

This study solely evaluated different versions of YOLO and does not encompass various deep learning architectures. Furthermore, it exclusively focused on the anatomical segmentation of the maxillary sinus and did not assess different sinus pathologies. Addressing these limitations and increasing the sample size in future studies could lead to more beneficial outcomes.

CONCLUSIONS

In conclusion, there are many studies in the literature on the examination of maxillary sinuses with artificial intelligence. The common goal of these studies is to improve the detection, segmentation, diagnosis and treatment planning of maxillary sinuses through the utilization of artificial intelligence techniques. Investigations employing deep learning models like YOLOv5, YOLOv7, and YOLOv8 stand as remarkable indicators of the advancements achieved in this domain. Notably, these models have exhibited significant success rates in maxillary sinus segmentation, with YOLOv7 and YOLOv8, the latest iterations, displaying particularly commendable outcomes. These studies emphasize the immense potential and influence of artificial intelligence in medical practices to improve the diagnosis and treatment processes of patients. The realm of oral and maxillofacial radiology has also greatly benefited from progress of the artificial intelligence, offering robust assistance in the identification of anatomical structures and pathological conditions. These findings suggest that the ongoing and future development of artificial intelligence holds the promise of further refining and enhancing precision and efficacy in healthcare diagnoses and treatments.

Informed Consent: The study is a retrospective study and was carried out by scanning the existing images in the archive system of our department.

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Original Research

Investigation of the Relationship between Adenoma Volume and Perioperative Hormone Levels in Patients with Acromegaly

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ABSTRACT

Objective: Current pituitary adenomas classifications and surgical treatment results are made only with two-dimensional radiological sections and hormonal measurements. This study investigated the relationship between hormone levels and volumetric tumor burden by measuring tumor volumes before and after surgery in patients with acromegaly.

Methods: In a retrospective clinical study, clinical and radiologically measured volumetric, hormonal and surgical results of 52 patients who were operated on with the diagnosis of acromegaly due to pituitary adenoma were examined. Radiological measurements were obtained using the ImageJ software package version 1.47 and the measure-stack plug-in. In statistical analysis, the relationship between tumor volumes, growth horomone (GH) and insulin-like growth factor (IGF-I) levels was analyzed during and after surgery.

Results: Of the 52 cases, 22 (42.3%) were male, 30 (57.7%) were female, and the mean age of the patients was 43.40±11.40 years. 45 cases (86.53%) were macroadenoma, 7 cases (13.47%) were microadenoma. All patients were operated by the transnasal-transseptal-transsphenoidal route. When the early preoperative and postoperative hormone results of the patients were compared, significant decreases were observed in GH (82.1%), volume (67%), and IGF-1 (50%) levels in the postoperative period. While there was a significant positive correlation between preoperative GH levels and tumor volumes (r: 0.516, $p<0.05$), there was also a significant positive correlation between postoperative GH levels and tumor volumes (r: 0.755, $p<0.05$). No correlation was observed between IGF-I levels and volume in the preoperative and postoperative period (r:-0.051, $p>0.05$) (r:0.259, $p>0.05$). A significant positive correlation was found between postoperative GH levels and IGF-1 levels (r: 0.303, $p<0.05$).

Conclusion: Both GH and IGF-I levels increase significantly as tumor volume increases in patients with pituitary adenoma before and after surgical treatment. Volumetric measurements may be necessary for classifying patients with acromegaly before and after surgery and in the more objective and quantitative determination of postoperative residual and/or recurrence. For this reason, we believe that it is more accurate to evaluate tumor tissues occupying a 3-dimensional volume with volumetric measurements.

Keywords: Pituitary adenoma, Acromegaly, Volume, Transsphenoidal

INTRODUCTION

Acromegaly is a rare disease often caused by pituitary tumor synthesizing growth hormone (GH) with increased circulating GH and insulin-like growth factor-1 (IGF-1) levels, somatic disorders, and systemic involvement [1-3]. Its prevalence is 40-70 per million, and its incidence is 3-4/year per million [1, 2].

In 98% of acromegaly cases, it develops due to a pituitary adenoma (somatotroph adenoma) that synthesizes GH, and these adenomas are usually benign. Somatotropic adenomas in the pituitary gland consist of sparse or dense granular cells [3, 4]. In 25% of cases, prolactin (PRL) is secreted in addition to GH, with the presence of mammosomatotroph or acidophilic stem cells [1]. Rarely, plurihormonal adenomas in which other anterior pituitary hormones [thyroid stimulating hormone (TSH), adrenocorticotropic hormone ACTH)] are secreted are seen [1]. Overt heart failure can be seen in the advanced stages of the disease in patients who remain untreated. As GH and IGF-1 levels decrease with treatment, improvement in cardiac mass and left ventricular functions is observed.

In this retrospective study, magnetic resonance imaging (MRI) and GH, IGF-1 levels of patients with acromegaly before and after transsphenoidal surgery were compared, and the relationship between pituitary adenoma volume and hormone levels was tried to be determined.

MATERIALS AND METHODS

This study included 52 patients who were operated on for acromegaly in our clinic between 2010 and 2016 and the relationship between preoperative-postoperative hormone profile and preoperative-postoperative tumor volume was investigated. In the endocrinological evaluation, all cases' anterior pituitary function data were obtained by examining preoperative and postoperative GH and IGF 1 levels. In the radiological evaluation, data on tumor volume in all cases were obtained by examining preoperative and postoperative pituitary MRIs (1.5 Tesla Dynamic Pituitary MRI cross-sectional interval 1mm) and calculating volume values with the ImageJ [5-7] computer program (Figure 1).

As a surgical procedure, the standard endonasal transsphenoidal intervention was performed in 52 patients.

Main Points;

- GH and IGF-I levels increase significantly as tumor volume increases in patients with pituitary adenoma.
- Postoperative volumetric evaluation may be important in the evaluation of postoperative residual and functional tumor remnants.
- For this reason, we believe that it is more accurate to evaluate tumor tissues occupying a 3-dimensional volume with volumetric measurements.

Statistical Analysis

The quantitative data obtained in the study were made with the SPSS statistical program (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp., our university license). When the data were evaluated in terms of homogeneity and normal distribution compatibility, it was observed that all the data obtained both preoperatively and postoperatively did not comply with the normal distribution (Kolmogorov-Smirnov test, $p<0.001$) (Table 1).

Because the data could not meet the parametric test assumptions, Wilcoxon signed-rank test (Table 2) was used for comparisons investigating within-group differences (dependent group). A bivariate correlation test (Pearson's Correlation) (Table 3) was used to determine the relationship between variables. Variable comparisons with a p-value below 0.05 in all tests were considered statistically significant.

RESULTS

Of the 52 patients included in the study, 30 (57.7%) were female, and 22 (42.3%) were male. The mean age of women was 42.10±11.53, and the mean age of men was 45.18±11.23. In the study, the preoperative tumor tissue volume measurement was 166.00±16.92 mm3, while the postoperative measurement was 55.50±10.65 mm3, and a volume decrease of 67% was detected in the postoperative period (Table 1, 2).

In the study, the preoperative GH level was 13.9±2.09 µg/L, while the postoperative level was 2.5±1.0 µg/L, and 82.1% low GH was detected in the postoperative period (Table 1, 2).

In the study, the preoperative IGF-1 level was 948±41 ng/ml, while the level was 474±42 ng/ml in the early postoperative period, and a 50% decrease in IGF-1 was detected in the postoperative period (Table 1, 2).

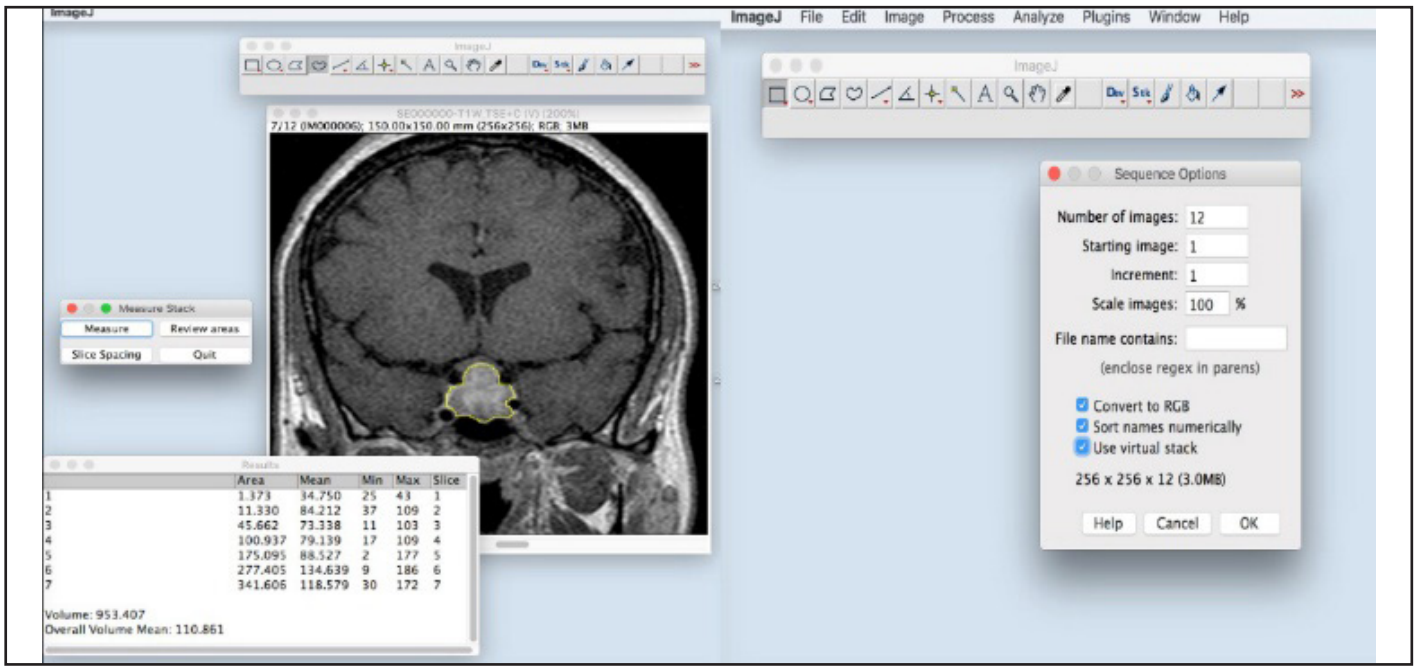

Figure 1. Volume measurement example

Table 1. The descriptive statistical results of preoperative-postoperative variables

	Preop. GH	Postop. GH	Preop. Volume	Postop. Volume	Preop. IGF-1	Postop. IGF-1
Median		2.53	166.00	55.50	948.50	474.00
Std. Error (S.E.)	2.09	1.00	16.92	10.65	41.26	42.45

Table 2. Wilcoxon signed rank test

Parameter	Median	SE	p-value*
Preop GH	13.95	2.09	
Postop GH (%82 decrease)	2.53	1.00	0.001
Preop volume	166.00	16.92	
Postop volume (%67decrease)	55.50	10.65	0.001
Preop IGF-1	948.50	41.26	
Postop IGF-1(%50 decrease)	474.00	42.45	0.001

* p value was considered as significant if less than 0.05

Table 3. The Correlation test results

Parameter-1	Parameter-2	r value	p value*
Preoperative tumor volume	Preop GH	0.516	0.001*
Preoperative tumor volume	Preop IGF-1	-0.51	0.722
Postoperative tumor volume	Postop GH	0.755	0.001*
Postoperative tumor volume	Postop IGF-1	0.259	0.064
Preoperative GH	Preop IGF-1	-0.90	0.524
Postoperative GH	Postop IGF-1	0.303	0.029*

* p value was

DISCUSSION

This study is one of the first retrospective studies investigating the effect of surgical treatment on volume and hormonal profile by measuring the tumor volume in the perioperative period before and after surgical treatment in patients with acromegaly. In this study, we investigated the relationship between volume measurements and hormone values, with the thought that adenoma volume would be more meaningful than the adenoma diameter used in previous studies and classifications [8, 9]. This is because current classifications attempt to classify a three-dimensional tumor in a two-dimensional environment. Because the fact that the classifications made by evaluating a three-dimensional structure that can show invasion and/or invagination to the surrounding structures only with coronal sections, where the sella and ICAs are best displayed, are insufficient is understood as different opinions have emerged over the years regarding these classifications and a common consensus has not been formed [8-10]. In addition, the operation's success is still evaluated with qualitative and not so objective methods, not quantitatively [11]. In this study, it is planned that these methods will play an important role in objectification and perhaps lead to an important way in terms of classification. Tumor volume assessment was performed with the help of a high-standard and high-resolution software application and software plug-in [12].

Colao et al. previously stated that tumor size increases and IGF-I levels are unrelated [13]. In this study, we also observed no relationship with IGF-1, which is consistent with this publication, but a positive relationship, or correctly put, a significant correlation between the volumetric load of the tumor and GH production, especially in the preoperative and postoperative periods. In addition, we observed a significant decrease in postoperative GH and IGF-1 in the postoperative period, which has not been reported in any study before and correlated with each other in the early postoperative period.

In many studies, volumetric evaluation of pituitary tumors has often been done using simple volume calculation formulas. For example, the volume is calculated with the Di Chiro and Nelson formula by assuming that the tumor is mainly in an approximately ellipsoid configuration. This is also not sensitive and descriptive enough [14]. Although this formula is still a reliable method for formula-based volume calculation, especially in smooth round or ellipsoid form tumors, they do not have sufficient scientific precision since they cannot be calculated by subtracting the volumetric indentations and protrusions in the volume [14]. In addition, this and similar methods are far behind today's technical possibilities, and today there are more advanced and sensitive techniques with ROI (Range of Interest) based volumetric feature. Moreover, they are better evaluated with ROI-based volumetry, especially if cavernous sinus invasion or invagination is in question [6, 15-17]. Although volume measurement programs in MR devices, which normally contain embedded software, are intended for clinical studies, they cannot be applied in daily practice because they are very time-consuming and economically expensive [18, 19].

An interesting issue in the literature is that most of the volumetric publications on the pituitary are related to schizophrenia and similar psychiatric diseases [20, 21]. Few publications are associated with a hormonal profile and are primarily growth-developmental publications [22-24]. There are very few publications on such 3-dimensional measurements in pituitary tumors [25-27]. In almost all of these three-dimensional studies published on acromegaly, except for a few related to surgery [25, 26], the relationship between medical treatment and reduction in tumor volume was investigated [28-30].

This study shows us a serious relationship between the volumetric load of the tumor and the hormonal increase, especially in terms of GH. In the current literature, schemes based on location, invasion pattern, size in two dimensions, and relations with the cavernous sinus have been used to classify pituitary tumors [31, 32]. One of the most critical issues to be discussed here is describing a structure that occupies space in three dimensions with two-dimensional criteria. Ideally, a more objective approach can be made by adding three-dimensional volume measurements to the existing classification and follow-up criteria. Thus, more meaningful decisions can be made in the follow-up of tumor tissue and preoperative planning. In addition, in cases of residual and recurrence, follow-up can be done not only on the hormonal level but also on the volumetric plan. The fact that the reduction rate in GH levels after surgery is tightly correlated with the percentage of tumors resected has a potential practical impact. For example, the success of radiotherapy and medical treatment for GH-producing tumors depends on the degree of GH elevation. This means that as the tumor burden increases, the chance of success will decrease [33, 34].

Limitations

These measurements can also be used predictively in estimating the chance of achieving a normal GH and IGF-1 level with post-

surgical medical or radiation therapy. Sometimes preoperative imaging will show whether the tumor can be removed entirely with surgery. In addition, knowing the relationship between the level of decrease in postoperative GH and the volume of the tumor fraction that can be surgically removed may provide additional information on whether the patient will need additional surgery. Although hormonally activated pathology was evaluated in our study, since it is known that pituitary tumors may not always be hormonally active, this causes the limitation of the study. Considering the evaluation of the hormone profile after effective pituitary surgery, the results of our study are also valuable in this respect. In our study, radiological studies will not replace endocrinological follow-up and evaluation in postoperative follow-up, but we think that surgical evaluation with endocrinological and 3D radiological studies may provide more advantages than 2D evaluation in the evaluation of space occupancy lesions."

CONCLUSIONS

Each GH-secreting tumor appears to have a unique level of GH production per tumor mass, and this level appears to be homogeneous over its tumor mass. Although the limitation of our study is that repetitive measurements of GH and IGF-1 values in the late postoperative period are not continued, if measurements can be made in a much larger case series, characteristic curves can be drawn between tumor volume and hormone levels. Therefore, when the volume or hormone levels are known, the quantity of the other parameter can be interpreted using this characteristic curve without measuring it. Expected hormone decline levels can be estimated from the volume removed after surgery. Volume measurements can be used as additional information in disease follow-up, residual, recurrence, and classification.

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Original Research

The Distribution of Missing Canals in Single-Rooted Teeth with Two Canals

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ABSTRACT

Objective: To determine whether the missing canals in endodontically treated single-rooted teeth with two-canals are present in either buccal or lingual/palatal canals for the Turkish subpopulation.

Methods: High-quality cone-beam computed tomography scans of 1297 endodontically treated single-rooted teeth belonging to 782 adults over the age of 18 were obtained from the archive of a dental clinic. Within this dataset, 129 single-rooted teeth had undergone endodontic treatment and possessed two canals, indicating the absence of one canal. These cases included 73 mandibular anterior teeth, 29 mandibular premolars, and 27 maxillary second premolars. We carefully documented both the tooth type and the location of the missing canal. To classify a missing canal as independent, we required it to have a separate orifice from the other canal or be connected to the other canal within 5 mm of its unsealed apex. The differences between categorical variables were tested with Chi-square analysis. $P \leq 0.05$ was chosen as the statistical significance level.

Results: The buccal canal was missing statistically more often in maxillary second premolars than in other teeth, and mandibular anterior teeth and premolars were statistically similar (p=0.001). The incidence of missing lingual canals was statistically similar in mandibular anterior teeth; and higher compared to maxillary second premolars (p=0.001). Overall, the most frequently missed canal was the lingual canal of the mandibular premolar teeth (96.6%).

Conclusions: The prevalence of a missing lingual canal is higher in mandibular anterior teeth and premolars, whereas a missing buccal canal is more frequently encountered in maxillary second premolars. It is essential for clinicians to be aware of these potential morphological variations to enhance the success of root canal treatment.

Keywords: Cone-beam computed tomography, endodontics, missed canal, prevalence, uninstrumented canal

INTRODUCTION

The success of root canal therapy, which involves the elimination of microorganisms from the infected root canal system, is a crucial factor [1]. Procedural errors during endodontic procedures in teeth with prior unsuccessful endodontic therapy can hinder the control of intracanal infections [2]. One common procedural mistake is the failure to identify a canal during endodontic treatment, typically resulting from a lack of awareness regarding tooth anatomy, the intricate configuration of canals, or inadequate access cavity design [3]. Overlooked canals that remain untreated can serve as reservoirs for sufficient bacteria to sustain or initiate infection, potentially leading to

reinfection [4]. Achieving the best prognosis necessitates the comprehensive identification of all canals within the root canal system. While magnification, conventional radiography, bur or ultrasonic devices, and illumination can be valuable aids in this regard, they do not guarantee the detection of all canals in every case [5-8]. Two-dimensional radiography serves as a valuable diagnostic and treatment tool but is limited in its ability to uncover missed canals due to its operational constraints [5]. In contrast, cone-beam computed tomography (CBCT) has emerged as a leading technique for addressing the shortcomings of traditional radiography, offering improved sensitivity and specificity in aligning images with actual anatomical structures [9]. When compared to periapical radiography, CBCT may offer more information that might affect the treatment planning of endodontic retreatment challenges. It is especially useful in the detection of apical periodontitis, determination of the affected and unaffected roots by the infection, vertical root fractures, and root resorption before apical surgery [10].

The purpose of this retrospective study was to ascertain if the buccal or lingual/palatal canals were seen in the endodontically treated single-root teeth with two canals in a Turkish subpopulation. The null hypothesis was that there was no difference between the tooth groups in terms of missing canal type.

MATERIALS AND METHODS

The ethical committee of the Gaziantep University approved the study (Decision No. 2022/292). This retrospective study included high-quality CBCT images taken from patients referred to a private dental clinic between March 2014 and July 2022 for implant surgery planning purposes. Furthermore, CBCT images were taken for large lesions of jaws such as cyst or other huge pathological structures. Preliminary radiographic evaluations of these patients were made with panoramic radiography, but CBCT was requested because further imaging was needed. CBCT images of 1,297 endodontically treated single-rooted teeth, which belonged to 782 subjects over the age of 18, were examined. Within this dataset, 129 endodontically treated single-rooted teeth featuring two canals (comprising 73 mandibular anterior teeth, 29 mandibular premolars, and 27 maxillary second premolars) representing one missing canal were included in the study. The type of tooth and the location of the missing canal were recorded. CBCT scans that exhibited teeth with root resorption or abnormal development were excluded from consideration. Before the assessment, all data were anonymized, with only the gender and age of the subjects being known.

Main Points;

- Mandibular premolars and anterior teeth with two canals tend to have a lingual canal missing.
- The buccal canals are **missed more often** in maxillary second premolars.
- The most frequently missed canal is the lingual canal of the mandibular anteriors.

CBCT images were obtained using an Orthophos XG 3D unit (Sirona Dental System, Charlotte, North Carolina, USA) configured with an 8x8 cm FOV and operated under standard settings of 85 kV and 7 mA. The voxel size was set at 0.4 mm. DICOM images were captured and displayed in a darkened environment using a 20-inch LED-backlit HP Compaq LE2002x LCD (HP, TX, US) with a resolution of 2560 x 1600 pixels. The images were assessed by two endodontists, each possessing at least 10 years of experience with CBCT, simultaneously. The assessment was carried out using CBCT software (Sirona Galaxis Galileos Viewer Version 1.9.2, Sirona Dental Systems GmbH, Bensheim, Germany) to identify missing canals. Disagreements among the examiners were discussed and resolved until a consensus was reached. Initially, axial views were examined by scrolling to identify unfilled canal spaces. Subsequently, sagittal and coronal views were used to validate the findings. A missing canal was defined as an independent canal if it had a separate orifice from the other canal or if it joined the other canal within 5 mm of the unfilled apex.

Statistical Analysis

Descriptive statistics, including measures such as the mean and standard deviation for numerical variables, as well as frequency and percentage analyses for both numerical and categorical variables, were employed. Furthermore, differences among categorical variables were assessed using Chi-square analysis. The statistical analyses were conducted using the SPSS 22.0 program (IBM, Chicago, USA). $P \leq 0.05$ was chosen as the statistical significance level.

RESULTS

The buccal canal could not be detected in 26 (20.15%) and the lingual canal in 103 (79.85%) teeth. The buccal canal was

missing statistically more often in maxillary second premolars in comparison to other teeth, and mandibular anterior teeth and premolars were statistically similar (p=0.001). Notably, the incidence of missing lingual canals was statistically similar in mandibular anterior teeth and premolars; but it was higher compared to maxillary second premolars (p=0.001). Mandibular premolars and anterior teeth were more likely to have a lingual canal missing (Table 1).

DISCUSSION

Many root canal treatments fail to yield positive outcomes because procedural errors often complicate the management of infections within the root canal system [11]. A critical technical issue in this regard is the inability to locate all of a tooth's root canals, with growing evidence suggesting that missing canals significantly contribute to the failure of endodontic procedures [4, 12]. Identifying all existing canals within the root system is essential for achieving the most favorable prognosis [13]. Even if initially uninfected, an untreated canal within an endodontically treated tooth can lead to permanent apical periodontitis or serve as a potential source of reinfection in the future, necessitating further dental treatment and potentially affecting the prognosis negatively. Therefore, determining the types and prevalence of missing canals can serve as a valuable guide for clinicians in their clinical practice.

Intraoral and panoramic radiographs provide information in only two dimensions, which can limit their ability to identify all root canals [9]. High-resolution CBCT (Cone-Beam Computed Tomography) images of tooth roots, with a relatively low radiation dose, prove beneficial in identifying these canals [5]. However, the effectiveness of CBCT in detecting canals depends on various imaging parameters [14, 15]. Additionally, the presence of restorative materials and metallic posts in the treated tooth can lead to artifacts and make the detection of canals challenging [16].

The prevalence of a second root canal in maxillary second premolars is reported to be 43.9% [17]. In contrast, the literature reports a range of 2.38% to 10.95% for the prevalence of missing canals in maxillary second premolars [3, 11, 13, 18, 19]. This discrepancy could be attributed to the dentists' familiarity with the anatomy of these teeth compared to other single-rooted teeth. Dentists may often assume that maxillary second premolars with two canals have only one canal, especially when the palatal canal is positioned near the center of the access cavity. Our findings suggest a higher probability of missing the buccal canal in these teeth compared to other tooth groups. Employing angled radiography techniques can aid in identifying possible second canals in maxillary second premolars.

The literature reports a prevalence of missing canals in mandibular first premolars ranging from 5.35% to 7.54% [3, 13, 18-20]. The lower incidence of anatomical variations could account for the lower prevalence of missing canals in mandibular premolars. Approximately 75% of mandibular first premolars and 97.5% of second premolars have only one canal in a single root [17].

Our study revealed that mandibular premolar teeth are more likely to have missing canals on the lingual side. While 23.6% of first mandibular premolars and 5.3% of second mandibular premolars featured second root canals, this prevalence was found to be influenced by factors such as ethnicity, age, and gender [21]. The inclination of the crown relative to the tooth root direction can make it challenging for dentists to locate the opening of the lingual canal.

Some studies utilizing CBCT have reported no missing canals in endodontically treated mandibular central incisors [11, 20]. However, a multicenter Portuguese study [3] found that 12.1% of mandibular central incisors had missing canals. This variation may be linked to the high prevalence of second canals, which can reach up to 20.4% in mandibular central incisors [17].

Table 1. Distribution of missing canals according to tooth groups

	Tooth groups			χ^2	p
	Mandibular anterior teeth	Mandibular premolars	Maxillary second premolars		
	N (%)	N (%)	N (%)		
Presence of missing buccal canal	8 (11.0)[a]	1 (3.4)[a]	17 (63.0)[b]	39.611	0.001*
Presence of missing palatal or lingual canal	65 (89.0)[c]	28 (96.6)[c]	10 (37.0)[d]		

*p<0.05; Chi-square test, [a b c d] Numbers followed by different lowercase letters in the same row indicate statistically significant differences.

The prevalence of a second canal in mandibular lateral incisors is reported as 25.3% [17]. Mandibular central and lateral incisors are comparatively smaller in size and have a reduced pulp cavity volume compared to other teeth [22]. These factors may complicate the identification of a second canal using periapical radiographs. Additionally, the small access cavity of mandibular incisors, which opens in the lingual direction due to their shape, can make it difficult to navigate during treatment. As a result, there was a statistically higher occurrence of missing canals on the lingual side of mandibular premolars and anterior teeth in our study. The design of the access cavity may obstruct access to the lingual canal orifices.

In previous studies, the prevalence of missing canals in mandibular canines has been reported to range from 1.6% to 9.5% [3, 11, 19]. This lower percentage may be attributed to the relatively infrequent occurrence of two canals rather than the technical skills of dentists, as only 5.9% of mandibular canines possess more than one canal [17].

Previous research has predominantly focused on the second mesiobuccal canal in maxillary molars when investigating missing canals [3, 11, 13, 18, 19]. To the best of our knowledge, the current study represents the first attempt to assess the prevalence and distribution of missing canals in single-rooted mandibular anterior teeth and premolars featuring two canals. To enhance the success rate of endodontic treatments, it is imperative for dentists to possess awareness regarding the presence of second canals, particularly in teeth with a history of missing canals and those commonly associated with missing canal types [20]. Furthermore, optimizing the access cavity and shaping the pulpal floor in a manner that facilitates the identification of canal openings is crucial. Ultrasonic systems have been recommended for precisely accessing small canal orifices [7]. Moreover, traditional radiographs may superimpose buccal and lingual canals, potentially obscuring their presence. In such cases, angled radiographs and three-dimensional radiographic imaging can offer comprehensive insights into the canal configuration [23]. Utilizing magnification and improved illumination can also aid in creating adequately wide access cavities to locate potential second canals in single-rooted teeth. Additionally, the use of an operating microscope can significantly enhance the identification of internal characteristics that influence canal localization, especially in teeth where external indicators may have been compromised due to prior restorative treatments [24].

Limitations

Several limitations need to be acknowledged in this study. Firstly, the absence of comprehensive data, such as the canal filling technique, type of root canal sealer, number of appointments, pre-treatment conditions of the teeth, and initial diagnoses, represents a limitation. The study's findings and analyses were constrained by the information available in the records. Additionally, this study's reliance on CBCT images from a single center constitutes another limitation. The outcomes may not be entirely generalizable to broader populations or settings. Furthermore, the scarcity of existing literature regarding which canals are frequently missing in single-rooted teeth in Turkiye and globally presented challenges in comparing our study's results. Future investigations involving multicenter and cross-sectional studies on this topic will be invaluable for a more comprehensive assessment of the prevailing circumstances. The final limitation of our study was the use of CBCT to detect missing canals. CBCT's important disadvantage is that the radiation dose is higher than periapical radiographs. As with any radiography device that emits ionizing radiation, the ALADA (As Low As Diagnostically Achievable) principle should be taken into account and the benefits should outweigh the risks when giving an indication for CBCT [25].

CONCLUSIONS

Despite the limitations within which this study operated, the prevalence of a missing buccal canal was primarily observed in maxillary second premolars, whereas a missing lingual canal was predominantly noted in mandibular anterior teeth and premolars. Greater awareness among clinicians regarding potential morphological variations could potentially enhance the overall success of root canal treatments. Future research endeavors may need to place a greater emphasis on this aspect for a more comprehensive understanding.

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Original Research

The Effect of Different Dentin Desensitizers and Self-adhesive Resin Cements on Shear Bond Strength: In Vitro Study

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ABSTRACT

Objectives: The aim of this study is to evaluate and compare the bond strength of different dentin desensitizers and self-adhesive resin cements to dentin surfaces.

Methods: The flat dentin surfaces of 72 wisdom molar teeth were randomly divided into six groups for bond strength analysis (n=12): Group CP: No desensitizer + Primer II A&B+ Panavia F 2.0, Group CM: No desensitizer+ Primer A&B+ Multilink N, Group TP: Tokuyama Shield Force+ Primer II A&B + Panavia F 2.0, Group TM: Tokuyama Shield Force + Primer A&B+ Multilink N, Group UP: Universal dentin sealant + Primer II A&B+ Panavia F 2.0 and Group UM: Universal dentin sealent + Primer A&B+ Multilink N. The shear bond strength test was performed using a universal testing machine (0.5 mm/min). ANOVA test was used to detect significant differences at a $p < 0.05$.

Results: The results indicated that bond strength values varied according to the desensitizing and resin cement materials ($p < 0.05$). The Tokuyama Shield Force desensitizer did not affect the bond strength of the resin cements to dentin ($p > 0.05$).

Conclusion: The different types of dentin desensitizer applications affected on the shear bond strength results of the self-adhesive resin cements.

Keywords: Desensitizer, self-adhesive resin cement, shear bond strength.

INTRODUCTION

Dentin hypersensitivity (DH) is commonly described as sensitive teeth and refers to short and sharp dental pain by patients in dentistry [1]. The mechanism of dentin sensitivity is expressed with the Brännström's hydrodynamic theory [2]. This theory is relied on the the innervation of nerves by differences in pulpal pressure with the movement of the liquid in the dentinal tubules [3].

The dentinal tubules are opened to the oral environment after cavity or tooth preparation and acted as channels that transfer thermal, chemical, mechanical, and bacterial stimuli to the pulp [4]. The exposed dentinal tubules can damage the pulp tissue [4]. Dentinal tubules can be blocked for the DH treatment, and then fluid shifts and dentin hypersensitivity sensitivity can be controlled [5].

In the literature, various treatment modalities were used for DH treatment, such as fluoride treatment [6], oxalate treatment [7], arginine treatment [8,9], dental adhesives [10], and laser therapies [11].

Recently, resin-containing agents have been the most commonly used for treating DH. Resin has a physical effect on sealing the

dentin tubules. Resin content occludes the dentin tubule-covered resin tags to help eliminate the hypersensitivity [12].

Self-adhesive resin cements were introduced to the markets to promote the procedures of prosthetic restoration cementation. This type of cement minimizes clinical stages and reduces postoperative sensitivity [13]. They also proposal pleasing aesthetics, adhesion of micromechanical, optimum mechanical attributes, and stability of dimensional [14]. Since these cements do not necessitate teeth surface pretreatment, they reduce application time and technical precision. These cements can diffuse and decalcify dentin because they do not contain water [15,16]. Their high hydrophilicity improves the wetting of the dentin and a low pH applying of the tooth [17].

Panavia F 2.O is a phosphate monomer-based (MDP) based resin cement and has a fluoride releasing fuction [18] and the monomer matrix of Multilink N is composed of dimethacrylate and HEMA and Multilink N Primer B contains HEMA, phosphoric acid and methacrylate monomers [19]. The bonding or sealing properties of these resin cements may be influenced by desensitizers, which contain ingredients that lead to chemical interactions with the tooth's organic tissue [20].

Desensitizing agents may be used on the tooth before cement application to avoid this hypersensitivity, but their effect on the adhesion is still suspicious [21,22].

There was limited research that assessed the effect of desensitizers on the dentin SBS of self-adhesive resin cement (SARC). Thus, the effect of dentin desensitizer (DDS) application and dentin-resin cement bond strength is still controversial, and moreover, there is no precise data about the dentin sealing protocols.

The aim of the present study was to investigate the effect of two different DDSs on the SBS of the SARCs to dentin. The null hypothesis was that DDS would not affect the bonding performance of the SARC.

Main Points;

It has been concluded that the interaction of the DDS and resin cement is the main point for the application procedures. This study will make it easier to reduce post cementation hypersensitivity and clarify the optimal indirect restoration treatment procedures.

MATERIALS AND METHODS

The Ethics Committee approved this in-vitro study of Gaziantep University (protocol 2023/453) with to the principles of the Declaration of Helsinki.

Sample Size

For SBS analysis, the minimum sample size was defined as 24, for an effect size of 0.5, 80% power (1-β), and a 5% (α) confidence interval with G * Power 3.1.9.4 software. The effect of Tokuyama Shield Force (TSF) and Universal dentin sealant (UDS) on the SBS performance of two SARCs was assessed. The details of the used materials are shown in Table 1.

The 72 non-carious wisdom molars were cleaned and collected in distilled water at room temperature after extraction. They were embedded in a self-cured acrylic resin (Meliodent; Bayer Dental Ltd, Newbury, UK) and the occlusal regions of the crowns were removed with a water-cooled slow-speed diamond saw sectioning machine (Isomet, Buehler Ltd., Lake Bluff, IL, USA). Then the specimens were polished under water cooling using 400-, 600-, and 1000-grit silicon carbide abrasive papers for 30 seconds to standardize the surfaces.

The flat-prepared dentin specimens (n=72) were randomly divided into three groups; the first group was the control group (n=24). Two DDSs were applied to the two experimental groups, respectively. The DDSs used were Tokuyama Shield Force Plus (Light-cured desensitizer, Tokuyama, Japanese) and Universal Dentin Sealent (Ultradent) (n=12).

The study groups

- Group CP (Control group): No desensitizer+ Primer II A&B+ Panavia F 2.0 (n=12)
- Group CM (Control group): No desensitizer+ Primer A&B+ Multilink N (n=12)
- Group TP: Tokuyama Shield Force+ Primer II A&B + Panavia F 2.0 (n=12)
- Group TM: Tokuyama Shield Force +Primer A&B+ Multilink N (n=12)
- Group UP: Universal dentin sealant + Primer II A&B+ Panavia F 2.0 (n=12)
- Group UM: Universal dentin sealent+ Primer A&B+ Multilink N (n=12) (Table 2).

Table 1. Compositions and brands for the used materials

Brand	AbbrevIatIon	Chemical compostIon	Manufacturer
Tokuyama Shield Force	TSF	Phosphoric acid monomer, Bis-GMA, TEGDMA, HEMA, camphorquinone, alcohol and purified water (The pH level immediately after dispensing is approximately 2.0).	Tokuyama Dental Corporation, Taitou-ku, Tokyo, Japan
Universal Dentin Sealent	UDS	Nonpolymerizable, high molecular weight resin in an ethanol	Ultradent, South Jordan, Utah, USA
Panavia F 2.0	PF	Paste A: Methacrylate, MDP, quartz-glass, microfillers, photoinitiator Paste B: Methacrylate, barium glass, sodium fluoride, chemical initiator	Kuraray, Noritake Dental, Kurashiki, Japan
Multilink N	MN	Dimethacrylate and HEMA, barium glass, ytterbium trifluoride and spheroid mixed oxide	Ivoclar,Vivadent, Schaan/Liechtenstein
Multilink Primer A	MPA	aqueous solution of initiators	Ivoclar, Vivadent Schaan/Liechtenstein
Multilink Primer B	MPB	HEMA, phosphonic acid and methacrylate monomers.	Ivoclar, Vivadent Schaan/Liechtenstein

Abbreviations: Bis-GMA: bisphenol A glycidyl methacrylate; TEGDMA: triethyleneglycol dimethacrylate; MDP: 10- methacrylate oxydecyl dihydrogen phosphate; HEMA: 2-hydroxyethylmethacrylate

Table 2. Study Groups

	Groups
Control	Group CP (No desensitizer+Primer II A&B+ Panavia F 2.0)
	Group CM (No desensitizer+ Primer A&B+ Multilink N)
Tokuyama Shield Force	Group TP (Primer II A&B + Panavia F 2.0)
	Group TM (Primer A&B+ Multilink N)
Universal dentin sealent	Group UP (Primer II A&B+ Panavia F 2.0)
	Group UM (Primer A&B+ Multilink N)

Applying Protocols

TSF application: The dentin surfaces were initially slightly dried. The dentin was not desiccated. One or two drops of desensitizer were dispensed into the dispenser. Generous amounts of the desensitizer were applied, left undisturbed for 10 seconds, and then wiped off. A light-blocking plate protected the dispensed desensitizer and the inserted applicator from ambient light before the application. The air drying was used by using an oil-free air/water syringe. Applying weak airflow continuously to the desensitizer surfaces until the runny desensitizer stayed in the same position without any movement (for 5 seconds). A strong airflow was used for 5 seconds or more. The curing light tip was cured (600 mW/cm2) on the surface for 10 seconds or more.

UDS application: Because of its high viscosity, the dentin surfaces were thoroughly isolated and dried, and Universal Dentin Sealant's flow was verified before applying it to the dentin surfaces. A thin coat of USD was used for the dentin and gently air-dried (5-10 seconds).

Bonding Protocols

Panavia F 2.0 application: Dentin surfaces were air dried for 5 sec, then mixed ED primer II A and B) were mixed in equal amounts, waited 30 sec, then gently air dried, dispensed, and mixed equal amounts of paste and applied.

Multilink N application: One drop of Primer A and, one drop of Primer B were mixed and applied to the dentin, then 30 sec waited. A silicone mold was positioned at each specimen's center of to provide standardization the adhesive area.

The Panavia F and Multilink N resin materials were placed into the to the silicone molds with a diameter of 5 mm and a length of 2.5 mm respectively, for each species and photopolymerized for 30 seconds (20 sec per, 5 sec per surface) using a light curing device (Lite Q LD-107; Monitex Industrial, Taipei, Taiwan) for each resin types of cement.

A scalpel was used to remove the silicone molds, and excess material was removed after polymerization. Then all samples were kept in distilled water at 37°C for 24 hours. After that, they were aged for 5000 thermalcycles (5 -55°C, 20s dwell, and transfer times) in each bath (Thermocycler; SD Mechatronik, Westerham, Germany).

Shear Bond Strength (SBS test)

The samples (n = 12) were positioned into a universal testing device (Shimadzu Corporation, Kyoto, Japan). SBS tests were applied at a 0.5 mm/min crosshead speed with a knife-edge-shaped apparatus between dentin and resin cement. The shear load was performed until the failure occurred, and the measurement was noted in Newtons (N). The shear-bond force was recorded digitally, and SBS was calculated according to the following formula and expressed in MPa: Stress= Failure Load (N) / Surface Area (mm^2).

Statistical Analysis

The Shapiro–Wilk test was used to check the conformity of continuous variables with normal distribution. One-way variance analysis and LSD tests were used to compare two independent measures across normally distributed variables. Analyses were conducted using IBM SPSS 22 at $p<0.05$.

RESULTS

SBS Results

The SBS's mean levels and standard deviations (SD) for all groups are shown in Table 3, and p values are shown in Table 4.

The highest SBS results among all groups were in Group CM (13.32 ± 1.47)(No desensitizer+ Primer A&B+ Multilink N)), and the lowest SBS results were in Group UP (0.76 ± 0.14) (Primer A&B+ Multilink N) ($p <0.05$). The UDS decreased the SBS results within the adhesive cement ($p < 0.05$).

Table 3. SBS values (MPa)

Groups	MEAN ± SD
Group CP	8.39 [a] ±1.12
Group CM	13.32 [b] ±1.47
Group TP	7.84 [a] ±1.47
Group TM	12.95 [b]±2.22
Group UP	0.76 [c] ±0.14
Group UM	0.80 [c] ±0.25

*Mean and standard deviations ± (SD)in megapascals (MPa) of shear bond strength (SBS) values and statistical differences between groups (n=12)

*Different letters within the lines indicate statistically significant differences.

Table 4. P values

Groups	P
Group CP - GROUP CM	**0.001**
Group CP – Group TP	0.317
Group CP – Group TM	**0.001**
Group CP – Group UP	**0.001**
Group CP – Group UM	**0.001**
Group CM – Group TP	**0.001**
Group CM – Group TM	0.505
Group CM – Group UP	**0.001**
Group CM– Group UM	**0.001**
Group TP – Group TM	**0.001**
Group TP – Group UP	**0.001**
Group TP – Group UM	**0.001**
Group TM – Group UP	**0.001**
Group TM – Group UM	**0.001**
Group UP – Group UM	0.940

*p<0.05 indicate statistically significant differences

Failure Type Results

After the shear load was applied to the specimens, the fractured areas were examined under a stereomicroscope (Leica model, Leica QWinV.3 software; Leica Microsystem Imaging Solutions,

Cambridge, UK) at 15× magnification. The failure type analysis revealed that the adhesive type of failure was the most predominant failure type within all groups. The adhesive failures were found on 80.55% of specimens. Cohesive 0 (0%) and mixed 19.44 % fractures have been seen for groups (Table 5).

Table 5. Frequency of types of bond failure for each group (%)

	Group CP	Group CM	Group TP	Group TM	Group UP	Group UM	Total Frequency and Percent
Adhesive	8/12 (66.66%)	9/12 (75%)	8/12 (66.66%)	9/12 (75%)	12/12 (100%)	12/12 (100%)	58/72 (80.55%)
Cohesive Dentin	0/0 (0%)	0/0 (0%)	0/0 (0%)	0/0 (0%)	0/0 (0%)	0/0 (0%)	0/0 (0%)
Mixed	4/12 (33.33%)	3/12 (25%)	4/12 (33.33%)	3/12 (25%)	0/12 (0%)	0/12 (0%)	14/72 (19.44%)

***Group CP:** Group CP (No desensitizer+Primer II A&B+ Panavia F 2.0)**; Group CM:** Group CM (No desensitizer+ Primer A&B+ Multilink N)**; Group TP:** Group TP (Primer II A&B + Panavia F 2.0)**; Group TM:** Group TM (Primer A&B+ Multilink N)**; Group UP:** Group UP (Primer II A&B+ Panavia F 2.0)**; Group UM:** Group UM (Primer A&B+ Multilink N) statistically significant differences.*

DISCUSSION

The aim of this study was to evaluate the effect of two different DDSs on the SBS of the SARCs to dentin, and the null hypothesis partially rejected. Following the application of TSF desensitizer and using SARC as Panavia F 2.0 and Multilink N, there was no increase/decrease in SBS. In contrast, using UDS decreased the SBS with the Panavia F 2.0 and Multilink N.

Many dentin coating methods are being investigated in the literature. Samartzi et al. found that resin-coated dentin lets dentin fluid pass through polymerized resins [20]. Therefore, the use of either adhesive of three-step or two-step self-etching primer adhesives is suggested by researchers [20].

Magne recommended using flowable composite over unfilled adhesives as an alternative to the use of filled adhesives and covering the resin-sealed preparation with glycerine gel [23].

In a clinical study, Sayed et all stated the clinical satisfaction results after tooth preparation due to the treatment with Gluma (Heraeus Kulzer, Hanau, Germany), Shield Force Plus (Tokuyama Dental America Inc., San Diego, CA, USA), and Telio CS (Ivoclar Vivadent, Schaan, Liechtenstein) desensitizing agents and all of these desensitizers were found to be effective in reducing the dentin sensitivity, as reported by the VAS scores throughout the pre and post-cementation visits [24].

Due to its simplicity and possibility of digital recording of the SBS test, it is most commonly used, and many conditions are investigated in the literature for dental adhesives [4,13].

On the other hand, the bond effect of newly produced desensitizers on dentin is still being investigated. From past to present, desensitizing agents are commonly treated for the aim of controlling pain, making much more comfortable making dental procedures for patients who need fixed prostheses; there are several in-vitro studies investigating the effect of the sensitizing agents or sealing materials on the SBS with different cements type [25,26,27].

Some studies examining the effect of desensitizing agents between the self-adhesive resin and dentin interface report conflicting results [4,22]. For example, Stawarczyk et al. investigated the effect of Gluma Desensitizer on SBS of conventional and self-adhesive resin cements after water storage and thermocycling, and they reported that Gluma Desensitizer showed increased SBS after aging conditions with self-adhesive resins (ranging from 7.4 ± 1.4 to 15.2 ± 3) and Panavia 21 and Gluma interaction showed a significant decrease thermocyling compared with initial values [4] and as a similar result Sailer reported that Gluma has a positive effect with Rely X Unicem [28] and in this study, SBS results were not effected for TSF groups and were decreased for UDS groups with the SARCs.

Dewan et all investigated the effect of desensitizing agents on the bond strength following cementation of zirconia crowns by applying self-adhesive resin and HEMA-containing sensitizers (Gluma, Shield Force Plus, and Telio CS) and Gluma served

better bond strength results [22].

Tokuyama Shield Force Plus is a resin-based light-cured, flüoride-releasing desensitizer designed to treat hypersensitive dentin [29]. It has been stated that the hydrophilic characterization provided by HEMA in Shield Force Plus desensitizer agent enhances bonding to structure [24]. A condensation reaction occurs between HEMA and water evaporation, resulting in a better bond [24]. In the present study, HEMA content was an advantage for TSF groups. TSF served similar results with the control groups with two SARCs and more successful results than all UDS groups, supporting the results of these studies.

The manufacturer explains the UDS as a biocompatible, non-polymerizable, high molecular weight resin in an ethanol carrier (UDS instructions) [30]. In the literature, limited studies have investigated UDS.

Milia et al. resin investigated the short-term response of these three materials, including UDS, and reported that the morphology of UDS on the tooth structure brought about a dense barrier-like structure with tag-like structures resembling demineralized tubular dentin [31].

Pinna et al. Vertise flow (self-adhesive composite) was considered a match for UDS sealant because the performance of flowable composites can be comparable to resin-based sealants [32].

When we applied the UDS and TSF to our species, we experienced that UDS had a higher viscosity than the TSF. Pashley et al. reported that adhesion efficiency is related to the viscosity and degree of conversion of the adhesive, which may negatively affect the penetration of the monomers into the interfibril spaces [33].

Furthermore, Zhang et al. reported that the permeability would be the critical consideration in incomplete infiltration of the bonding and effecting the SBS to dentin [34].

Therefore, we assume that viscosity and insufficient permeability had negatively affected the bond strength for all UDS groups, and TSF and UDS groups served statistically different bond strengths because of the differences in their ingredients or chemical activation, dissolution resistance capacity, and the different dissolving quality and precipitation level in the dentinal tubules.

Adhesive failures were the primary failure type observed for all UDS and TSF groups. This has led to apparent results similar to Dewan et al. [22]. They reported adhesive failure was seen for 80% in the Gluma and Telio groups, 70% in the no-treatment group, and 90% in the ‘’Shield Force “ group for Rely X U-200. Various factors affect in vitro SBS, including the type and age of the dentin, mineralization degree, adhered dentin surface, test conditions, and environment of storage [35]. Desensitizing agents are different in ingredients, functional or cross-linking monomers, chemical activation, and solvents, including inhibitors and activators, and can affect the bonding quality of resin-type cement. All these factors may have an effect on the SBS.

Limitations

This study has limitations in that it can be mentioned that not using distilled water instead of artificial saliva to mimic intra-oral conditions fully. With the limitations of this in vitro study, further investigation is required about desensitizing agents’ ability to bond and seal to tooth surfaces.

CONCLUSIONS

Although the use of DDS before applying the bonding agent may reduce postoperative sensitivity, it was observed that the SBS may decrease depending on the bonding agent used.

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Original Research

In-vitro Diagnosis of Approximal Caries in Teeth Periapical Radiography with Different Exposure Parameters

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ABSTRACT

Objective: The aim of this study was to evaluate periapical radiographs of enamel caries, dentin caries, and deep caries with exposed pulp and intact teeth obtained in vitro using photo-stimulated phosphor plates (PSP) under different exposure parameters.

Methods: 3 non-carious extracted molars were selected. The obtained molars were embedded in the wax created from pink wax by ensuring approximal contact and a base was created. 14 different imaging protocols were used with 60 kVp, 4 mA 0.02-0.1 second and 70 kVp 7 mA, 0.25-1.25 second exposure parameters. Intact teeth were imaged with these various imaging protocols. Artificial cavities were then created for enamel caries, dentin caries and deep caries with exposed pulp and imaged according to the same protocols. The images were evaluated by 3 clinicians who were blind to the exposure protocol and caries status. Inter-observer agreement with actual situations was examined with Kappa statistics.

Results: In the low-dose group, the kappa values of observer 1, observer 2, and observer 3 were 0.905, 0.952, 0.952, respectively. The kappa values of observer 1, observer 2, and observer 3 in the ultralow-dose group were 0.833, 1, 1, and the kappa values of observer 1, observer 2, and observer 3 in the high-dose group were 1, 1, 0.833, respectively. The results obtained in all groups showed a statistically significant-excellent agreement ($p<0.001$).

Conclusion: Approximal caries can be diagnosed with intraoral radiography obtained with low radiation doses with PSP in dentistry. Thus, patients could be exposed to less ionizing radiation.

Keywords: Approximal Caries, Periapical Radiography, Photo-stimulated Phosphor Plate

INTRODUCTION

One of the most frequently encountered problems in clinical dentistry is dental caries [1]. In today's standard clinical practice, visual examination, probing, and radiography are the most commonly employed methods for the diagnosis of caries [2]. Radiographic examination should be used in conjunction with clinical examination, especially as proximal caries lesions can be difficult to detect [2]. In dental clinics, panoramic radiographs are commonly used. Panoramic radiography, which is achieved by simultaneously rotating the X-ray source and image receiver around the patient in a fixed position, is a simple technique for image acquisition. The distances between the radiation source, the object, and the image receiver add to the magnification factor associated with image formation, and the projection geometry results in image distortion and a noticeable overlap of teeth [3]. Panoramic radiographs from patients do not completely eliminate

the necessity of intraoral imaging for the definitive diagnosis of dental disease. Therefore, it is essential to supplement information obtained from panoramic radiography with intraoral radiographs to allow a comprehensive examination of the teeth and surrounding bone with a low radiation dose and without loss of diagnostic information [4].

Demineralization of hard tissues in teeth is a result of the caries process. Because the X-ray absorption of the demineralized region of teeth is less than that of the unaffected area, the lesion appears as a radiolucent area on radiographic images as a result of the caries process [5]. Significant advances in computer technology have enabled the rapid advancement of digital radiologic systems and different digital radiologic methods have been developed [6]. These systems allow for measurements and improvements that are not possible using conventional films. Images can be electronically transmitted to different healthcare providers without any change in the original image quality, and digital intraoral sensors require fewer rays than film, thus lowering the radiation dose to the patient [6-8].

Dental practices use a variety of sizes and shapes of digital image receptors that support various technologies. These are divided into two categories: solid-state detectors and photo-stimulated phosphor plates (PSP) [9]. Although solid-state detectors are subdivided into charge-coupled devices (CCD) and complementary metal oxide semiconductors (CMOS), they share certain common features and the ability to create digital images on a computer without the need for other devices [9]. PSP systems do not have a cable connection between the sensor and the computer, and the size and flexibility of the plates used are very similar to periapical films, thus ensuring ease of use [10-12]. Additionally, the main advantages of these systems are decreased radiation and a larger dynamic range [12].

Main Points;

- Periapical radiographs are complementary radiologic examination methods in the diagnosis of approximal caries.
- In dental routine, photo-stimulated phosphor plates are frequently used as an intraoral imaging tool.
- While intraoral imaging is provided with photo-stimulated phosphor plates for caries detection, imaging can be performed with lower radiation doses by changing the exposure parameters.

Despite the reduction in radiation dose with the transition from conventional to digital radiography, exposure to X-rays has serious consequences. The effects of X-rays on living organisms are the result of direct and indirect interactions at atomic levels [5]. Patients are likely to be exposed to dental X-rays numerous times. Although the radiation dose related to dental radiography is low, it has been acknowledged that there is no safe level of ionizing radiation exposure [13]. Given this lifetime prevalence and high frequency of exposure, additional efforts should be made to minimize the risks from exposure to X-rays.

This study's objective is to evaluate radiographic images of artificial approximal caries created in extracted teeth using PSP with different irradiation values.

MATERIALS AND METHODS

This study was conducted in vitro in the Department of Oral and Dentomaxillofacial Radiology at Inonu University between 2021 and 2022. The Inonu University Scientific Research and Publication Ethics Committee gave its approval for the study (2021/2454).

Three extracted caries-free molars were selected from the Department of Oral and Maxillofacial Surgery of the Faculty. Blood and soft tissues on the extracted teeth were cleaned on the same day and stored in 0.1% thymol solution at room temperature for one day. Then, the molars obtained were templated by providing approximal contact and embedding them in a wax wall formed from pink wax used in the construction of prosthetic bases up to the enamel-cementum borders of the teeth. To mimic soft tissue, the transparent cup was filled with water to the level determined during exposure.

Exposure Protocols

When radiographs were taken, the PSP (Digora Optime, Soredex, Finland) was placed on a flat surface under a transparent container. The distance between the focal spot and the template was set to 40 cm. To ensure standardization, film holders (Kerr X-ray sensor holders, USA) were used while taking periapical radiographs.

To obtain periapical radiographs, 14 different exposure protocols were used on a dental X-ray machine (Belmont Takara. Phot X, Osaka Japan). These are;

Ultralow-dose
1.60 kVp, 4 mA, 0.02 s
2.60 kVp, 4 mA, 0.03 s

Low dose
1. 60 kVp, 4 mA, 0.04 s
2. 60 kVp, 4 mA, 0.05 s
3. 60 kVp, 4 mA, 0.06 s
4. 60 kVp, 4 mA, 0.08 s
5. 60 kVp, 4 mA, 0.1 s

High Dose
1. 70 kVp, 7 mA, 0.25 s
2. 70 kVp, 7 mA, 0.4 s
3. 70 kVp, 7 mA, 0.5 s
4. 70 kVp, 7 mA, 0.6 s
5. 70 kVp, 7 mA, 0.8 s
6. 70 kVp, 7 mA, 1 s
7. 70 kVp, 7 mA, 1.25 s

Periapical radiographs were obtained to obtain images for the intact tooth group in all the above exposure protocols before obtaining artificial caries.

In the second stage, artificial caries was obtained for the enamel caries group by abrading the enamel tissue and ¼th of the dentin adjacent to the enamel with a round tipped diamond bur (Dimei Royal, China). The radiographs obtained by visualizing enamel caries in all exposure protocols were recorded.

In the third stage, controlled artificial caries was obtained for cases belonging to the dentin caries group by abrading between the ¼th of the dentin adjacent to the enamel and the ¼th of the dentin close to the pulp. In all exposure protocols, dentin caries was visualized, and the radiographs obtained were recorded.

In the fourth stage, ¼ of the dentin adjacent to the pulp was abraded using a round-tipped diamond bur (Dimei Royal, China) to obtain controlled artificial caries in cases deep caries with pulp exposed. Radiographs obtained by visualizing the deep caries with exposed pulp in all exposure protocols were recorded.

Evaluation of Periapical Radiographs
The resulting images were scrambled and coded by a clinician (7 years of experience DCO) based on the exposure protocols and caries status. Images were evaluated individually on a monitor (HP Compaq LA2205wg) by three clinicians (23 years of experience (OA), 15 years of experience (ND) and 10 years of experience (SBD)), each with a minimum of 15 years of experience. They were blinded to the exposure protocol and caries status and instructed to select a suitable option from the list below. All three clinicians evaluated the images on the same day. Since the clinicians had more than 10 years of experience, they responded as soon as they looked at the image. Therefore, it took an average of 10 minutes for the three clinicians to evaluate the images.

A1. Dental tissues can be identified radiographically.
A2. Dental tissues cannot be identified radiographically.
If the answer A1 is given,
B1. Intact: No carious lesions are present on the tooth.
B2. Enamel caries: There is a carious lesion in the enamel extending up to ¼th of the dentin.
B3. Dentin caries: There is a carious lesion in the enamel and dentin extending to about ¼th of the pulp.
B4. Deep caries with exposed pulp**:** There is a carious lesion extending from the enamel and dentin to the pulp.

In addition, if the answer B1 was given, it was classified as intact, and if any of the answers B2, B3, and B4 were given, it was classified as carious and a carious-intact comparison was also made.

In the first grouping, periapical radiographs obtained with 70 kVp, 7 mA were grouped as high doses, while those obtained with 60 kVp, 4 mA were grouped as low doses.

In the second grouping, images obtained with 60 kVp, 4 mA, 0.02 s and 60 kVp, 4 mA, 0.03 s were compared by grouping them as ultralow-dose and others.

Statistical Analysis
Data was analyzed using IBM SPSS V23 (Chicago, USA). Inter-observer agreement with actual situations was analyzed with the Kappa statistic. The results of the analysis were presented as frequency (percentage). The level of significance was set at $p<0.050$.

RESULTS
A total of 56 periapical radiographs were evaluated by 3 observers. In 12 (21.4%) of these radiographs, dental tissues could not be observed and therefore were not evaluated. Of the exposure factors used in periapical radiographs without dental

tissues, 4 were 70 kVp, 7 mA, 0.8 s, 4 were 70 kVp, 7 mA, 1 s, and the other 4 were 70 kVp, 7 mA, 1.25 s.

Inter-observer Agreement with the Actual Situation without Group Distinction

The kappa value between the actual situation and observer 1 was 0.939; the kappa value between the actual situation and observer 2 was 0.970; the kappa value between the actual situation and observer 3 was 0.909. A statistically significant and a very good level of agreement was obtained between the actual situation and all three observers (p<0.001) (Table 1).

Inter-observer Agreement with the Actual Situation within Dose Groups

In the low-dose group, the kappa value between the actual situation and observer 1 was 0.905; in the high-dose group, the kappa value between the actual situation and observer 1 was 1. In both cases, a statistically significant and a very good level of agreement was obtained between the actual situation and observer 1 (p<0.001). In the low-dose group, the kappa value between the actual situation and observer 2 was 0.952 and in the high-dose group, the kappa value between the actual situation and observer 2 was 1. A statistically significant and a very good level of agreement was obtained between the actual situation and observer 2 (p<0.001). The kappa value between the actual situation and observer 3 in the low-dose group was 0.952, and the kappa value between the actual situation and observer 3 in the high-dose group was 0.833. A statistically significant and excellent agreement was obtained between the actual situation and observer 3 (p<0.001) (Table 2).

Inter-observer Agreement with the Actual Situation at Ultralow-dose and Others (High and Low Dose)

The kappa value between the actual situation and observer 1 in the ultralow-dose group was 0.833, and the kappa value between the actual situation and observer 1 in the "others" group was 0.963. A statistically significant and excellent agreement was obtained between the actual situation and observer 1 (p<0.001). The kappa value between the actual situation and observer 2 in the ultralow-dose group was 1, and the kappa value between the actual situation and observer 2 in the "others (high and low dose)" group was 0.963. A statistically significant and a very good level of agreement was obtained between the actual situation and observer 2 (p<0.001). The kappa value between the actual situation and observer 3 in the ultralow-dose group was 1, and the kappa value between the actual situation and observer 3 in the "others" group was 0.889. A statistically significant and a very good level of agreement was obtained between the actual situation and observer 3 (p<0.001) (Table 3).

Table 1. Evaluation of the inter-observer agreement with the actual situation without making a group distinction

	Actual Situation				Kappa	p
	Intact	Enamel caries	Dentin caries	D.c.e.p*		
Observer1						
Intact	11 (100)	0 (0)	0 (0)	0 (0)	0.939	**0.000**
Enamel caries	0 (0)	11 (100)	1 (9.1)	0 (0)		
Dentin caries	0 (0)	0 (0)	10 (90.9)	1 (9.1)		
D.c.e.p*	0 (0)	0 (0)	0 (0)	10 (90.9)		
Observer2						
Intact	11 (100)	0 (0)	0 (0)	0 (0)	0.970	**0.000**
Enamel caries	0 (0)	10 (90.9)	0 (0)	0 (0)		
Dentin caries	0 (0)	1 (9.1)	11 (100)	0 (0)		
D.c.e.p*	0 (0)	0 (0)	0 (0)	11 (100)		
Observer3						
Intact	10 (90.9)	0 (0)	0 (0)	0 (0)	0.909	**0.000**
Enamel caries	1 (9.1)	9 (81.8)	0 (0)	0 (0)		
Dentin caries	0 (0)	2 (18.2)	11 (100)	0 (0)		
D.c.e.p*	0 (0)	0 (0)	0 (0)	11 (100)		

*: Deep caries with exposed pulp

Table 2. Evaluation of inter-observer agreement with actual situation within dose groups

Observation	Dose groups		Actual Stuation				Kappa	p
			Intact	Enamel caries	Dentin caries	D.c.e.p *		
Observer1	Low dose	Intact	7 (100)	0 (0)	0 (0)	0 (0)	0.905	**0.000**
		Enamel caries	0 (0)	7 (100)	1 (14.3)	0 (0)		
		Dentin caries	0 (0)	0 (0)	6 (85.7)	1 (14.3)		
		D.c.e.p*	0 (0)	0 (0)	0 (0)	6 (85.7)		
	High dose	Intact	4 (100)	0 (0)	0 (0)	0 (0)	1.000	**0.000**
		Enamel caries	0 (0)	4 (100)	0 (0)	0 (0)		
		Dentin caries	0 (0)	0 (0)	4 (100)	0 (0)		
		D.c.e.p *	0 (0)	0 (0)	0 (0)	4 (100)		
Observer2	Low dose	Intact	7 (100)	0 (0)	0 (0)	0 (0)	0.952	**0.000**
		Enamel caries	0 (0)	6 (85.7)	0 (0)	0 (0)		
		Dentin caries	0 (0)	1 (14.3)	7 (100)	0 (0)		
		D.c.e.p *	0 (0)	0 (0)	0 (0)	7 (100)		
	High dose	Intact	4 (100)	0 (0)	0 (0)	0 (0)	1.000	**0.000**
		Enamel caries	0 (0)	4 (100)	0 (0)	0 (0)		
		Dentin caries	0 (0)	0 (0)	4 (100)	0 (0)		
		D.c.e.p *	0 (0)	0 (0)	0 (0)	4 (100)		
Observer3	Low dose	Intact	7 (100)	0 (0)	0 (0)	0 (0)	0.952	**0.000**
		Enamel caries	0 (0)	6 (85.7)	0 (0)	0 (0)		
		Dentin caries	0 (0)	1 (14.3)	7 (100)	0 (0)		
		D.c.e.p *	0 (0)	0 (0)	0 (0)	7 (100)		
	High dose	Intact	3 (75)	0 (0)	0 (0)	0 (0)	0.833	**0.000**
		Enamel caries	1 (25)	3 (75)	0 (0)	0 (0)		
		Dentin caries	0 (0)	1 (25)	4 (100)	0 (0)		
		D.c.e.p *	0 (0)	0 (0)	0 (0)	4 (100)		

*:Deep caries with exposed pulp

Table 3. Evaluation of inter-observer agreement with actual situation in ultralow-dose and others

Observer	Dose-subgroup		Actual Situation				Kappa	p
			Intact	Enamel caries	Dentin caries	D.c.e.p*		
Observer 1	Ultralow-dose	Intact	2 (100)	0 (0)	0 (0)	0 (0)	0.833	**0.000**
		Enamel caries	0 (0)	2 (100)	1 (50)	0 (0)		
		Dentin caries	0 (0)	0 (0)	1 (50)	0 (0)		
		D.c.e.p*	0 (0)	0 (0)	0 (0)	2 (100)		
	Others	Intact	9 (100)	0 (0)	0 (0)	0 (0)	0.963	**0.000**
		Enamel caries	0 (0)	9 (100)	0 (0)	0 (0)		
		Dentin caries	0 (0)	0 (0)	9 (100)	1 (11.1)		
		D.c.e.p*.	0 (0)	0 (0)	0 (0)	8 (88.9)		

Observer 2	Ultralow-dose	Intact	2 (100)	0 (0)	0 (0)	0 (0)	1.000	**0.000**
		Enamel caries	0 (0)	2 (100)	0 (0)	0 (0)		
		Dentin caries	0 (0)	0 (0)	2 (100)	0 (0)		
		D.c.e.p*	0 (0)	0 (0)	0 (0)	2 (100)		
	Others	Intact	9 (100)	0 (0)	0 (0)	0 (0)	0.963	**0.000**
		Enamel caries	0 (0)	8 (88.9)	0 (0)	0 (0)		
		Dentin caries	0 (0)	1 (11.1)	9 (100)	0 (0)		
		D.c.e.p*	0 (0)	0 (0)	0 (0)	9 (100)		
Observer3	Ultralow-dose	Intact	2 (100)	0 (0)	0 (0)	0 (0)	1.000	**0.000**
		Enamel caries	0 (0)	2 (100)	0 (0)	0 (0)		
		Dentin caries	0 (0)	0 (0)	2 (100)	0 (0)		
		D.c.e.p*	0 (0)	0 (0)	0 (0)	2 (100)		
	Others	Intact	8 (88.9)	0 (0)	0 (0)	0 (0)	0.889	**0.000**
		Enamel caries	1 (11.1)	7 (77.8)	0 (0)	0 (0)		
		Dentin caries	0 (0)	2 (22.2)	9 (100)	0 (0)		
		D.c.e.p*	0 (0)	0 (0)	0 (0)	9 (100)		

*: Deep caries with exposed pulp

Inter-observer Agreement with Intactness Regardless of Group

The kappa value between real intactness and observer 1 was 1, the kappa value between real intactness and observer 2 was 1, and the kappa value between real intactness and observer 3 was 0.938. A statistically significant and a very good level of agreement was obtained between real robustness and all three observers (p<0.001) (Table 4).

Table 4. Evaluation of robustness and inter-observer agreement without group distinction

	Intactness		Kappa	p
	Intact	Carious		
Intactness O1*				
Intact	11 (100)	0 (0)	1.000	**0.000**
Carious	0 (0)	33 (100)		
Intactness O2#				
Intact	11 (100)	0 (0)	1.000	**0.000**
Carious	0 (0)	33 (100)		
Intactness O3"				
Intact	10 (90.9)	0 (0)	0.938	**0.000**
Carious	1 (9.1)	33 (100)		

*:Observer 1; #:Observer 2; ":Observer 3

Inter-observer Agreement with Intactness within Ultralow-dose and Others(High and Low Dose)

In the ultralow-dose group, the kappa value between real intactness and observer 1 was 1, and a statistically significant and a very good level of agreement was obtained between real intactness and observer 1 (p=0.000). In the "others" group, the kappa value between real intactness and observer 1 was obtained as 1, and a statistically significant and a very good level of agreement was obtained between real intactness and observer 1 (p<0.001). In the ultralow-dose group, the kappa value between real intactness and observer 2 was 1, and a statistically significant and a very good level of agreement was obtained between real intactness and observer 2 (p=0.000). In the "others" group, the kappa value between real intactness and observer 2 was obtained as 1, and a statistically significant and a very good level of agreement was obtained between real intactness and observer 2 (p<0.001). In the ultralow-dose group, the kappa value between real intactness and observer 3 was 1, and a statistically significant and a very good level of agreement was obtained between real intactness and observer 3 (p=0.000). In the "others" group, the kappa value between real intactness and observer 3 was 0.923, and a statistically significant and a very good level of agreement was obtained between real intactness and observer 3 (p<0.001) (Table 5).

Tablo 5. Evaluation of intactness and inter-observer agreement in ultralow-dose and others

Observer	Dose-subgroup		Intactness		Kappa	p
			Intact	Carious		
Intactness O1*	Ultralow-dose	Intact	2 (100)	0 (0)	1.000	**0.000**
		Carious	0 (0)	6 (100)		
	Others	Intact	9 (100)	0 (0)	1.000	**0.000**
		Carious	0 (0)	27 (100)		
Intactness O2#	Ultralow-dose	Intact	2 (100)	0 (0)	1.000	**0.000**
		Carious	0 (0)	6 (100)		
	Others	Intact	9 (100)	0 (0)	1.000	**0.000**
		Carious	0 (0)	27 (100)		
Intactness O3"	Ultralow-dose	Intact	2 (100)	0 (0)	1.000	**0.000**
		Carious	0 (0)	6 (100)		
	Others	Intact	8 (88.9)	0 (0)	0.923	**0.000**
		Carious	1 (11.1)	27 (100)		

*:Observer 1; #:Observer 2; ":Observer 3

DISCUSSION

The main goal in radiology is to produce images that have sufficient detail to reveal important diagnostic information while ensuring that patients are exposed to the minimum amount of radiation required due to the potential risks of ionizing radiation. Tissues and organs can be harmed by exposure to radiation even at the low doses that are effective from intraoral radiographs [14]. To protect tissues and organs from ionizing radiation, effective dose estimation and decrease are therefore important.

Several studies have compared different imaging modalities for caries diagnosis [2, 15-17]. Abesi et al. contrasted the diagnostic efficacy of intraoral films, CCD, and PSP in detecting non-cavitated caries. The sensitivity and specificity of film, CCD and PSP for the detection of enamel caries were 38% and 98%, 15% and 96% and 23% and 98%, respectively; while the sensitivity and specificity of dentin and enamel caries were 55% and 100%, 45% and 100%, and 55% and 100%, respectively. These findings show that the diagnostic accuracy of digital images was similar to that of traditional intraoral films in identifying non-cavitated approximal caries [15]. In this study, we only used PSP with high diagnostic efficiency, which is frequently preferred intraorally.

Strong inter-observer agreement was found in a study comparing the diagnostic efficacy of visual inspection, film, PSP, CCD, and cone beam computed tomography (CBCT) in the detection of proximal caries. The detection methods' kappa coefficients ranged from 0.631 to 0.811, and the methods that were chosen demonstrated similar results [2].

In another study, at 50, 65, and 70 kVp, there was no variation in the diagnostic efficacy of PSP, CDD, and film images of proximal caries in deciduous teeth. However, at 50 kVp, a significant difference was noted in favor of PSP images [16].

The current study is the first to compare the detection of intact and carious teeth with 14 different exposure parameters in PSP images. Actual and inter-observer agreement was statistically significant for all three observers in the identification of enamel caries, dentin caries, deep caries with exposed pulp, and intact teeth imaged with high dose parameters (70 kVp; 7 mA; 1.25–0.25 s). However, at high-dose exposure factors, PSPs imaged with application times of 0.8–1.25 s were not visualized, and no interpretation could be made. For PSPs imaged with low-dose (60 kVp; 4 mA; 0.04–0.1 s) and ultra-low-dose (60 kVp; 4 mA; 0.02–0.03) parameters, a statistically significant and good agreement between the three observers and the actual situation was obtained ($p<0.001$).

In a previous study CCD, PSP, and intraoral films were compared for caries diagnosis with 60 and 70 kVp parameters. For enamel lesions, the PSP with 70 kVp and an exposure time of 0.03 s was reported to have the highest sensitivity; for the detection of lesions with and without cavitation, the PSP with 60 kVp and an exposure time of 0.07 s was reported to have higher sensitivity and less radiation dose to the patient [17]. Compared to this study, in our study, where we used the same voltage parameters (60 and 70 kVp), observers were able to detect the presence or absence of caries and the level of caries even at shorter exposure

times Since the low-dose and even ultralow-dose parameters obtained with 60 kVp do not cause a change in image quality that would prevent accurate diagnosis, the use of 70 kVp in intraoral imaging indicates that the patient will be unnecessarily exposed to a high radiation dose.

De Melo et al. reported that PSPs were sensitive to tube setting changes when the range of use was 50–80 kVp and reported that the best results were obtained using 70 kVp [18]. In this study, the kappa values of the three observers in the identification of intact teeth and caries imaged at 70 kVp, 7 mA, and different application times grouped as high-dose were 1, 1, and 0.833, respectively. The kappa values of the three observers at 60 kVp, 4 mA, and different application times grouped as ultra-low-dose were 0.833, 1, and 1, respectively. Consequently, in our study, decreasing the tube potential (kVp), current, and time did not change the diagnostic accuracy and provided imaging with a lower radiation dose.

Selecting the appropriate exposure parameters is crucial in achieving an image of diagnostic quality. The average photon energy used to create the image is one of the factors affecting contrast, which is determined by the choice of X-ray tube voltage and the amount of X-ray beam filtering. Accordingly, less energy is usually associated with higher contrast [19]. Of all the exposure parameters, tube potential is very important for caries detection, and high contrast is a precondition for the accurate identification of radiographic approximal caries [7]. Studies have found that caries lesions are easier to identify on high-contrast images, so lower tube potential values are generally suggested for this objective [16, 20].

One study showed that reducing the tube voltage from 70 kVp to 60 kVp did not compromise image quality for the evaluation of carious lesions. The same study reported that the patient was exposed to an absorbed dose that was approximately 40–50% higher when a tube voltage of 70 kVp was used [21]. Dehghani et al. suggested the use of PSP with 60 kVp to comply with the As Low As Reasonably Achievable (ALARA) rule, especially when a tooth has clinical signs or discoloration indicative of caries, and they attributed this to the higher sensitivity and lower exposure time of PSP compared to intraoral film [17]. In the present study, one of the three observers incorrectly detected only one enamel caries, and the other two observers incorrectly detected one dentin caries, while all intact teeth and all other caries were correctly detected at low doses. At ultra-low doses, intact teeth and other caries were correctly detected by the three observers, while only one observer incorrectly detected dentin caries. Based on the results of this study, caries diagnosis at ultra-low doses seems possible.

Limitations

A limitation of this study is that it was an in vitro study, and consequently, the accuracy of the imaging modalities tested could not be evaluated in a clinical setting. However, in in vitro radiographic studies, the imaged objects can be repeatedly exposed to X-rays and optimal positioning seems to be possible. By using these advantages of an in vitro environment, effective doses can be calculated based on radiographic studies, and patients' exposure to X-rays can be minimized.

CONCLUSIONS

Even at the lowest dose used in this study (60 kVp 4 ma 0.02 s), images were obtained that enabled accurate diagnosis. Based on this result, it is necessary to revise the routine exposure parameters used in dentistry when obtaining intraoral radiographs with PSP, and it should be recognized that approximal caries diagnosis can be made safely at lower radiation doses. Moreover, it is anticipated that this study will shed light on future clinical studies.

Conflict of interest The authors declare that they have no confict ofinterest.

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Informed consent Informed consent form was not obtained because the study was retrospective.

Ethical Approval Statement This study was carried out with the approval (Protocol no: 2021/2454) from the University Health Sciences Non-Interventional Clinical Research Ethics Committee.

Author Contributions: Conception: D, N - Design: D, N – Supervision: A, O - Materials: E, G; D, K – Data Collection and/or Processing: T, E; Ö, B – Analysis and/or Interpretation: D, ŞB - Literature Review: ÇÖ, D – Writing: ÇÖ, D – Critical Review: A,O

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Original Research

The Relationship Between Odontogenic Cyst and P53 Codon 72 And P53 Codon 175 Variants in Turkish Patients

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ABSTRACT

Objective: Odontogenic cysts that cause bone destruction can exhibit various types of metaplasia. Inherited genetic variants in codons 72 and 175, the hotspot codons of p53, known as the guardian of the genome, can cause a wide variety of cancers. We aimed to investigate the effects of the p53 codon 72 and p53 codon 175 variants on odontogenic cyst formation.

Methods: This research encompassed 71 individuals with odontogenic cysts and 90 without any conditions as a control group. After DNA was extracting, the p53 codon 72 was detected using PCR techniques, while p53 codon 175 was identified through allele-specific amplification-PCR.

Results: The presence of the p53 codon 72 GG genotype and its G allele was less frequent in the group with odontogenic cysts compared to the healthy participants. Conversely, the C allele was found more often in the cyst-afflicted group. For the p53 codon 175, the AA genotype and A allele were more common in the affected group, while the G allele was more predominant in the control group.

Conclusion: The p53 codon 175 AA genotype and A allele, p53 codon 72 C allele, and p53 codon 72/codon 175 CCAA combined genotype may be associated with odontagenic cyst formation. Individuals with this allele and genotype can be considered at risk for odontagenic cyst formation.

Keywords: Odontogenic cysts; Gene; Metaplasia

INTRODUCTION

Odontogenic cysts, which develop within the regions of the mandible and maxilla that bare teeth, are pathological cavities lined with epithelium and encased in fibrous connective tissue derived from odontogenic tissue. These cysts, which are capable of causing bone deterioration, can lead to resorption or displacement of neighboring teeth. Such cysts can be of either inflammatory or developmental origin [1]. It is widely recognized that odontogenic cysts, whether inflammatory or developmental, contain linings of squamous epithelial cells. These linings may undergo various forms of metaplasia, transforming from stratified squamous cells to highly differentiated ciliated columnar or mucous cells [2].

The role of p53, which is the most frequently mutated gene across diverse cancer types, has evolved since it was discovered four decades ago. Initially believed to be an oncogene, p53 was subsequently identified as an essential tumor suppressor [3]. P53 stringently controls cellular growth, induces apoptosis, and facilitates DNA repair under stress conditions. Mutations in p53

can disrupt these functions, contributing to tumor advancement and unchecked cellular proliferation. Mutations in p53 have been shown to cause protein aggregation, thereby inhibiting its function [4].

Two polymorphic variants arise because of an amino acid residue shift from arginine to proline at the 72nd codon of the p53 protein, each exhibiting distinct biological and biochemical attributes. The proline variant of p53 acts as a more robust activator of transcription factors but exhibits less potent apoptotic properties than the arginine variant, which is more prone to degradation [5]. This polymorphism at p53's 72nd codon is posited to play a crucial role in cancer development and pathogenesis [6]. However, a recent study suggested that this polymorphism selectively modulates functional metabolic pathways without influencing cell fate pathways, such as apoptosis and growth arrest under genotoxic stress [7].

Mutations in p53 are prevalent in solid tumors, with missense mutations being particularly common at codons 273, 248, and 175, which are known hotspots for mutations [8]. Among these, codon 175 is situated in the DNA-binding domain of p53 and is associated with global denaturation of the protein [9].

There is no original study in the literature on the effect of the variants observed in the p53 codon 72 and p53 codon 175 gene regions on odontogenic cyst development in the Turkish population. In the light of this information, this study aimed to investigate the effects of p53 codon 72 and p53 codon 175 variants on odontogenic cyst development in the Turkish population. The frequencies of the alleles and genotypes of p53 codons 72 and p53 codon 175 in individuals with odontogenic cysts were compared with those in healthy individuals. Additionally, the association between the demographic and clinical data of the participants and the distribution of genotypes was examined.

Main Points;

- The AA genotype and A allele frequency in the p53 codon 175 variant was high in individuals with odontogenic cysts.
- The C allele frequency in the p53 codon 72 variant was high in individuals with odontogenic cysts.
- The p53 codon 72/codon 175 CCAA combined genotype may be associated with odontagenic cyst formation.
- The p53 codon 72/codon 175 CCAA combined genotype can be considered to be at risk for odontogenic cyst formation.

MATERIALS AND METHODS

Study Design

This study is a single-center case-control study. All procedures conducted during the study strictly adhered to the ethical guidelines outlined in the Declaration of Helsinki. For the purpose of this study, the patient group consisted of 71 individuals diagnosed with odontogenic cysts, who sought treatment at the Oral and Dental Surgery outpatient clinic. In addition, a control group comprising of 90 healthy individuals was established. The participants provided 2 ml of venous blood collected in tubes pre-filled with ethylenediaminetetraacetic acid. DNA was subsequently extracted from these samples using the QIAamp DNA Extraction Kit (Qiagen, Valencia, California, United States).

Genotyping

Primers used to identify rs1042522 (p53 codon 72) in the Arg72 variant (5′- TCCCCCTTGCCGTCCCAA-3′; 5′-CTG GTGCAGGGGCCACGC-3′) and Pro72 variant (5′- GCCAGAGGC TGCTCCCCC-3′; 5′-CGTGCAAGTCACAGACTT-3). Genomic DNA (100ng) was amplified by polymerase chain reaction (PCR). A final volume of 25 µL was used for the PCR. The following protocol was used for the PCR amplification on a Thermo Cycler thermal cycler: 4 min at 95°C, 35 s at 94°C, 35 s at 60°C, 50 s at 72°C for the Pro72 variant and 4 min at 95 °C, 40 s at 94 °C, 40 s at 58 °C, 50 s at 72 °C for the the Arg72 variant, and for 35 cycles, with an extra min at 72 °C after the last cycle. The amplified products were visualized on a 3 % agarose gel. T100 Thermal Cycler (Bio-Rad Laboratories - Dubai Branch) device was used for this procedure.

We employed allele-specific amplification (ASA)-PCR to test for codon 175 (CGCCAC) variants in the p53 gene. This technique entails the amplification of particular alleles or DNA sequence variations at the same locus. Designing one or both PCR primers so that they partially overlap the location of sequence differences between the generated alleles is how specificity in ASA-PCR examination is achieved. Three primers were used for amplification. The third was created particularly for the mutant gene, whereas the other two were used to amplify the

wild-type allele. The primer sequences were as follows: the 3' end of the primer 1:5'-GCAGCGCTCATGGTGGGGGCAGT-3' contained a T residue. The 3' end of the primer, 2:5'-GCGCTCATGGTGGGGGCAGC-3, 'has a C residue. Unspecific primer 3:5'- TTGATTCCACACCCGCCCG. T100 Thermal Cycler (Bio-Rad Laboratories - Dubai Branch) device was used for this procedure.

Each sample under investigation underwent two PCR amplifications, one of which amplified the wild type (with primers 2 and 3), while the other amplified the p53 codon 175-point mutation using primers 1 and 3, yielding a 105 bp fragment. We detected two bands for heterozygotes and one band for homozygotes of the wild type in the electrophoretic pattern.

100 ng of genomic DNA, 1 unit of Taq polymerase (Thermo Fisher Scientific Inc., Porto Salvo), 0.1 mM oligonucleotide primers, 100 mM dNTPs, and 1.5 mM MgCl2 made up the PCR mixture. Both the mutant alleles and the wild type underwent the same thermal cycle protocols, which included denaturation at 94°C for 40 seconds, annealing at 66°C for 40 seconds, extension at 72°C for 40 seconds, and amplification for 30 cycles. Polymerase chain reaction (PCR) was performed using a Thermo Thermocycler. Template DNA was changed using PCR-grade water as a negative control. The amplified products were visualized on a 3% agarose gel.

Data Evaluation

We utilized the IBM's SPSS 22.0 22 for Windows (IBM, New York, USA) software for our statistical evaluations. For categorical information, we presented results in terms of percentage frequencies. For continuous information, results were given as the average plus or minus the standard deviation. To compare groups, we employed the chi-square test for categorical data and the independent t-test for continuous data.

RESULTS

This research evaluated 71 participants, with ages ranging from 14 to 72 years, who had confirmed diagnoses of odontogenic cysts. In contrast, the control group incorporated 90 healthy individuals, aged between 19 and 70 years. The mean age for those with odontogenic cysts stood at 35.99±14.08 years, while the average for the control group was 34.84±12.31 years. Among the cyst-diagnosed participants, 39 (54.93%) were female, and 32 (45.07%) were male. The control set consisted of 39 females (43.33%) and 51 males (56.67%). There was no significant difference between the control group and the participants with odontogenic cysts in terms of mean age and gender ratio.

The distribution frequencies for the genotypes and alleles of p53 codon 72 and p53 codon 175 variants across both sets of participants presented in Table 1.

For the genotype distribution related to p53 codon 72, a reduced frequency of the GG genotype was noted among the cyst-afflicted group compared to the controls, as detailed in Table 1.

Considering the allele distribution for p53 codon 72, those diagnosed with cysts showed a diminished presence of the G allele and an elevated presence of the C allele relative to the control group, as depicted in Table 1.

When assessing the genotype distribution associated with p53 codon 175, there was an evident increase in the frequency of the AA genotype among the patient group compared to the control participants, as illustrated in Table 1.

Regarding the allele distribution of p53 codon 175, the patient group demonstrated a decreased prevalence of the G allele and an augmented prevalence of the A allele in contrast to the controls, as outlined in Table 1.

A detailed examination was conducted on the combined genotype frequencies for the variants of p53 codons 72 and 175 across both sets of participants, and these findings are presented in Table 2.

From this combined assessment, the GGGA genotype appeared less frequently in the cyst-diagnosed group, while the CCAA genotype's frequency was more predominant among them, as shown in Table 2.

Participants' age, gender, cyst diagnostic features, cyst sizes were analyzed according to different genotypes of p53 codons 72 and 175. These detailed results are provided in Table 3.

After comprehensive evaluation, no direct association was found between the genotype distributions of the p53 codon 72 and p53 codon 175 variants and the factors like participant age, gender, cyst diagnosis details, cyst measurements, and locations, as specified in Table 3 ($p>0.05$).

Table 1. Genotype and allele distribution of p53 codon 72 and p53 codon 175 variants of the groups

Gene region Genotype/Allele	Patient n=71(%)	Control n=90 (%)	p*	OR (CI 95%)
p53 codon 72	**Genotype**			
GG	18 (25.35)	40 (44.44)	**0.012**	2.356 (1.197-4.637)
GC	29 (40.85)	31 (34.44)	>0.05	0.761 (0.400-1.447)
CC	24 (33.80)	19 (21.11)	>0.05	0.524 (0.259-1.061)
p53 codon 72	**Allele**			
G	65 (45.77)	111 (61.67)	**0.004**	0.525 (0.336-0.820)
C	77 (54.23)	69 (38.33)		
Gene region Genotype/Allele	**Patient n=71(%)**	**Control n=90 (%)**	**p**	**OR (CI 95%)**
p53 codon 175	**Genotype**			
GG	24 (33.80)	35 (38.89)	>0.05	1.246 (0.651-2.385)
GA	20 (28.17)	37 (41.11)	>0.05	1.780 (0.914-3.465)
AA	27 (38.03)	18 (20.00)	**0.011**	0.407 (0.201-0.824)
p53 codon 175	**Allele**			
G	68 (47.89)	107 (59.44)	**0.039**	0.627 (0.402-0.977)
A	74 (52.11)	73 (40.56)		

* Chi-square test

Table 2. Combined genotype distribution of p53 codon 72 and p53 codon 175 variants of the groups

Combined Genotype p53 codon 72/p53 codon 175	Patient n=71 (%)	Control n=90 (%)	p*
GGGG	7 (09.86)	14 (15.56)	>0.05
GGGA	6 (08.45)	19 (21.11)	**0.028**
GGAA	5 (07.04)	7 (07.78)	>0.05
GCGG	10 (14.08)	12 (13.33)	>0.05
GCGA	8 (11.27)	11 (12.22)	>0.05
GCAA	11 (15.49)	8 (08.89)	>0.05
CCGG	7 (09.86)	9 (10.00)	>0.05
CCGA	6 (08.45)	7 (07.78)	>0.05
CCAA	11 (15.49)	3 (03.33)	**0.007**

* Chi-square test

Table 3. The relationship between descriptive and clinical information of the patient group and genotype distribution of p53 codon 72 and p53 codon 175 variants

p53 codon 72					
Parameter		**GG n=18 (%)**	**GC n=29 (%)**	**CC n=24 (%)**	**p***
Gender n(%)	Male	7 (38.89)	13 (44.83)	12 (50.00)	>0.05
	Woman	11 (61.11)	16 (55.17)	12 (50.00)	
Age X±SD		38.28±12.84	37.10±13.86	32.92±5.25	>0.05
Diagnosis n(%)	Radicular cyst	17 (94.44)	20 (68.97)	21 (87.50)	>0.05
	Dentigerous cyst	1 (05.56)	3 (10.34)	2 (08.33)	
	Odontogenic keratocystic cyst	0 (00.00)	6 (20.69)	1 (04.17)	
Region n (%)	Maxilla Anterior	5 (27.78)	6 (20.69)	6 (25.00)	>0.05
	Maxilla Posterior	3 (16.67)	2 (06.90)	2 (08.33)	
	MandibulaAnterior	1 (05.56)	8 (27.59)	5 (20.83)	
	Mandibula Posterior	9 (50.00)	13 (44.83)	11 (45.83)	
Dimension (mm^2) X±SD		20.94±18.72	40.55±38.41	26.58±40.69	>0.05
p53 codon 175					
Parameter		**GG n=24(%)**	**GA n=20(%)**	**AA n=27(%)**	**p***
Gender n(%)	Male	8 (33.33)	11 (55.00)	13 (48.15)	>0.05
	Woman	16 (66.67)	9 (45.00)	14 (51.85)	
Age X±SD		32.33±13.21	34.75±14.60	40.15±13.88	>0.05
Diagnosis n(%)	Radicular cyst	22 (91.67)	17 (85.00)	19 (70.37)	>0.05
	Dentigerous cyst	1 (04.17)	1 (05.00)	4 (14.81)	
	Odontogenic cyst	1 (04.17)	2 (10.00)	4 (14.81)	
Cyst location n (%)	Maxilla Anterior	7 (29.17)	2 (10.00)	8 (29.63)	>0.05
	Maxilla Posterior	1(04.17)	2 (10.00)	4 (14.81)	
	MandibulaAnterior	1 (04.17)	6 (30.00)	7 (25.93)	
	Mandibula Posterior	15 (62.50)	10 (50.00)	8 (29.63)	
Cyst size (mm^2) X±SD		23.04±20.59	23.85±29.78	43.00±46.92	>0.05

* Chi-square test for categorical data and the independent t-test for continuous data

DISCUSSION

The P53 protein, renowned as a tumor suppressor, plays an indispensable role in deterring malignant transformations and is colloquially labeled as the genome's sentinel. An intriguing observation is that mutations in the P53 gene manifest in almost 50% of all human tumor cases. In the instances where such mutations are absent, the P53 regulatory network tends to be incapacitated. Most mutations linked to P53 are of the missense type, with a staggering 90% affecting specific codons, denoted as "hotspot codons", predominantly located in the DNA-binding region. Among the p53 gene's significant hotspot codons, codons 248, 245, and 175 are found within the DNA-binding domain, while codon 72 is positioned externally [10]. Genetic alterations within these specific codons seem to have a more profound impact on the susceptibility to various cancers than mutations in alternative genetic routes. Interestingly, the genetic features of cancer-associated variations within the P53 pathway share considerable similarities with the attributes of widely researched mutations in the same pathway [11].

Among the different polymorphisms within the p53 gene, the p53 codon 72 variant has garnered substantial scientific attention. This specific variant triggers a morphological change in the protein and is strategically located within the proline-rich domain, a segment instrumental in p53's apoptotic functionality [12]. A substantial study encompassing 27,958 individuals hinted that

the p53 codon 72 polymorphisms might have a subtle influence on predisposition to lung cancer, particularly accentuated in adenocarcinoma patients and those who have never smoked [13]. A separate comparative analysis between Asian and Caucasian females deduced that individuals with the GG genotype associated with p53 codon 72 polymorphisms generally had tumors of smaller dimensions compared to their counterparts with the CC genotype [14]. Delving into a broad literature review that amalgamated findings from 11 distinct case-control studies and an additional 10 standalone publications, there emerged a proposition that the p53 codon 72 variants might impart a protective shield against the development of glioblastoma [15].
A comprehensive review encompassing 13 studies with a total of 2,413 cases and 3,201 controls highlighted that the p53 codon 72 variants, in conjunction with Human Papilloma Virus infections, can collectively modify an individual's predisposition to oral cancer. Additionally, the p53 codon 72 variants may play a role in the progression and onset of oral cancer. However, the association between susceptibility to oral cancer and the p53 codon 72 variant appears to be subject to variation among diverse ethnic populations [16]. Research conducted on Taiwanese subjects revealed that individuals possessing the Arg/Arg (GG genotype) at p53 codon 72 were at a risk 2.68 times greater risk of oral cancer than those possessing the CC genotype (Pro/Pro) [17]. Conversely, a study involving Iranian subjects determined that the CC genotype (Pro/Pro) was a contributing risk factor for oral squamous cell carcinoma, particularly in the anatomical location of the tumor and in individuals below 50 years [18]. Additionally, another investigation of Taiwanese participants indicated that p53 codon 72 Pro homozygosity (CC genotype) was associated with an elevated likelihood of hypopharyngeal tumor development [19].

In the present study involving Turkish participants, the ratio of patients with odontogenic cysts carrying the GG genotype in the p53 codon 72 variant was found to be less than the ratio observed in their healthy counterparts. Moreover, the occurrence of the G allele was diminished in patients with odontogenic cysts compared to the healthy population, while the frequency of the C allele was higher in the patient group.

An investigation in a Thai cohort revealed that the CC genotype of the P53 gene codon 72 increased the probability of developing sporadic keratocystic odontogenic tumors [20]. In contrast, another study in the same Thai demographic determined that the GG genotype of the P53 gene codon 72 could play a crucial role in the genesis of ameloblastoma, suggesting that the heightened risk associated with the P53 codon 72 GG genotype may remain independent of the tumors' clinical characteristics [21].

Participants were categorized into three distinct diagnostic groups: radicular cysts, dentigerous cysts, and odontogenic keratocystic cysts. No associations were observed between the frequencies of the GG, GC, and CC genotypes of the p53 codon 72 variant and factors such as patient age, sex, type of cyst, cyst dimensions, and cyst location among these individuals.

Over 8000 mutations in p53 have been documented. The diversity of these mutations is subject to variation across different tumor types, with more than half concentrated in three predominant codons:175, 248, and 273. The polymorphism observed in codon 175, which is among the key codons, may potentially serve as an indicator of the progression of colorectal cancer. Furthermore, it could prove beneficial in evaluating the confines of surgical excision [22]. Mutations of p53 in glioblastoma multiforme, the most prevalent primary brain tumor, are commonly found within the DNA-binding domain, indicating that the region encompassing p53 codon 175 is a mutation hotspot. These alterations often lead to a variety of effects, including gain of function, loss of function, and dominant negative mutations in p53 [23].

No existing studies have investigated p53 codon 175 variants in individuals presenting with oral symptoms. This study, pioneering in its nature, discovered that the proportion of patients diagnosed with odontogenic cysts possessing the AA genotype in the p53 codon 175 polymorphism surpassed that of healthy individuals with the same genotype. Additionally, the prevalence of the A allele was elevated in individuals with odontogenic cysts compared to healthy subjects, while the frequency of the G allele was diminished in patients with odontogenic cysts relative to the healthy population. Moreover, no associations were identified between the frequencies of the GG, GA, and AA genotypes of the p53 codon 175 variant and variables such as patient age, sex, cyst classification, cyst dimensions, and cyst location among these participants.

Our study is a single-center study conducted with Turkish patients. This can be considered as a limitation of our study. On the other hand, it can also be considered as a reference study for future multicenter studies.

CONCLUSIONS

In conclusion, individuals diagnosed with odontogenic cysts exhibited a decreased prevalence of the GG genotype for the p53 codon 72 variant and an increased prevalence of the AA genotype for the p53 codon 175 variant compared to their healthy counterparts. Additionally, the C allele for p53 codon 72 polymorphism and the A allele for p53 codon 175 polymorphism were more frequently observed in these patients. When evaluating both p53 codon 72 and p53 codon 175 variants in tandem, a higher proportion of the combined CCAA genotype was observed. The presence of the C allele in the p53 codon 72 variant and the A allele in the p53 codon 175 variant may be indicative of an elevated risk for the development of odontogenic cysts. However, no significant correlations were found between the genotype distributions of p53 codon 72 and p53 codon 175 variants and factors such as patient age, sex, cyst type, cyst dimensions, and cyst position.

Conflict of interest: The authors declare that there is no potential conflict of interest.

Patient consent: Written and verbal consent was obtained from the participants.

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Original Research

The Effect of Biogel Using Biomagnetic Energy in the Treatment of Acute Pain in the Upper Extremity and Spine: A Randomized Controlled Trial

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ABSTRACT

Objective: Pain is a public health problem, which is caused by various etiological factors and leads to diminished quality of life and decreased workforce. The aim of this study was to determine whether Biogel has an effect in the treatment of pain.

Methods: Patients due to acute pain in the upper extremity and/or trunk were divided into two groups as treatment and placebo by randomization method. For the patients in the treatment group,the non-interventional Biogel was applied for 10 minutes. For the control group, a non-interventional placebo was applied for 10 mins. A record was made of patient demographic data,the region of the pain, and mean arterial pressure (MAP) values before and after the application. All the patients in both groups were administered a Visual Analogue Scale (VAS) to evaluate pain severity,and the Nottingham Health Profile (NHP) before and after the applications. The data obtained were compared.

Results: In the biogel group, a statistically significant decrease was determined in the NHP-P values after treatment compared to before treatment ($P<0.001$). In the placebo group,no statistically significant difference was determined in the NHP-P values before and after treatment ($P=0.104$). In the Biogel group,a statistically significant decrease was determined in the VAS values after treatment compared to before treatment ($P<0.001$). In the placebo group,no statistically significant difference was determined in the VAS values before and after treatment ($P=0.157$).

Conclusion: These types of complementary medicine applications focussed on pain treatment can reduce the disease burden and can probably reduce costs.

Keywords: Pain, Biogel, Complementary Medicine, Traditional Medicine, Integrative Medicine

INTRODUCTION

Pain is defined as an unpleasant sensory and emotional experience which identifies a type of damage or is associated with actual or potential tissue damage [1, 2]. Pain can occur for many different reasons. Acute pain is triggered by a specific disease or injury, serves a beneficial biological purpose, is associated with skeletal muscle spasm and sympathetic nerve system activation, and is self-limiting. Acute pain is short-lasting and can generally be easily described by the patient [3]. In contrast, chronic pain is a common and uncomfortable condition caused by pain which persists despite the normal healing process or which lasts for a period of longer than 3 months [4]. When pain is experienced,

it is perceived in different anatomic regions such as the head and neck region, the upper and lower back, abdomen, and chest. Abnormal signal transmission and processing in the nervous system is the true explanation of this condition [5]. Treatment of acute pain aims to treat the underlying cause and cut nociceptive signals [3].

Complementary Medicine, is a branch of science, which treats the patient holistically, providing healthcare services to patients with a patient-focussed and evidence-based approach. Traditional and Complementary Medicine (T&CM) is defined by the World Health Organisation (WHO) as "the entirety of knowledge, skills and practices based on theories, beliefs and experiences specific to different cultures, which can be explained or not, used in the prevention, diagnosis, improvement, or treatment of physical and mental diseases as well as in maintaining good health" [6]. In recent years, the interest of patients and researchers in T&CM has increased. In parallel with this, different methods have been accepted throughout the world, including phytotherapy, mesotherapy, larva application, prolotherapy, cupping applications, music therapy, hypnotherapy, homeopathy, leech therapy, ozone applications, osteopathy, reflexology, acupuncture, apitherapy, and chiropractice [6]. Another complementary medicine method that is applied worldwide is bioenergy [7].

Main Points;

- Pain is a significant public health concern, arising from a multitude of etiological variables and resulting in a notable decline in both quality of life and workforce productivity.
- Complementary medicine is a field of research that uses an evidence-based, patient-centered approach to care for patients by treating them holistically.
- Bioenergy is regarded as a therapeutic modality that promotes the principles of holistic medicine, acknowledging the potential for comprehensive treatment of ailments encompassing the physical, mental, and spiritual aspects of an individual. This approach emphasises the enhancement of the immune system and the harmonious flow of energy within the body, particularly targeting energy centres.
- The findings of this study indicate that biogel may be considered a viable adjunctive therapeutic approach for managing acute pain in the upper extremities and/or trunk.

Bioelectromagnetism is a discipline related to how the human body produces electromagnetic energy and what sort of response is given when exposed to this energy from outside. The energy area around the heart is the bio-area that was first measured in humans. Research related to this subject almost a century ago led to the invention of the electrocardiogram device. After a further twenty five years, Berger measured the bio-area around the brain and that study led to the development of electroencephalography. The studies conducted created bioelectromagnetic areas of organs such as the heart and brain and proved that the energy produced by these areas could be measured with electrodes attached to the body [7, 8].

Bioenergy is a concept coming from the words bio and energy, which have the meanings of living and life in Latin. Energy healing is currently applied for the provision of general health, well-being, and relaxation, the elimination of symptoms of several chronic diseases, for strengthening the immune system, and in the resolution of several health problems such as stress, depression, anxiety, fatigue, asthma, hypertension, cancer, arthritis, acute or chronic pain, and wound healing [9].

Treatment is applied with the harmonisation of chakras, which are accepted as aura and energy centres in bioenergy. Thus, the energy balance of the body is restored and physical and psychological diseases are treated through the activation of chakras [9]. Treatment in terms of energy applications is defined as the return of the body to a process of balance and harmony as a result of determining the causes of physical disease in the body, eliminating these and thereby regaining physical health. Energy therapies are known to have been used as healing methoods since ancient times. Current modern energy therapy is known to be based on the Einstein paradigm. According to this paradigm, just as matter is composed from energy and vibrations, so the human body is also thought to be composed from energy and vibrations. In contrast to traditional drugs and surgical interventions, this approach advocates that treatment can be made with pure energy. At the same time, understanding and resolving the molecular organisation of the physical body is primarily accepted in this treatment approach, and it is believed that diseases occur when the balance of energy systems is disrupted, and thus pathological symptoms occur in physical, emotional, mental, and spiritual planes [9-11].

Bioenergy is seen as a treatment technique that advocates the understanding of holistic medicine, and it is accepted that the

treatment of diseases can be applied holistically to the body, mind, and spirit, especially strengthening the immune system and directing energy in the body in a balanced way to energy centres [12]. In diseases originating from a local weakness, acute and chronic pain, allergies, varices, constipation, and similar disorders, it is aimed to increase the energy level of cells with bioenergy and prevent recurrence of the same disease in the same area by increasing their resistance. The aim of treatment with bioenergy is not treatment of the disease itself, but to strengthen and activate the natural defence mechanisms of the body [12].

Pain is a public health problem, which is caused by various etiological factors and leads to diminished quality of life and decreased workforce. Drugs taken for treatment purposes do not always provide the desired results and this causes patients to seek different methods [13]. Pain related to the musculoskeletal system and rheumatological diseases are among the leading reasons for presentation at healthcare facilities worldwide [14]. Since ancient times many T&CM methods have been used in the treatment of pain, primarily Chinese medical acupuncture. The aim of this study was to determine whether or not there was any effect in the treatment of pain of biogel formed using amino acids and trace elements of gold, platinum, silver, and other semi-precious metal minerals processed with nano technology.

MATERIALS AND METHODS

Biogel

The biogel application performed in our study is shown in Figure 1a, and the image of the used biogel is shown in Figure 1b. The biogel (Biomagnetic Compress gel, BiogelyTM, Hitit University Technopolis Campus, Çorum, Turkey) was produced using amino acids and trace elements of gold, platinum, silver, and other semi-precious metal minerals processed with nano technology [15].

Clinical Application

The study was conducted in the Orthopaedics and Traumatology Department of Hitit University Erol Olçok Training and Research Hospital and in Mimar Sinan Family Health Centre. The study included voluntary patients aged 18-65 years who presented with the complaint of acute pain in the upper extremity and/or trunk. Patients were excluded from the study if they had a chronic disease such as diabetes mellitus, if they had a cardiac pacemaker, were pregnant, or had any psychiatric disease. Approval for the study was granted by the Local Ethics Committee of Hitit University (decision no: 2022-28, dated: 09.01.2023).

Figure 1. a. An example application showing Biogel therapy for the treatment of pain in the shoulder area **b.** Biogel used in the research

The patients were randomly separated into two groups using randomisation software (https://www.randomizer.org) [16] as the treatment group and placebo group. With the categorisation and block randomisation method, sample size was equal between the groups and in distribution of age and gender (4 age group categories were formed of 18-29, 30-42, 42.53, and 54-65 years). The randomisation was performed by a biostatistician, using the computer-generated random numbers and the sealed envelope method.

People who met the trial inclusion criteria were invited to receive detailed written information before their written informed consent was obtained.

The study was designed to be single blind, and the patients were not aware of whether they were in the treatment or placebo group. For the patients in the treatment group, the non-interventional biogel was applied for 10 minutes. For the control group, a non-interventional placebo was applied for 10 mins (fluid not containing any substance). After the application of biogel and placebo, necessary medical treatments for pain were given to both patient groups.

After the data were obtained, routine treatments were

administered to all the patients. A record was made of patient demographic data, the region of the pain, and mean arterial pressure (MAP) values before and after the application. To determine the effect of the biogel treatment, all the patients in both groups were administered a Visual Analogue Scale (VAS) to evaluate pain severity, and the Nottingham Health Profile (NHP) before and after the applications. The data obtained were compared between and within the groups.

The NHP, developed by Hunt et al. (1980), is a scale which measures 6 areas of mobility, pain, energy, sleep, emotional reactions, and social isolation, with 38 true/false items, and contains an optional second section with items about sexual life, work, hobbies, and social relationships [17]. The NHP was adapted to Turkish by Küçükdevi et al. [18]. It is a simple, comprehensive scale, which is widely used, especially in Europe. In some conditions, the NHP can be more sensitive than the SF-36 to treatment-related changes, and compared to the SF-36, it contains a specific sleep scale and more pain items [19]. In this study, the general health profile of the patients was measured with the NHP and pain levels with the NHP- Pain (NHP-P).

Sample Size Estimations (Priori Power Analysis)

Before starting the research, power analysis was performed using Student's t-test to test the main hypothesis. In order to reach 90% power with α=0.05 error, it was decided to include a total of 88 patients, with a minimum of 44 in each group, as a result of the power analysis using the Cohen d=0.70 effect size, which was calculated by using the literature knowledge and expert opinion. However, considering that there would be loss of patients during the research process, the sample size was increased by 10% - 20%, and as a result of randomization, a total of 100 patients, with a minimum of 50 patients in each group, were included in the study (Figure 2).

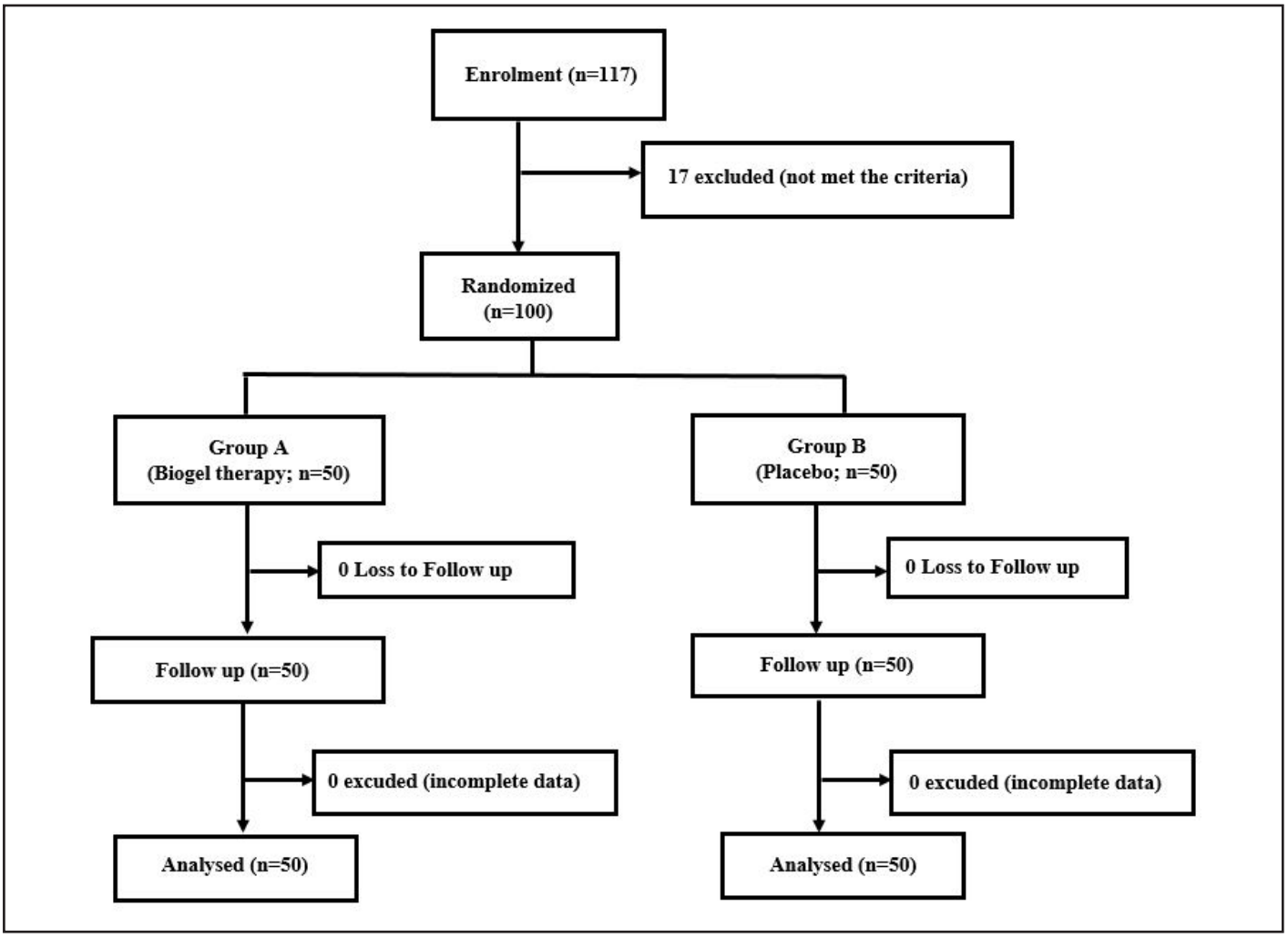

Figure 2. Consort Diagram of the block-randomized controlled trial of biogel therapy

Statistical Analysis

Statistical analyses of the data collected from the patients in our study were performed with the SPSS (Version 22.0, SPSS Inc., Chicago, IL, USA, program usage license: Hitit University) software. The normal distribution test of the data was tested with Shapiro-Wilks, Kolmogorov Smirnov and some graphical methods (Histogram and Q-Q plot). The assumption of homogeneity of variances was evaluated with the Levene test. Descriptive statistics of numerical variables were reported using mean±standard deviation or median (min-max) depending on the normal distribution of data. Descriptive statistics of categorical variables were reported using numbers (n) and percentages (%). Ratio comparisons between study groups were performed with either the Chi-square test or Fisher's exact test, depending on the sample sizes in the crosstab cells. Comparison of numerical data between research groups was performed with Student's t-test depending on parametric test assumptions. Comparison of numerical data between research groups before and after treatment was performed with the Paired t-test or Wilcoxon signed rank test, depending on parametric test assumptions. The statistical significance level was accepted as $P<0.05$.

RESULTS

The data of a total of 100 patients were analysed, as 50 in the biogel treatment group and 50 in the placebo group. The descriptive statistics of the sociodemographic characteristics of the patients are presented in Table 1. When the patients were assigned to the groups, randomisation was performed according to age and gender, so that gender distributions and mean ages were similar in the two groups (P=1.000, P=0.114, respectively) (Table 1). The mean age was determined as 45.28±7.13 years (range, 24-65 years) in the biogel treatment group and 47.67±7.85 years (range, 25-65 years) in the placebo group. The mean body mass index (BMI) was 29.18±4.64 (range, 18.99-36.57) in the biogel group and 28.29±4.36 (range, 19.43-40.70) in the placebo group, with no significant difference determined between the groups (P=0.325).

The distributions of education levels and marital status were similar in the two groups (P=0.773, P=0.373, respectively). The smoking status, presence of chronic disease, and history of surgery were found to be similar in both groups (P=0.488, P=0.202, P=0.295, respectively). The diagnoses of acute pain in the biogel group were arm pain in 14 patients (28%), neck pain in 6 (12%), low back pain in 20 (40%), and shoulder pain in 10 (20%). In the placebo group, the diagnoses of acute pain were arm pain in 11 patients (22%), neck pain in 9 (18%), low back pain in 18 (36%), and shoulder pain in 12 (24%). No significant difference was determined between the groups in respect of the diagnosis distribution rates (P=0.742).

In the first section of the NHP, the total mean points were 238.8±108.2 [median (min-max): 239 (60.3-497.6)] in the biogel group and 256.8±105.7 [median (min-max): 278 (32.1-400.7)] in the placebo group. In the second section of the NHP, the mean total points were 1.59±1.7 [median (min-max): 1 (0-7)] in the biogel group and 1.55±1.24 [median (min-max): 2 (0-5)] in the placebo group. No statistically significant difference was determined between the groups in respect of the points of the first and second sections of the NHP (P=0.517, P=0.781, respectively).

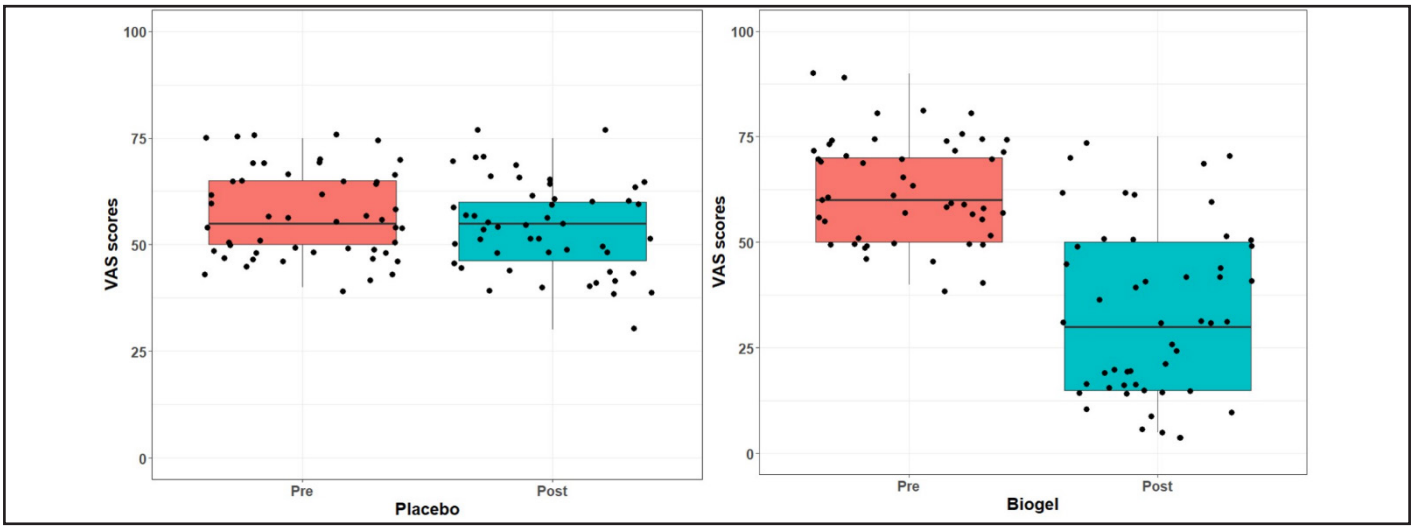

Figure 3. Line graph showing the change of VAS scores before and after biogel therapy

Table 1. Comparison of socio-demographic characteristics between research groups

		Biogel (n=50)	Placebo (n=50)	P values
Gender	Female	25 (50%)	25 (50%)	1.000[a]
	Male	25 (50%)	25 (50%)	
Age		45.28±7.13 (24-65)	47.67±7.85 (25-65)	0.114[b]
BMI		29.18±4.64 (18.99 – 36.57)	28.29±4.36 (19.43 – 40.70)	0.325[b]
Education	Primary school	5 (10%)	7 (14%)	0.773[a]
	Middle school	13 (26%)	10 (20%)	
	High school	20 (40%)	23 (46%)	
	University	12 (24%)	10 (20%)	
Marriage status	Married	38 (76%)	34 (68%)	0.373[a]
	Single	12 (24%)	16 (32%)	
Smoking	Yes	11 (22%)	14 (28%)	0.488[a]
	No	39 (78%)	36 (72%)	
Chronic disease	Yes	7 (14%)	12 (24%)	0.202[a]
	No	43 (86%)	38 (76%)	
Surgical history	Yes	15 (30%)	20 (40%)	0.295[a]
	No	35 (70%)	30 (60%)	
Diagnosis	Arm pain	14 (28%)	11 (22%)	0.742[a]
	Neck pain	6 (12%)	9 (18%)	
	Backache	20 (40%)	18 (36%)	
	Shoulder pain	10 (20%)	12 (24%)	

[a]Chi square test with n (%)

[b]Student's t-test with mean±standard deviation (min-max)

BMI: Body Mass Index

Table 2. Comparison of blood pressure, VAS, NHP-P values between research groups pre and post therapy

		Pre	Post	P values
Mean arterial pressure	**Biogel**	87.37±11.58	87.13±10.43	0.793[a]
	Placebo	95.45±8.65	93.36±8.36	0.467[a]
NHP-P	**Biogel**	59.4 (22.9 - 100)	26.01 (0 - 79.52)	**<0.001[b]**
	Placebo	59.4 (9.99-100)	50.44 (9.99 - 100)	0.104[b]
VAS	**Biogel**	62.50±12.46	32.70±20.05	**<0.001[a]**
	Placebo	57.00±10.35	54.30±10.49	0.157[a]

[a] Paired t-test with mean±standard deviation

[b] Wilcoxon signed rank test with median (min-max)

VAS: Visual Analog Scale

NHP-P: Nottingham Health Profile-Pain

The statistical findings of the within-group comparisons of MAP, VAS, and NHP-P values between the groups and within the groups before and after the application of biogel are shown in Table 2. No statistically significant difference was determined in the MAP values before and after treatment in both the biogel and placebo groups (P=0.793, P=0.467, respectively). In the biogel group, a statistically significant decrease was determined in the NHP-P values after treatment (26.01 [0-79.52]) compared to before treatment (59.4 [22.9-100]) (P<0.001). In the placebo group, no statistically significant difference was determined in the NHP-P values before and after treatment (P=0.104). In the biogel group, a statistically significant decrease was determined in the VAS values after treatment (32.70±20.05) compared to before treatment (62.50±12.46) (P<0.001). In the placebo group, no statistically significant difference was determined in the VAS values before and after treatment (P=0.157). The change in VAS scores from before to after the application of biogel is shown in graph form in Figure 3.

In the biogel treatment group, the patients were asked, "Have you benefitted from biogel?", and the responses were reported as definitely agree by 19 (38%) patients, agree by 21 (42%), undecided by 6 (12%), disagree by 3 (6%), and definitely disagree by 1 (2%).

DISCUSSION

Acute or chronic pain is one of the most common reasons for adults presenting for medical care. Many people worldwide experience pain. In a limited number of studies, the prevalence of chronic pain has been estimated to vary between 11% and 40%. According to a CDC report, it was estimated that in 2016, 1 in every 5 (20.4%) adults in the USA had chronic pain, and 8% had a chronic pain with a high impact, defined as restricting work activities on most days or every day for a period of 6 months [20, 21]. In a recent meta-analysis, the prevalence of chronic pain in developed countries was calculated as 18% (95% CI: 10-29%) [22]. A review by Gregory and McGowan (2016) reported that up to 84% of hospitalised adult patients reported acute pain and up to 36% severe pain (acute pain prevalence:37.7%-84%, severe pain prevalence: 9-36%) [23]. Acute and chronic pain have a great effect on the economy of a country. Therefore, medical and traditional treatment of pain has long been one of the most researched subjects. Since the earliest recorded times, doctors and other healing experts have applied many traditional and complementary treatments to prevent, alleviate, or cure pain [24].

In the current study, it was investigated whether or not biogel had an effect in the treatment of acute pain in the upper extremity and/or trunk. The findings demonstrated that the majoority of patients stated that they benefitted from the biogel treatment applied, and there was a significant decrease in the VAS and NHP-P scores in the biogel treatment group compared to the placebo group. No study could be found in lierature that has examined the effect on pain of a biogel directly obtained with these elements. However, there are several studies in literature about the effect of biomagnetic energy on pain.

Magnets and magnetic therapy have been used for hundreds of years in the treatment of different types of pain. Magnetic therapy is applied with static magnets which produce a therapeutic magnetic field for pain relief and healing in various problems. There are natural magnetic and electrical fields in the body, and there is a small amount of magnetic energy in all the molecules of these. The thinking behind magnetic field therapy is that some problems occur because the magnetic fields are unbalanced [25]. Ions such as calcium and potassium assist cell signal transmission and magnets have been reported to change the behaviour of these ions [26].

Conflicting results have been obtained in literature about the effect of magnetic therapy. Pawluk W. (1998) reported that magnetic therapy provided improvements in muscle strains and sprains and joint pain [25]. A systematic review published in 2020 included 21 studies (1101 patients) which focussed on electromagnetic therapy for musculoskeletal pain conditions and reported that electromagnetic therapy reduced pain and improved functions in patients with different musculoskeletal system diseases. The 21 studies examined comprised 8 which focussed on knee osteoarthritis, 2 on shoulder impingement syndrome, 1 on chronic mechanical neck pain, 4 on low back pain, 3 on fibromyalgia, 1 on patellofemoral knee pain, 1 on plantar fasciitis (heel pain), and 1 on hand osteoarthritis. This systematic review showed that electromagnetic field therapy alleviated pain and improved function in patients with various painful musculoskeletal system diseases. Studies which have analysed electromagnetic field therapy have stated that it is well tolerated without any negative side-effects reported. Thus, it has been concluded that electromagnetic therapy may be a useful component during treatment with drugs for chronic and acute pain in musculoskeletal diseases [27].

Magnetic therapy using pulsating electromagnetic field (PEMF)

treatment has been approved by the FDA for certain conditions, including postoperative oedema and pain in superficial soft tissues and the treatment of fractures that have not healed with standard medical treatment. The FDA has also approved a certain type of magnetic therapy known as transcranial magnetic stimulation, using magnetic fields to stimulate brain cells for severe migraine, depression, and obsessive-compulsive disorder. The use of FDA-confirmed magnetic therapy for these conditions is accepted as traditional medicine [28]. It has been reported in literature that PEMF has analgesic and anti-nociceptive efficacy similar to the opioid analgesic effect, although the biological and biochemical mechanism of magnetic therapy on pain is not fully understood [29]. Some researchers have shown that short-term exposure to electromagnetic fields is effective on inflammatory cellular and neurological processes such as cortical activation and inhibition models and various neurotransmitter activities [30]. In a systematic examination and meta-analysis to evaluate the clinical evidence obtained from randomised experiments of static magnets for the treatment of pain, Pittler et al. (2007) concluded that despite the widespread use of static magnets to eliminate pain, there was no evidence to suggest that static magnets could be effective in pain relief [4].

Although there is no definitive consensus in literature about the effect of biomagnetic energy on pain treatment, the positive effects of magnetic energy have been reported in many studies. As a result of the current randomised, controlled clinical study of patients with pain in the upper extremity region or trunk, the biogel treatment was determined to have made a statistically significant improvement in both the VAS scores and the NHP-P scores. In addition, there was no statistically significant change in the MAP values.

Limitations

A limitation of this study could be said to be that only patients with acute pain were included. An investigation of the effect of biogel on patients with chronic pain could be supportive of the current study. Another limitation was that the effect of biogel was only investigated on pain in the upper extremity and/or trunk. It can be recommended that further studies are planned to evaluate the efficacy of biogel on acute and chronic pain in more specific regions with larger and different patient groups.

CONCLUSIONS

The results of this study demonstrated that biogel can be accepted as a complementary medicine method in the treatment of acute pain in the upper extremity and/or trunk. As a result of the burden of acute and chronic pain and the associated suffering, there are great costs to society. These types of complementary medicine applications focussed on pain treatment can reduce the disease burden and can probably reduce costs. It can be predicted that these types of biogels will become more widely used in the future due to the ease-of use, rapid effect, and reduction in costs of public services. The optimal treatment for acute pain is a function of the desire of an individual to choose between the side-effects of treatment and pain control. The results of this study suggest that T&CM applications with no side-effects should be supported in addition to evidence-based medicine.

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Conflict of Interest: The authors declare no conflicts of interest.

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

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Ethical Approval: Approval for the study was granted by the Local Ethics Committee of Hitit University (decision no: 2022-28, dated: 09.01.2023).

Author Contributions: Conception: T, K; E, D; T, A - Design: S, K - Supervision: E, D - Fundings: T, K -Materials: T, A; T, K - Data Collection and/or Processing: T, A; T, K - Analysis and/or Interpretation: E, D; S, K - Literature: T, K; E, D; S, K - Review: T, A; T, K - Writing: E, D; S, K - Critical Review: S, K; T, A.

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Original Research

Blocking the Apelin Receptor (APJ) Attenuates TNBS-Induced Colitis in Rats

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ABSTRACT

Objective: The apelinergic system, consisting of apelin, ELABELA, and the apelin receptor (APJ), has a wide range of roles in physiological and pathophysiological processes in tissues. The effects of increased apelin and APJ as an indicator of damage in inflammatory conditions or as a compensatory mechanism are not fully clear in inflammatory bowel disease (IBD). This study was designed to assess the role of APJ in 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis model.

Methods: Colitis in adult male Wistar rats were induced by intrarectally administered TNBS (30 mg b.w. in 50% ethanol). While the control group was treated with only saline to the colon, the TNBS+F13A and F13A groups received the APJ antagonist F13A (30 µg/kg/day, i.v.) for 3 days, starting immediately after TNBS or saline administration, respectively.

Results: A decrease in body weight and an increase in colon weight/length ratio and stool consistency score were observed in the TNBS group. TNBS caused an increase in the myeloperoxidase (MPO) activity and the number of proinflammatory cytokines (TNF-α, IL-1β, and IL-6), as well as apelin production, leading to mucosal ulceration, necrosis, and submucosal edema in the colon. While F13A administration to the control did not cause any change in the colon, F13A administration immediately after TNBS greatly reduced the effects of TNBS.

Conclusion: APJ is involved in the development of damage in colitis induced by TNBS. F13A reduces the level of damage, inflammatory cell infiltration, and MPO enzyme activity. APJ may be a therapeutic target in IBD.

Keywords: APJ, F13A, TNBS, ulcerative colitis, rat

INTRODUCTION

Inflammatory bowel disease (IBD), a chronic global health problem, is a disease that reduces the quality of life of patients and has a medical cost burden due to its acute and chronic complications [1]. Genetic and environmental factors, poor host defense against microorganisms, or inappropriate immune responses may cause IBD [2, 3]. The disease is characterized by mucosal inflammation in the digestive system, diarrhea, hematochezia, weight loss, and abdominal pain, and has a rather complex etiology [4]. Inflammation, which is very important for the modulation of tissue repair, is a complex process in which various factors and cells play a role. However, uncontrolled inflammation directly contributes to the development of various chronic diseases such as IBD.

IBD mainly includes Crohn's disease (CD) and ulcerative colitis (UC) [2, 5]. CD, which can affect all segments of the gastrointestinal (GI) tract, is characterized by transmural inflammation. UC occurs in the colon and mostly the rectum and is classically a superficial mucosal disease. However, when the disease becomes severe, deep and transmural ulcerations may occur. Although the clinical manifestations of UC and CD are different, dysregulation of the immune response and chronic inflammation in the GI system play a role in the pathogenesis of both subtypes [2, 3]. Treatment strategies for IBD include reducing inflammation and microbial load, suppressing inappropriate immune responses, and neutralizing inflammatory mediators [6].

The apelin receptor (APJ), isolated in 1993, is a G protein-coupled receptor [7]. APJ consists of 380 amino acids and its gene is in the chromosome 11 (q12). The APJ, which has complex effects because it can activate different G proteins and is expressed in all the tissues, has a high sequence homology of approximately 54% with the angiotensin II (Ang II) type 1 receptor (AT1R), the dominant receptor of Ang II [7, 8].

Apelin, the specific ligand of APJ is broken down into bioactive peptides such as apelin-12, -13, -17, and -36 by various enzymes shortly after it is produced as preproapelin with 77 amino acids [9]. Apelin exerts its effects only by activating APJ and has various physiological and pathophysiological roles such as modulation of the immune system, eating and drinking behavior, cardiac contractility, blood pressure, cell proliferation and apoptosis [8]. Recent clinical and experimental studies suggest that the apelin/APJ system modulates inflammation and oxidative processes and thus is involved in the regulation of various diseases [10, 11]. It is known that apelin expression increases in various tissues during the inflammatory process. Although increased colonic apelin production has been demonstrated in patients with IBD and an animal model of sodium dextran sulfate (DSS)-induced colitis, the role of apelin in this process is not fully understood [12]. The regulatory effects of apelin on intestinal physiology are mostly related to motility, absorption, and enzyme secretion, and the number of studies to elucidate its role in IBD is very limited [13]. Pro-inflammatory cytokines, such as IL-6 and IFN-γ, play critical roles in the pathogenesis of IBD [14, 15]. These cytokines are known to increase apelin expression in the ileum of rats via the Jak/STAT pathway [16]. Findings from studies suggest that the apelin/APJ system plays an important role in IBD. In this study, the role of apelin in the inflammatory process in the colon was investigated in a TNBS-induced colitis model.

Main Points;

- Apelin receptor (APJ) is involved in the development of damage in colitis.
- Trinitrobenzene sulfonic acid (TNBS) application enhanced the level of apelin in colon.
- The APJ blocker F13A reduced the level of damage, inflammatory cell infiltration and MPO enzyme activity in rats with TNBS-induced colitis.

MATERIALS AND METHODS

Experimental Animals

In the experiments, 72 male, adult Wistar rats, weighing 200-250 g, were used. The Wistar rats were obtained from the Akdeniz University Experimental Animals Application and Research Center. All animal procedures were following the European Community Council Directive of 24 November 1986. Animals were treated in accordance with the Guide for the Care and Use of Laboratory Animals and were approved by the Akdeniz University Animal Experiments Local Ethics Committee (approval code: 1174/2020.08.001). The rats were allowed to adapt to the laboratory environment for at least 1 week before the experiment. They were housed in a room with a constant temperature (22 ± 1°C) and 12:12 hour light/dark cycle and fed with standard laboratory chow and tap water.

Drugs and Chemicals

TNBS (Picrylsulfonic acid, cat. no. P2297), hexadecyltrimethylammonium bromide (HTAB, H-5882), and O-dianisidine dihydrochloride (cat. no. D9154) from Sigma (St. Louis, Missouri, USA); phosphate-buffered saline (PBS) tablets (cat. no. 3002) from Invitrogen (Carlsbad, California, USA) and ethanol (cat. no. 100983), formaldehyde (cat. no. 818708), hydrogen peroxide (H2O2, cat. no. 107298), and sodium chloride (cat. no. 106406) were purchased from Merck (Darmstadt, Germany). F13A was synthesized by GenScript (Piscataway, New Jersey, USA).

Experimental Procedure

The animals were randomly divided into groups as control, F13A, TNBS, and TNBS+F13A (n=18 in each one). 6 animals in each group were used for histological parameters and 12

animals were used for other parameters. The weights of the rats were measured both at the beginning and at the end of the experiments. Experimental procedures are summarized in Figure 1. The rats were anesthetized with ether after fasting for 24 hours.

Control group: 500 µl of saline was intrarectally given into 8 cm of (well-formed pellet); grade 1, pasty consistency (not adherent to the anus); grade 2, pasty consistency (adherent to the anus); grade 3, pasty the colon from the anus with the aid of a catheter (diameter 2 mm) attached to a syringe (1 ml).

F13A group: 500 µl of saline was given intrarectally and then apelin receptor antagonist F13A (30 µg/kg/day in saline, i.v.) was administered for three days (saline was given only on day 0, while F13A was given on days 0, 1, and 2).

TNBS group: TNBS (30 mg/kg b.w.) was given intrarectally [17]. TNBS was diluted 1:1 v/v with 50% v/v ethanol solution to give a total volume of 500 µl.

TNBS+F13A group: F13A (30 µg/kg/day in saline, i.v.) was applied for three days following TNBS injection (TNBS was given only on day 0, while F13A was given on days 0, 1, and 2) [18].

Three days after TNBS or saline administration, the stool consistency in the subjects was evaluated at 9 am according to the following criteria (on day 3): grade 0, normal consistency (adherent to the anus, containing blood); grade 4, diarrhea (watery stools sticking to the anus, containing blood) [19]. Then, the subjects under anesthesia (10 mg/kg xylazine and 90 mg/kg ketamine, i.p.) were euthanized by draining blood from the isolated abdominal aorta. The total colons of the subjects were removed, and the lesion areas were evaluated macroscopically. After the colon weight and length were determined, the weight/length ratio, an indirect indicator of inflammation, was calculated [20]. Distal colon tissues were divided into pieces of appropriate size according to the number of parameters and stored at -80°C for biochemical analysis (n=12 in each group) or placed into 10% formalin for histological staining studies (n=6 in each group).

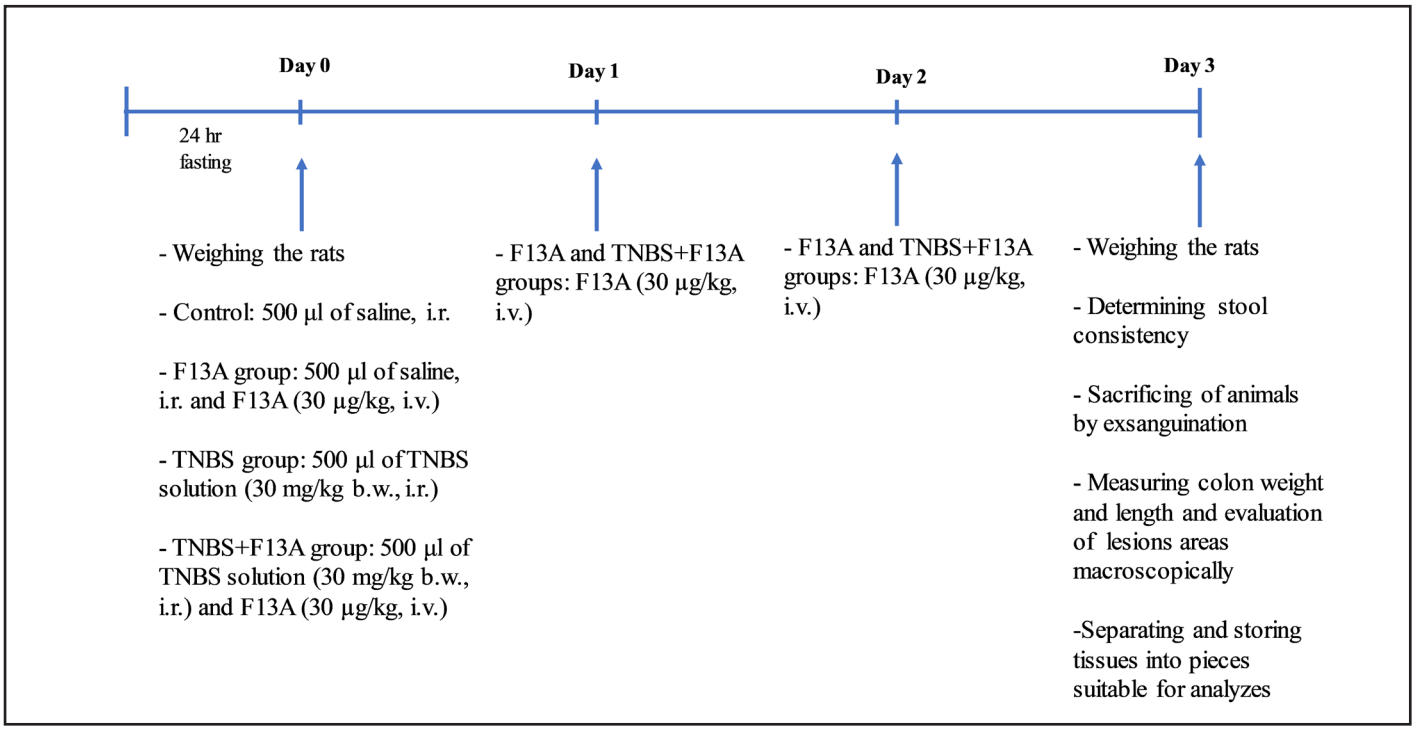

Figure 1. Summary of experimental procedures. On day 0, 500 µl of saline was administered intrarectally (i.r.) to the control group. TNBS (30 mg/kg in 500 µl, 50% ethanol v/v) was administered to TNBS and TNBS+F13A groups. F13A (30 µg/kg/day) was intravenously given to the F13A group after saline administration and to the TNBS+F13A group after TNBS administration. F13A applications were repeated on days 1 and 2. Rats were euthanized on day 3 and colons were removed for various testing (n=18 in the groups).

Macroscopic Evaluation of Damage

After the subjects' colons were opened longitudinally from the mesenteric margin and the mucosa was exposed, it was washed with physiological saline to clear the luminal content. Then damaged areas on the mucosa were examined macroscopically using a scoring system [21]. Morphological damage scoring criteria are as follows grade 0, no ulcer, no inflammation; grade 1, local hyperemia without ulcers; grade 2, hyperemia and ulcers without inflammation; grade 3, hyperemia and ulcers with inflammation at one side; grade 4, ulcers and/or inflammation in two or more areas; grade 5, ulcers and inflammation extending more than 1 cm in 2 or more areas; grade 6-10 if the ulcer size was larger than 2 cm, the score was increased by "1" for each additional cm.

Histopathology

For the microscopic evaluations, samples taken from the colon were fixed in 10% formalin fixative. Then the tissues were embedded in paraffin and 5 μm thick sections were taken from the blocks. After hematoxylin-eosin (H-E) staining of the sections, changes in the colon tissue were examined by light microscopy. In addition, based on previous studies, inflammatory cell infiltration, submucosal edema, mucosal necrosis, and mucosal ulceration were semi-quantified based on a scoring system [22]. The following criteria were used for scoring: Based on cell infiltration, submucosal edema, and mucosal necrosis as grade 0, no damage; grade 1, limited; and grade 2, diffuse; for mucosal ulceration grade 0, no ulceration; and grade 1, ulceration is present.

Enzyme Immunoassays

The homogenates of the colons obtained from the experimental groups were tested for apelin and the proinflammatory cytokines (TNF-α, IL-1β, and IL-6) using enzyme immunoassay kits (Bioassay Technology Laboratory, cat. numbers respectively: E1026Ra E0764Ra and E0119Ra, E0135Ra).

Briefly, the colon tissues were rinsed in ice-cold PBS (pH 7.4) and weighed before homogenization. Then tissues were homogenized in PBS (tissue (g)/PBS (mL)=1:9) with an ultrasonic cell disrupter on ice. The homogenates were centrifuged for 15 minutes at 12 000 rpm at 4°C. The analysis of the parameters in supernatants was carried out according to the instructions in the kit. After measurement of the protein concentrations with the Bradford method, supernatants were assayed in well plates and the absorbance of the samples was determined at 450 nm. The cytokine and apelin levels were expressed as nanograms or picograms per milligram of protein.

MPO Enzyme Activity

MPO enzyme activity was measured as an indicator of neutrophil infiltration into the colon tissues [23]. Briefly, the colon tissues were homogenized in 50 mM PBS, pH 6.0, containing 0.5% HTAB (1 ml solution per 50 mg wet tissue). Then, homogenates were centrifuged at 12 000 g for 15 min (+4°C). One unit of enzyme activity in the clear supernatants was expressed as the quantity of product formed in 3 minutes. MPO enzyme oxidizes O-dianisidine dihydrochloride in the presence of H2O2 and absorbance of the produced compound is measured at 460 nm. After the measurement of protein concentration in the samples, MPO activity was determined by mixing 100 μl of the sample with 2.9 ml of reaction buffer containing 0.167 mg/mL O-dianisidine dihydrochloride and 0.0005% H2O2 in 50 mM PBS, pH 6.0. The change in absorbance for 3 min was measured at 37°C and enzyme activity was expressed as U/mg protein.

Statistical Analysis

The number of subjects was calculated according to the power analysis performed in the light of the information in the literature, assuming 95% confidence (1-α) interval, 95% test power (1-β) and effect size d = 0.5. The data were analyzed with SPSS program version 23.0 software (Chicago, Illinois, USA). The suitability of the data to the normal distribution was evaluated with the Kolmogorov-Smirnov and Shapiro-Wilk tests. One-way ANOVA followed by Tukey's multiple comparisons test was used to compare the percent change in body weight, stool consistency score, colon weight/length ratio (g/cm), macroscopic injury score, and histopathological changes in the groups. Kruskal-Wallis and Mann-Whitney U tests were used to compare apelin, proinflammatory cytokines, and MPO activity in the colon of the groups. Values were expressed as the mean ± standard error of the mean (SEM). A value of $p<0.05$ was considered statistically significant.

RESULTS

Body Weight, Stool Consistency Score, Colon Weight/Length Ratio, and Macroscopic Colonic Injury

As seen in Figure 2A, the percentage change in body weight was found to be 0.4 in the control, 2.1 in the F13A group, 17.7 in the TNBS group, and 10.2 in the TNBS+F13A group. Compared to the control, the body weight loss was significantly increased in the TNBS group ($p<0.001$). However, administration of F13A

following TNBS significantly prevented this TNBS-related loss in body weight ($p<0.001$). It was observed that diarrhea developed in the TNBS group and there was a significant increase in the stool consistency score compared to the control ($p<0.001$, Figure 2B). F13A reduced the enhancing effect of TNBS on stool consistency score.

The ratio of colon weight to length (g/cm) was 0.79 ± 0.04 g/cm in the control group, 0.78 ± 0.02 g/cm in the F13A group, 1.57 ± 0.08 g/cm in the TNBS group, and 1.13 ± 0.07 g/cm in the TNBS+F13A group (Figure 2C and 2D). Compared with the control group, in the TNBS-induced colitis group, colon weight increased about inflammation, and therefore the weight/length ratio significantly increased ($p<0.001$). Administration of F13A following TNBS caused the weight/length ratio to decrease ($p<0.001$).

When the degree of damage in the colon wall of the subjects was evaluated macroscopically, no damage was observed in the control group and F13A groups (Figure 2C and 2E). While significant damage was detected in the colon tissue of rats treated with TNBS ($p<0.001$), these lesions were significantly reduced by F13A injection following TNBS administration ($p<0.001$).

Histopathology

In the microscopic examination of the colons of the control group rats, crypts with the normal histological structure were seen (Figure 3A, 3A1, and Table 1). Similarly, it was observed that the normal histological structure was preserved in the F13A group (Figure 3B, 3B1 and Table 1). In the TNBS group (Figure 3C, 3C1, and Table 1), the borders of the histological layers became indistinguishable with severe damage/deformation in the crypts, lesions in the epithelium (mucosal necrosis), intense mucosal/submucosal inflammatory cell infiltration and increased submucosal edema were determined. On the other hand, in the TNBS+F13A group, submucosal edema decreased, and some improvements were observed in the crypts and layers. In the TNBS+F13A group, intense mucosal/submucosal inflammatory cell infiltration and epithelial necrosis were milder than in the TNBS group (Figure 3D, 3D1, and Table 1).

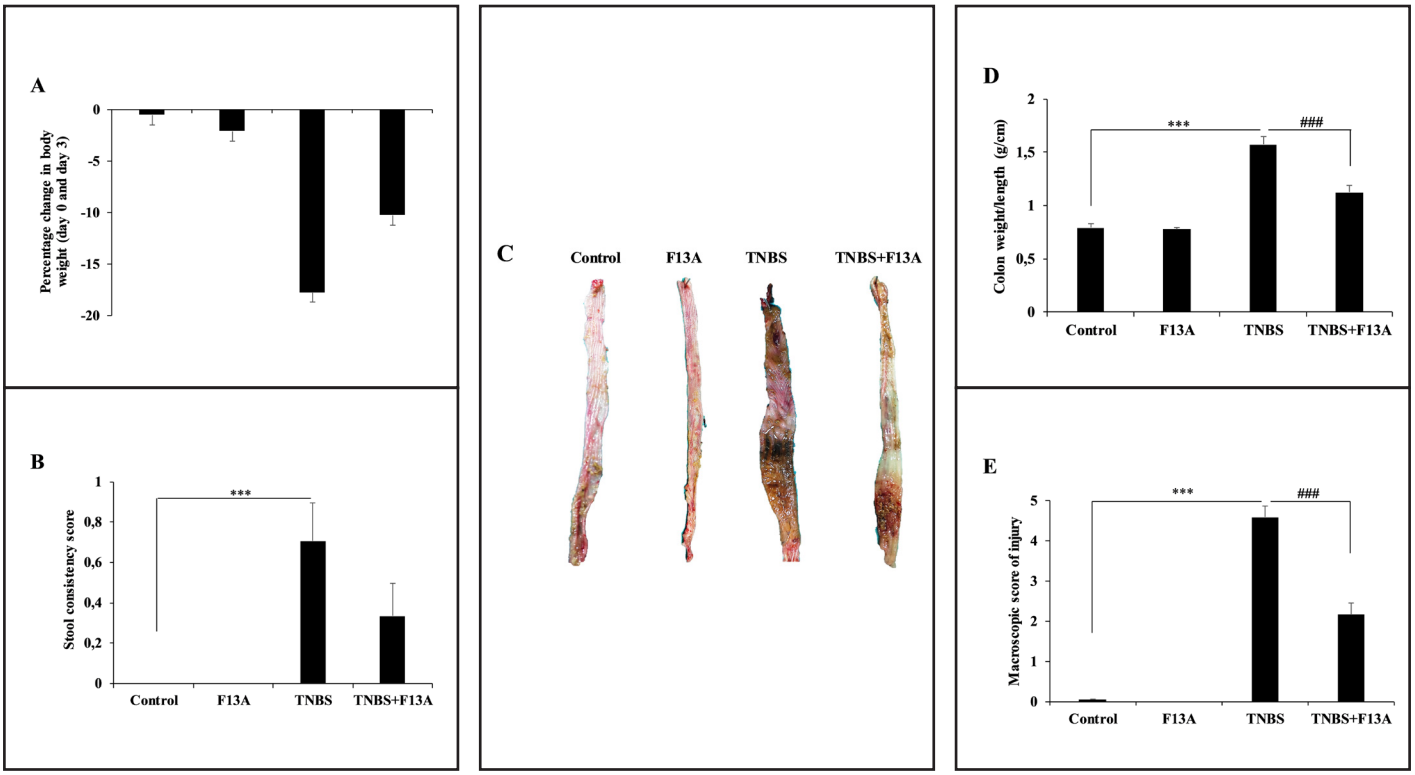

Figure 2. Effect of TNBS and F13A on the percentage change in body weight, stool consistency score, the colon weight/length ratio (g/cm), and macroscopic colonic injury score. The percent change in body weight from day 0 to day 3. B The consistency of the stool was calculated according to a scoring system on day 3. C The representative colon images. D The ratio of the total weight to length of the colons of the groups and E The macroscopic injury calculated according to a scoring system on day 3. Data was presented as mean ± S.E.M (n=18 in groups). ***P<0.001 vs. control group. ###P<0.001 vs. TNBS-induced colitis group.

Table 1. Semi-quantitative scoring of histopathological changes in the colon.

	Control	F13A	TNBS	TNBS±F13A
Focal mucosal ulceration	0	0	1; 1±0*	0.5; 0,5±1#
Inflammatory cells infiltration in mucosa	0.3; 0±1	0.5; 0.5±1	2; 2±0*	1.2; 1±2#
Submucosal edema	0.2; 0±1	0.5; 0.5±1	2; 2±0*	1; 1±2#
Mucosal necrosis	0	0.2; 0±1	2; 2±0*	1.2; 1±2#

Data are expressed as mean; median ± range and analyzed by One-Way ANOVA followed by Tukey's multiple comparisons test which was used for comparing histological results between different selected groups (* P < 0.05 vs. control group, # P <0.05 vs. TNBS group, n=6 in each group).

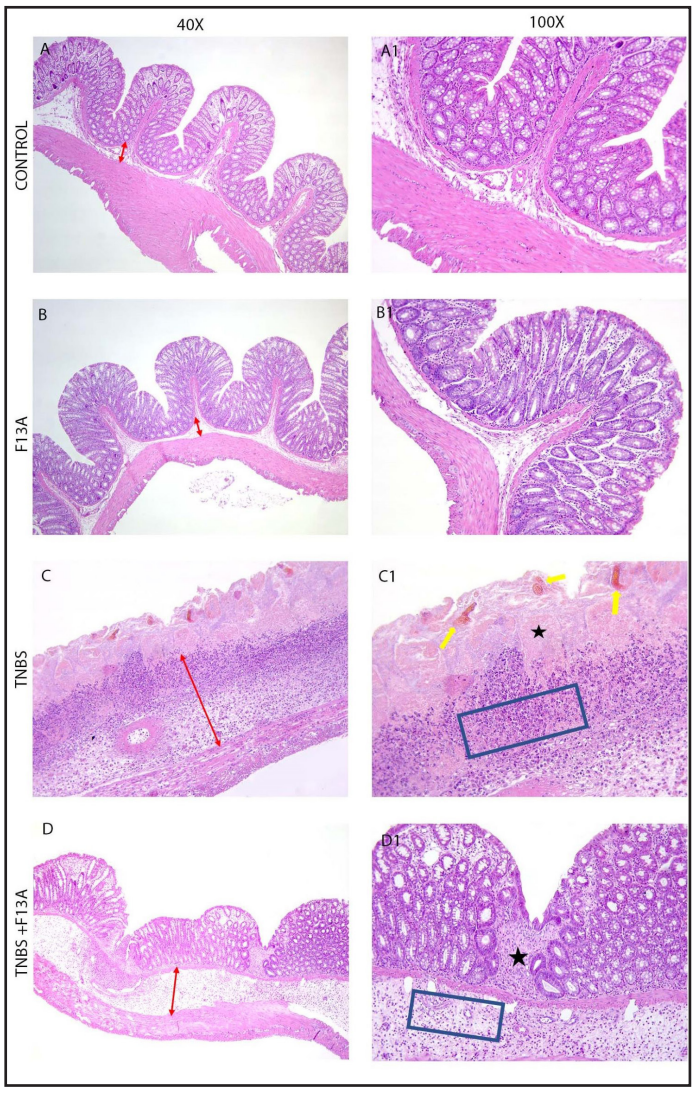

Figure 3. Representative H-E staining images of colon sections (40X and 100X) (n=6). While the tissue sections of the control and F13A groups exhibited a normal tissue appearance (A-A1, B-B1), the tissue sections in the TNBS group had mostly submucosal edema (red arrow), inflammatory cell infiltration (rectangular frame), necrotic areas (asterisk) and ulceration (yellow arrow) was observed (C-C1). Colon tissues of the TNBS+F13A groups were similar to those of the control groups. (D-D1).

Apelin

Compared to the control group (2.27 ± 0.72 ng/mg protein), blocking the APJ receptor with F13A did not significantly affect the apelin level in colon tissue (1.54 ± 0.12 ng/mg protein) (Figure 4). The rats treated with TNBS exhibited a significant increase in apelin level (16.43 ± 2.02 ng/mg protein, p<0.001). But F13A administered following the application of TNBS prevented the increase in colonic apelin level due to TNBS (2.51 ± 0.25 ng/mg protein, p<0.001).

Proinflammatory Cytokines

As observed in Figure 6. in the control group, the TNF-α (Figure 5A), IL-1β (Figure 5B), and IL-6 (Figure 5C), the important proinflammatory cytokines, were 30.12 ± 2.85 ng/mg protein, 313.85 ± 23.40 pg/mg protein and 1.36 ± 0.18 ng/mg protein, respectively. F13A treatment did not cause any change in these parameters (42.70 ± 5.64 ng/mg, 224.73 ± 12.12 pg/mg, and 2.24 ± 0.23 ng/mg, respectively). In rats treated with TNBS, a significant increase in TNF-α levels (329.15 ± 32.59 ng/mg protein, p<0.001), IL-1β (2222.11 ± 225.12 pg/mg protein, p<0.001) and IL-6 (14.08 ± 1.21 ng/mg protein, p<0.001). However, F13A administration immediately after TNBS treatment decreased the changes due to TNBS in these parameters (35.93 ± 4.61 ng/mg protein, p<0.001; 289.04 ± 16.59 pg/mg protein, p<0.001; 1.98 ± 0.28 ng/mg protein, p<0.001, respectively).

MPO Enzyme Activity

As seen in Figure 6, MPO activity, which is an indicator of neutrophil infiltration into the tissue, was found to be 80.62 ± 9.08 U/mg protein in the colon tissue in the control group, while it was 68.61 ± 6.97 U/mg protein in the F13A group. The rats treated with TNBS exhibited a significant increase in MPO activity (148 ± 16.88 U/mg protein, p<0.05). However, blocking the APJ receptor immediately after TNBS treatment decreased the changes due to TNBS (87.92 ± 8.74 U/mg protein, p<0.05).

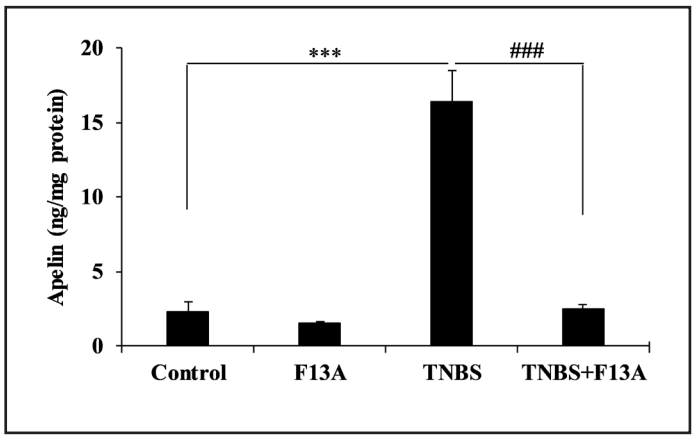

Figure 4. Effect of TNBS-induced colitis on the apelin level in the colon. Data was presented as mean ± S.E.M (n=12 in groups). ***P<0.001 vs. control group. ###P<0.001 vs. TNBS-induced colitis group.

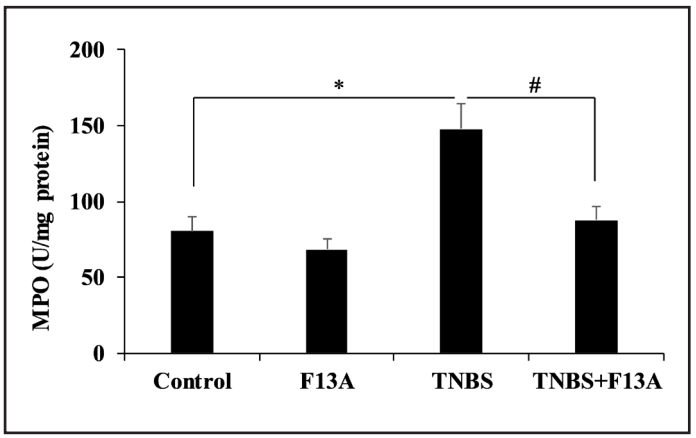

Figure 6. Effect of TNBS and F13A on the MPO enzyme activity in colon tissue. Data was presented as mean ± S.E.M (n=12 in groups). *P<0.05 vs. control group. #P<0.05 vs. TNBS-induced colitis group.

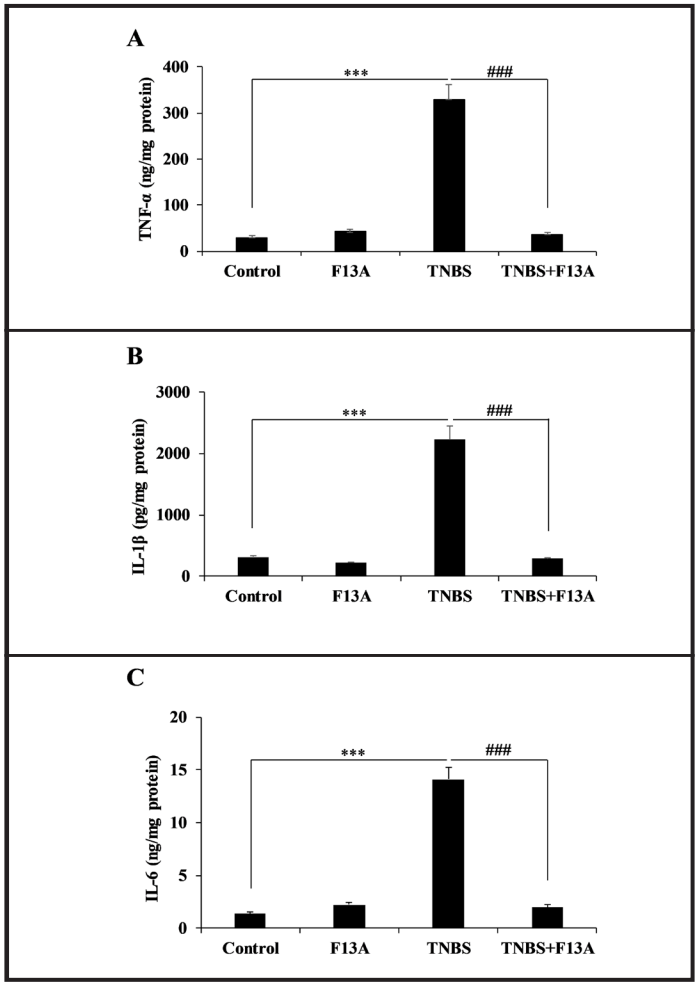

Figure 5. Effect of TNBS and F13A on the proinflammatory cytokines in colon tissue. A TNF-α level; B IL1β level; and C IL-6 level. Data was presented as mean ± S.E.M (n=12 in groups). ***P<0.001 vs. control group. ###P<0.001 vs. TNBS-induced colitis group.

DISCUSSION

The role of the apelinergic system, which is known to modulate inflammatory processes and oxidative stress, in the pathophysiology of IBD has not been clarified to date [10, 11]. In our study, by blocking APJ with F13A, the effects of apelin, whose expression is increased during TNBS-induced colitis, were prevented. Thus, the role of endogenous apelin in the pathophysiology of IBD was shown for the first time. Our major findings were as follows: (i) Blocking the APJ prevented macroscopic and microscopic injury induced by TNBS in the colon. (ii) Blocking the APJ suppressed inflammation by decreasing MPO activity and the proinflammatory cytokines. (iii) Blocking the APJ ameliorated TNBS-induced changes in body weight and colon weight/length ratio.

Immunological, genetic, and environmental factors play a role in the pathogenesis of IBD. It has been thought that it may occur because of excessive activation of the immune system and abnormal interactions. In this study, colitis mimicking UC was induced by intrarectal administration of TNBS dissolved in diluted ethanol [24]. Ethanol acts as a barrier breaker and increases intestinal permeability. TNBS passes into the subepithelial space, causing a rabid immune response [24]. Immune cells that migrate to the site of inflammation release cytokines [25]. Overproduction of cytokines damages tight junctions and causes tissue injury. Therefore, bacterial penetration into the submucosa is increased [26]. In this study, we focused on stool consistency, weight loss, the ratio of colon weight to length, macroscopic and microscopic damage levels in the colon, TNF-α, IL-6, and IL-1β levels, and MPO activity as

indicators of inflammation. Weight loss accompanying diarrhea was observed in the TNBS group. In addition, it was observed that TNBS caused an increase in colonic apelin production, apart from mucosal damage, proinflammatory cytokines, and MPO activity. Similar findings related to inflammation have been reported in previous studies in which colitis was induced by TNBS [27, 28]. Inflammation-related findings approached control values with the administration of F13A, thus our data suggest that F13A, an APJ antagonist, may be a therapeutic approach for IBD.

The apelin/APJ system has important functions for physiological and pathophysiological mechanisms in humans and animals, and these roles vary according to the bioactive peptides of apelin, localization of APJ expression, and activated different signaling pathways [13, 29]. In the GI tract of young rats, apelin and APJ mRNA levels are highest at birth and progressively decline into adulthood. The APJ is expressed on epithelial cells, goblet cells, and smooth muscle cells, as well as neurons of the enteric nervous system [30, 31]. It is also known that apelin mRNA expression decreases in the GI tract from the stomach to the colon [32]. In the present study, it was observed that the production of apelin, whose expression is low in the colon under normal conditions, was high in TNBS-induced colitis. While proinflammatory factors can affect the expression of apelin, conversely, apelin can affect the level of proinflammatory factors [10]. It is known that apelin expression is significantly increased in various pathological processes such as liver damage, fibrosis, cirrhosis, non-alcoholic fatty liver disease, pancreatitis, and gastroesophageal and hepatocellular cancers [13]. In general, apelin, which has its receptor on immune cells, reduces inflammation by suppressing immune cell migration and secretion of proinflammatory cytokines and inhibits apoptosis by modulating caspases [10, 11, 29, 33]. In our previous studies, we observed that apelin-13 had a protective effect against ischemia reperfusion injury in the gastric mucosa by activating vago-vagal reflex mechanisms [34, 35]. Also, it was observed that blocking apelin receptors after ischemia delays the healing process in stomach [36]. Similarly in the colon, the application of synthetic pyro-apelin-13 for 3 days in the recovery phase after colitis was induced with DSS in mice, increased cell proliferation [12]. In another study, it was determined that chronic (4-week) application of apelin-13 diminished the disease activity index, and inflammatory score, and induced lymph angiogenesis in mice that spontaneously developed colitis [37]. In these last two studies, the effects of exogenously applied apelin after the development of colitis were examined. However, in our present study, the effect of endogenous apelin during the inflammatory process was abolished by blocking the APJ. Thus, it was shown for the first time that the pathology caused by TNBS in the colon tissue was reduced when the effects of APJ were inhibited by F13A.

The apelinergic system can regulate inflammation in tissues by different mechanisms. Administration of Fc-apelin, which has a long half-life, reduces lipopolysaccharide-mediated liver damage by suppressing macrophage infiltration, oxidative stress, and apoptosis [38]. Although the activation of APJ is thought to reduce oxidative stress inflammation and apoptosis, some studies say the opposite [10, 39]. Apelin stimulates the expression of NADPH oxidase through the APJ and contributes to atherosclerosis by inducing proliferation dependent on oxidative stress in vascular smooth muscle cells [40]. Jo2-induced damage and apoptotic changes in the liver were less observed in APJ-/- mice [41]. Plasma apelin levels and hepatic APJ expression were increased in cirrhotic rats [42]. It was observed that hepatic fibrosis decreased with F13A application. These studies suggest that the apelinergic system exerts regulatory effects through different mechanisms in various tissues in different situations.
Intestinal permeability plays a critical role in the development of digestive system diseases such as IBS and IBD [43, 44]. Disturbances in the intestinal barrier cause bacterial translocation, impaired absorption of nutrients and motility, and visceral hypersensitivity [26, 45]. It was reported that accelerated colonic transit caused by acute stress is reduced by intraperitoneally administered APJ antagonists [46]. It is also thought that APJ contributes to corticotrophin-releasing factor (CRF)-mediated changes in colonic motility. Furthermore, the APJ agonist activates the CRF-TLR4-proinflammatory cytokine signal, causing visceral hypersensitivity and increased epithelial permeability in the colon [47]. In the GI tract, CRF signaling regulates not only motility, but also permeability, inflammation, and immunity [48]. CRF signaling which may also play a role in the regulatory effect of the apelinergic system in IBD, was not evaluated in the present study. Moreover, it was reported that LPS or CRF activates the AT1 system to cause colonic hyperpermeability and injury [47]. AT1 receptor expression increases in the colon with colitis [49]. AT1 signaling activates NF-κB and induces inflammation [50]. There has been a report suggesting the existence of signal interaction between apelin-APJ and the AT1 system. Non-activated APJ via apelin may suppress AT1 receptor activation [51]. In this regard, the effects

due to the blockade of APJ may also be due to the suppression of AT1 signaling. The relationship between CRF and AT1R signals during APJ blockade was not evaluated in this study. It would be useful to reveal these mechanisms in elucidating the role of APJ during colitis.

CONCLUSIONS

In conclusion, in this study, it was shown that blocking the APJ in a TNBS-induced colitis model alleviated inflammation, edema, and infiltration of inflammatory cells, and decreased cytokine and damage levels (Figure 7). Our findings have shown that the apelin/APJ system contributes to the pathophysiology of IBD, further studies are needed to explain how the apelinergic system acts. Clarifying the signaling mechanisms between the apelinergic system and CRF or AT1 will also be beneficial in terms of the approach to ulcerative colitis treatment.

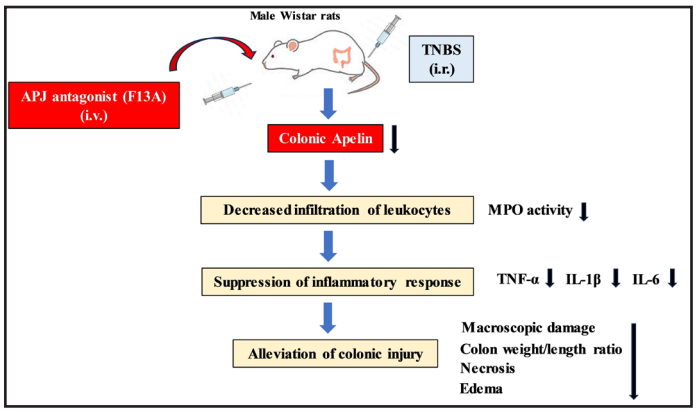

Figure 7. TNBS increased apelin production in colon tissue and also caused leukocyte infiltration into the tissue and pro-inflammatory cytokine response. APJ antagonist F13A administered after TNBS reduced these effects of TNBS.

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Conflict of interest statement: The authors declare that they have no conflict of interest.

Ethics Statement: All animal procedures were following the European Community Council Directive of 24 November 1986. Animals were treated in accordance with the Guide for the Care and Use of Laboratory Animals and were approved by the Akdeniz University Animal Experiments Local Ethics Committee (approval code: 1174/2020.08.001).

Author Contributions: Conception – V.N.I.U.; Design – V.N.I.U., I.U.; Supervision – V.N.I.U.; Fundings – V.N.I.U.; Materials – V.N.I.U., I.U.; Data Collection and/or Processing – I.B., O.S., S.S.A.; Analysis and/or Interpretation - I.B., O.S., S.S.A.; Literature Review – I.B., V.N.I.U.; Writing – I.B., V.N.I.U; Critical Review – O.S., S.S.A., I.U.

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Original Research

The Effect of MitoTEMPO on Rat Diaphragm Muscle Contraction Parameters in an Experimental Diabetes Model Induced with Streptozotocin

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ABSTRACT

Objective: Diabetes Mellitus (DM) not only causes hyperglycemia but also leads to clinical challenges involving respiratory functional impairments. The contraction of the diaphragm reduces pleural pressure, thereby contributing significantly to the process of breathing. This study examines the functional impairments in diaphragm muscle isometric contraction parameters due to increased reactive oxygen species (ROS) associated with DM, as well as the effects of MitoTEMPO, a mitochondria-specific antioxidant, on these impairments.

Methods: Wistar Albino male rats at 12-14 weeks of age were randomly divided into three groups: the control group (CON, n=6), the diabetes group (DM, n=6), and the diabetes + MitoTEMPO (MT, n=6) group. A single dose of 50 mg/kg streptozotocin (STZ) was administered to the rats in the DM and MT groups. When the rats in the MT group reached a blood glucose level of 300 mg/dl, they were administered MitoTEMPO at a dose of 0.7 mg/kg/day for 28 days. Isometric contraction recordings were obtained from diaphragm muscle preparations isolated from the experimental animals at the end of the 28-day period.

Results: Although the effectiveness of mitochondria-specific antioxidants in reducing blood glucose levels in DM is debated in the literature, results for the MT group were interestingly indicative of a statistically significant decrease in blood glucose levels following MitoTEMPO administration at the end of the fourth week. Furthermore, MitoTEMPO exhibited therapeutic effects on diaphragm muscle contraction parameters impaired by DM.

Conclusion: The findings suggest that in DM patients, MitoTEMPO could be utilized for blood glucose control and might also be effective in the treatment of DM-induced diaphragm muscle mechanical dysfunction.

Keywords: Diabetes Mellitus, Diaphragm muscle, Isometric contraction, MitoTEMPO

INTRODUCTION

Hyperglycemia caused by Diabetes Mellitus (DM), which is known as a group of metabolic disorders, is a result of impaired insulin secretion from the pancreatic β-cells, insulin sensitivity, or a combination of both [1]. Chronic hyperglycemia in DM leads to damage and dysfunction in various tissues and organs. On the other hand, in recent years, there has been a significant increase in the prevalence of DM, and World Health Organization (WHO)

statistics indicate that this upward trend is likely to continue [2].

Strong evidences support that oxidative stress plays important roles in the pathogenesis of damage and dysfunction caused by DM in many tissues and organs [3]. Reactive oxygen species (ROS) are recognized as the predominant origin of oxidative stress for cells. The increased amount of ROS may disrupt the physiology of intracellular signal transduction mechanisms, stimulate inflammatory responses, and lead cells to apoptosis [4]. As a result, diseases that increase ROS production, such as DM, can cause distant tissue and organ damage through these free radicals entering the bloodstream.

Although NADPH-oxidase, xanthine oxidase, immune reactions, arachidonic acid metabolism mechanisms contribute to ROS production, it is mostly produced in the electron transport chain localized to the inner membrane of the mitochondria [5]. The expected results from traditional applications, such as vitamin E and vitamin C, against mitochondria-induced oxidative stress cannot be achieved in clinical practice [6]. According to this information, researchers have focused on the idea of targeting antioxidants to mitochondria in recent years, thereby inactivating ROS at the production site before they enter the bloodstream. The mostly used molecule when targeting mitochondria is triphenylphosphonium (TPP), which is a lipophilic cation [7]. Another molecule bounds to TPP for drag into the intracellular matrix. It is more negative than the extracellular matrix, creating electrical force thanks to the cationic property of TPP. Then it continues to drag into the mitochondrial matrix, which is also more negative than the intracellular matrix, by the same mechanism. TPP can easily pass the membrane layers it encounters during this movement, thanks to its lipophilic feature [8].

Main Points;

- This study investigates the relationship between respiratory function impairments observed in patients with DM and the parameters of diaphragm muscle contraction, as well as the effects of MitoTEMPO, a mitochondria-specific antioxidant, on these functional impairments. Through this and similar studies, the effectiveness of mitochondria-specific antioxidants against secondary diseases observed in DM patients can be determined, potentially leading to higher quality of life standards for DM patients in the future.

The diaphragm is a skeletal muscle that plays a crucial role in respiration by contracting to decrease pleural pressure, enabling inspiration. The contraction and relaxation activities of skeletal muscles are determined by the ion concentrations on both sides of the cell membrane [9]. Increased ROS levels can lead to lipid peroxidation, potentially disrupting the physiological balance of the cell membrane potential [10]. Therefore, protecting respiratory functions against the increased amount of ROS in the tissues of patients with DM is vital for quality of life.

The aim of this study is to determine the dysfunctions that the amount of ROS, which is likely to increase with DM, may cause on the contraction-relaxation activity of the diaphragm muscle. In addition, it is another aim to determine whether possible dysfunctions can be treated with MitoTEMPO, a mitochondria-specific antioxidant.

MATERIALS AND METHODS

Experimental Animals and Groups

Wistar Albino male rats (12-14 weeks old) weighing 200-300 grams were used in the experiments. Rats were housed with a maximum of 5 animals per cage, on 12h/12h light/dark cycles, and with no restrictions on their access to feed and water. Experimental animals were randomly divided into three groups: control group (CON, n=6), diabetes group (DM, n=6) and diabetes + MitoTEMPO (MT, n=6) group. A single dose (50 mg/kg) intraperitoneal (i.p.) streptozotocin (STZ) (dissolved in 0.1 M sodium citrate, pH 4.5) was administered to the DM and MT groups, while the same dose of citrate vehicle injections were administered to the CON group. One week after the STZ injection, blood glucose measurements greater than 300 mg/dl were accepted as diabetic [11]. Starting one week after the STZ application, 0.7 mg/kg MitoTEMPO (cat. no: SML0737, Sigma-Aldrich, Darmstadt, Germany) was applied to the MT group [12] and vehicle gavage was applied to the other groups at the same dose for 4 weeks. The MT dose was determined in our previous studies as the value that maintains the oxidative stress index at the level of the control group [12,13]. At the beginning and subsequently for 4 weeks, the blood glucose (mg/dl) and body weight (g) values of the experimental animals were measured and recorded weekly. All procedures of this study were approved by the Animal Experiments Local Ethics Committee of Necmettin Erbakan University with the approval no 18-007.

Diaphragm Muscle Isolation and Isometric Contractility Recordings

Diaphragm tissue was quickly isolated from rats decapitated using a guillotine, and placed in a petri dish with a fresh modified solution of Krebs (composed of mM: 119 NaCl, 4.8 KCl, 1.8 $CaCl_2$, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 20 $NaHCO_3$ and 10 glucose, pH 7.4, and gassed with a mixture of 95% O_2 and 5% CO_2). The muscle strips prepared by trimming 20x5mm were placed in an isolated tissue bath with the costal side up, connected to a force transducer (FT03 Force Displacement Transducer, Grass Instruments) and the other side connected to a micromanipulator. The isolated tissue bath was continuously perfused with the modified Krebs solution and the temperature was kept constant at 37 ^oC. Diaphragm muscle preparations suspended in this way were placed in a 30-minute equilibration period under a tension of 2 grams. After the equilibration period, a rectangular field stimulation of 1 ms duration was given via a stimulator (S48, Grass Instruments). To determine the maximal contraction response, the voltage value was gradually increased and fixed at 12 Volts. Directly applying a field stimulus to the muscle tissue allowed for obtaining contraction data without the effects of neuromuscular junction and neural transmission disorders. Basic contraction recordings were obtained with a 1 Hz stimulus frequency. From these recordings, contraction force, maximum speed of force development ($+dF/dt_{max}$), and maximum speed of force decrease ($-dF/dt_{max}$) parameters were evaluated. Additionally, for the assessment of frequency-dependent contraction force, stimuli at 0.2, 0.5, 1, 2, 3, 4, and 5 Hz were applied [14]. Furthermore, for the responses to the post-rest potentiation protocol, pre-rest intervals of 10, 20, 30, 40, 50, 60, 70, and 80 seconds were implemented before each 100-second recording [15]. Isometric contraction data were simultaneously recorded using a data acquisition unit (MP45, Biopac) via software (BSL Pro 3.7.5, Biopac) for further analysis. Finally, the muscle masses were weighed, and the contraction data were normalized by dividing the wet muscle mass [16]. The presented data on isometric contraction were created by averaging the 10 data obtained from the recording of each rat.

Data Analysis and Statistics

All data were presented as mean ± standard error of the mean (SEM), and the normal distribution of data was tested with Kolmogorov-Smirnov. The significance of the rats' body weight and blood glucose data compared to the previous measurements was analyzed using a nonparametric paired t-test. For all other data, a statistical analysis was performed using One-way ANOVA, followed by the Tukey post-hoc test.

RESULTS

Both the blood glucose and body weight parameters were statistically evaluated for inter-group comparison at the same measurement time as well as for the intra-group changes over the weeks. In the DM group, following the STZ injection, statistically significant differences were observed in blood glucose values in all measurements compared to both the initial value and the CON group. In an interesting manner, the 2nd-week blood glucose value in the MT group was found within the range of values for both the DM and CON groups. Furthermore, in the 4th-week measurement, the MT group did not show statistical significance compared to the initial and CON group values but differed significantly from the DM group value. Regarding the body weight parameter, while no statistically significant differences were found between groups in all measurements, the differences detected in comparison to the initial value are as indicated in the table. No other statistical significance was observed between weeks for both blood glucose and body weight values within the same group, except for the significance marked in the table compared to the initial measurements (Table 1).

According to the contraction recordings taken at a basic stimulus frequency of 1 Hz, the contraction force did not show significant differences between the experimental groups (Figure 1-A). While the $+dF/dt_{max}$ parameter from the same recordings did not show differences between the groups (Figure 1-B), the $-dF/dt_{max}$ parameter increased in the DM group compared to the CON group, whereas the MT group remained at the CON level (Figure 1-C). The maximum tetanic force value obtained at a 100 Hz stimulation frequency increased in the DM group, whereas the MT group remained at the same level as the CON group (Figure 1-D).

It was observed that there was no statistically significant change in the contraction force across groups with varying stimulus frequencies (Figure 2-A). Similarly, the contraction force values obtained through the post-rest potentiation protocol did not show significant differences between the groups (Figure 2-B).

Table 1. Blood glucose and body weight measurements of the rats.

Blood glucose (mg/dl)					
	Initial	**1st week**	**2nd week**	**3rd week**	**4th week**
CON	109.83±3.66	101.83±3.72	99.67±6.78	100.17±5.03	100.67±1.65
DM	107.50±5.77	330.83±16.43[a,*]	345.50±28.23[a,*]	413.33±41.70[a,*]	390.33±27.22[a,*]
MT	103.67±2.79	313.00±12.32[a,*]	235.83±18.56[a,b,*]	218.17±42.57[b,*]	161.83±23.97[b]
p	0.5966	<0.0001	<0.0001	<0.0001	<0.0001
Body weight (g)					
	Initial	**1st week**	**2nd week**	**3rd week**	**4th week**
CON	240.83±6.99	273.67±23.95	289.33±25.32	301.83±25.68	316.67±20.33*
DM	249.50±18.33	282.33±25.33*	287.83±27.32*	297.83±28.53*	280.00±32.44
MT	216.67±8.20	266.17±20.88*	270.67±20.56*	282.50±19.41*	286.33±21.05*
p	0.1808	0.8887	0.8385	0.8462	0.5592

[a] p<0.05 vs CON, [b] p<0.05 vs DM, * p<0.05 vs initial measurement.

Abbreviations: control group (CON, n=6), diabetes group (DM, n=6) and diabetes + MitoTEMPO (MT, n=6). Between-group comparisons were conducted using One-way ANOVA with Tukey post-hoc test, while within-group measurements across weeks were compared using nonparametric paired t-tests. p<0.05 was considered statistically significant. Values are given mean ± SEM.

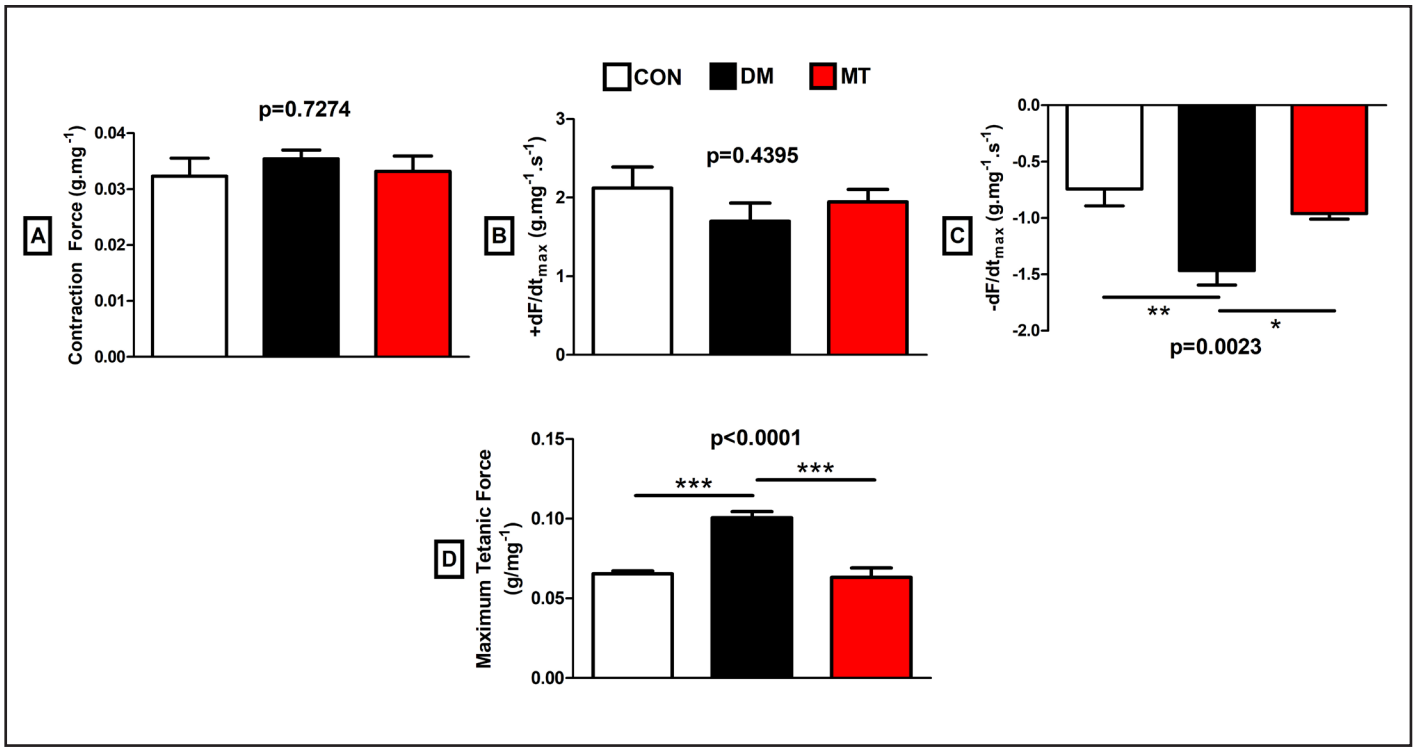

Figure 1. A, B, C- 1 Hz basic contractility parameters, D- maximum tetanic force. Abbreviations: control group (CON, n=6), diabetes group (DM, n=6) and diabetes + MitoTEMPO (MT, n=6). Between-group comparisons were conducted using one-way ANOVA with Tukey post-hoc test. Values are given as mean ± SEM.* p<0.05, ** p<0.01, *** p<0.0001.

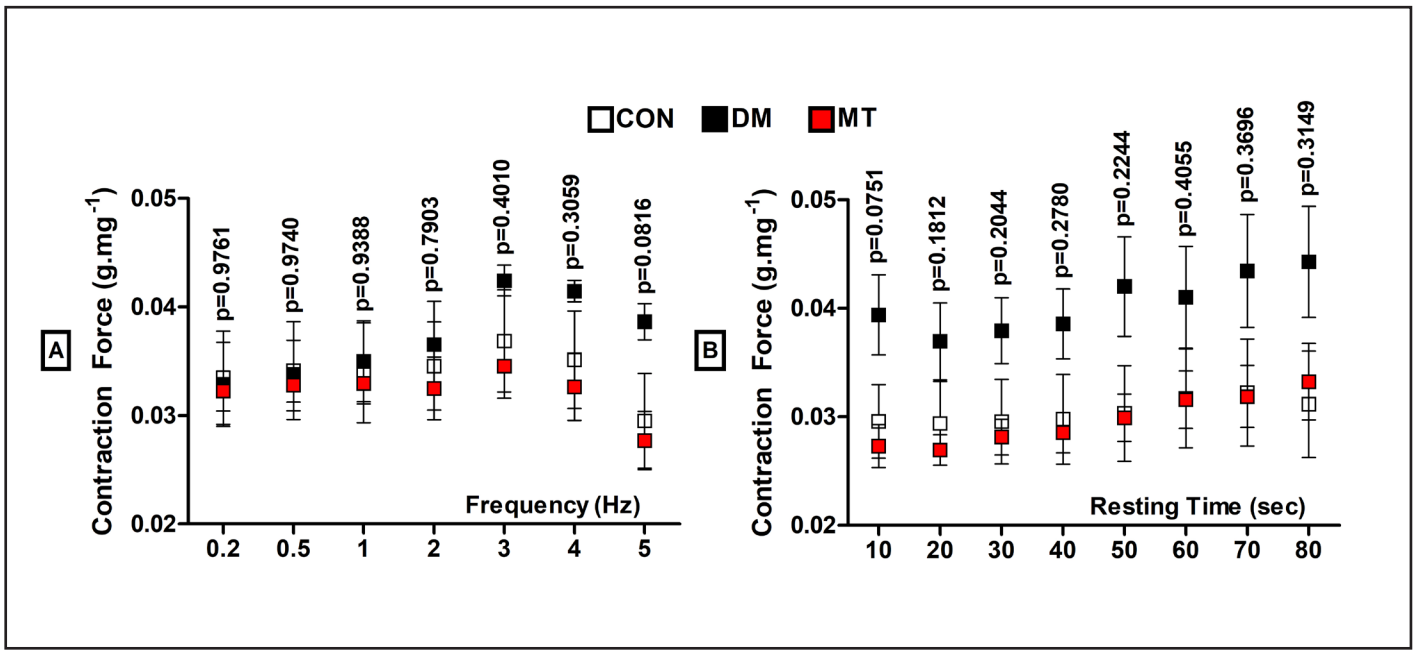

Figure 2. A- frequency dependent contraction force, B- postrest-potentiation protocol contraction force. Abbreviations: control group (CON, n=6), diabetes group (DM, n=6) and diabetes + MitoTEMPO (MT, n=6). Between-group comparisons were conducted using one-way ANOVA with Tukey post-hoc test. $p<0.05$ was considered statistically significant. Values are given as mean ± SEM.

DISCUSSION

DM has been considered an important comorbidity that affects the natural course of various respiratory system diseases. One of the significant causes of damage occurring in various biological structures in diabetic patients is the increased production of ROS due to hyperglycemia [17]. In line with the undisputed findings in the literature, we had previously identified the regulation of oxidative stress parameters following the application of MitoTEMPO in our studies [13,18]. Kabitz et al. have suggested that the impairment in diaphragm muscle functions resulting from DM is largely responsible for respiratory function impairment [19]. Clinical studies show that diaphragmatic breathing exercises prevent oxidative damage and regulate glycemic parameters in patients with type 2 diabetes [20]. Conversely, impairment in diaphragm muscle functions in these patients could become a source of various pathophysiologies due to oxidative damage and impaired glycemic parameters. In this study, we examined the contraction parameters of the diaphragm muscle preparation isolated from diabetic rats and the effects of MitoTEMPO on these parameters.

Mason et al. proposed that, contrary to our findings, the application of mitochondria-specific antioxidants to DM patients did not significantly alter glycemic parameters [21]. On the other hand, Jeong et al. found that in the glucose tolerance test, conducted by intraperitoneal glucose injection on the experimental animals, the high-fat diet group exhibited significantly higher blood glucose levels compared to the control group. However, the blood glucose levels of the group treated with MitoTEMPO were like those of the control group, in line with our findings [22]. Virgana et al. found in their DM model created with STZ that the group treated with mitochondria-specific antioxidants had a significantly lower fasting blood glucose level compared to the diabetic group [23]. In their study, Xiao et al. found a significant decrease in blood glucose levels in the mitochondria-specific antioxidant group compared to the DM group starting from the 12th week [24] (Table 1). The decrease in blood glucose levels may be associated with the improvement in β-cell functions, as indicated in the studies by Plecitá-Hlavatá et al., highlighting the potential involvement of mitochondria-specific antioxidants [25]. Based on this information, we believe that the role of MitoTEMPO in regulating blood glucose levels in DM patients' needs to be confirmed by further research. The body weights of the experimental animals did not show any significant differences between the groups in all measurements. However, while there was a significant weight gain in the CON and MT groups compared to the initial measurement at the end of the 4th week, there was no statistically significant weight gain

in the DM group (Table 1). Lin et al. did not find a significant difference in the body weights of the experimental animals belonging to the control, DM, and MitoTEMPO groups in a study similar to this study's findings [26]. In contrast, Xing et al. found that the body weights of diabetic mice were significantly higher compared to the control group, and they also observed that MitoTEMPO treatment had no effect on weight gain [27]. It appears that there is a lack of a study elucidating the mechanisms of weight gain or loss in experimental animals following the administration of MitoTEMPO.

Brotto et al. did not find a significant difference between the groups in diaphragm muscle contraction force on the 4th day, similar to ours; however, unlike ours, they observed a significant decrease in the DM group compared to the control in the data at the 4th week [28]. On the other hand, De Jong et al. did not find a significant difference in the isometric contraction parameters of the diaphragm muscle in diabetic rats compared to the control group, parallel to our findings [29]. RodríGuez-Reyes et al. also found that the normalized contraction force value of the skeletal muscle did not change in the DM group, in line with our findings [30] (Figure 1-A). Lamberts et al. indicated diastolic function impairments in cardiac muscle contraction parameters of diabetic patients, parallel to the diaphragm muscle contraction parameters in our study [31]. In another study, the diaphragm muscle contraction parameters of diabetic fatty rats did not show a difference between the groups in $+dF/dt_{max}$, which is in line with this study. However, unlike ours, $-dF/dt_{max}$ did not show a significant difference in the diabetic fatty group [29] (Figure 1-B,C). Peixoto et al. found the maximum tetanic force of the diaphragm muscle isolated from diabetic rats to be high, similar to our findings. They suggested that this might be due to increased resistance to fatigue [32]. This could be associated with the increased ATP levels in diabetes [33-35]. The injection of MitoTEMPO resulted in the treatment of oxidative damage, thereby maintaining the data of this group at the level of the CON group (Figure 1-D).

Laitano et al. did not observe any significant difference between the groups in terms of frequency-dependent contraction force of the diaphragm muscle in their study on a chronic heart failure model, which is in line with our findings [36]. Eshima et al. demonstrated an increase in blood glucose levels in mice in a high-fat diet model and, parallel to our findings, did not detect any significant difference in the frequency-dependent contraction parameters of skeletal muscle in these animals [37] (Figure 2-A). In our data, where the post-rest potentiation protocol was applied to test the sarcoplasmic reticulum Ca^{++} reuptake mechanism without altering the extracellular Ca^{++} influx, no differences were observed between the groups [38] (Figure 2-B). Eshima et al., in contrast to our findings, reported the disruption of Ca^{++} homeostasis in diabetes [39]. Perhaps this situation could be a phenomenon involving different diabetic durations.

Limitations

This study was conducted only in male rats, aged 12-14 weeks, without evaluating gender- and age-dependent parameters. For instance, a difference in the rate of occurrence of cardiac diseases in diabetic patients has been identified in relation to age and gender [40]. Furthermore, only the mechanical activity of the diaphragm muscle was identified for respiratory dysfunctions related to DM. Another limitation of our study is the lack of investigation into the molecular mechanisms associated with the regulatory effects of MitoTEMPO treatment on blood glucose and diaphragm muscle mechanical activity. Among these mechanisms, oxidative stress parameters, intracellular Ca^{++} concentration as a contraction-relaxation regulator, insulin levels, etc., can be mentioned.

CONCLUSIONS

In conclusion, it is essential for DM patients to maintain the physiological working capacity of the diaphragm muscle for healthy respiratory functions. Our findings demonstrated that MitoTEMPO treatment regulates blood glucose levels in diabetic rats, thereby correcting the impairment in the maximum tetanic force and $-dF/dt_{max}$ values of the diaphragm muscle. Thus, MitoTEMPO can be used as an alternative therapeutic agent in patients with DM.

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Original Research

Red Cell Distribution Width Is an Independent Predictor of 1-Year Mortality in a Turkish Patient Population with Acute Decompensated Heart Failure

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ABSTRACT

Objective: Heart failure (HF) is a significant public health issue in Turkey. The goal of this study was to look into how red cell distribution width (RDW) affected patients with acute decompensated HF (ADHF) patients' prognoses.

Methods: A total of 101 ADHF patients above the age of 18 were enrolled in the study. Venous blood was drawn to measure the serum rdw. After a year of follow-up, the patients' survival status was determined.

Results: The patients' mean age was 72. Forty-nine patients had heart failure (HF) with a reduced ejection fraction (EF), 8 had HF with a mildly reduced EF, and 44 had HF with a preserved EF. The median RDW value was 15.9%. In the hospital, nine patients passed away, and 92 others were discharged. 14 patients were lost to follow-up after one year, 87 patients completed the trial, and 40 patients passed away. Inotropic medication use, and serum RDW value were identified as independent predictors of 1-year death in ADHF patients by multivariate logistic regression analysis. According to this data, there was a 44% increase in 1-year mortality for every 1% increase in RDW.

Conclusion: In Turkish patients hospitalized for ADHF, red cell distribution width represents an independent prognostic predictor for 1-year mortality.

Keywords: Red cell distribution width, mortality, heart failure

INTRODUCTION

Heart failure (HF) is still a clinical illness with a significant morbidity and mortality rate in the twenty-first century. In a study on the prevalence of HF in Turkey, it was shown that among 4650 persons above the age of 35, absolute HF prevalence was 2.9%, compared to a predicted prevalence of 6.9%, and absolute asymptomatic left ventricular dysfunction prevalence was 4.8%, compared to a predicted prevalence of 7.9% [1]. HF thus remains a significant public health issue in Turkey. Despite the most recent developments in evidence-based therapies and device technology being employed in HF, fatality rates are still significant. In a more recent study, the mortality rate for Turkish HF patients at one year was 19.9% (13.7% for patients with chronic heart failure and 32.6% for those with acute heart failure) [2]. In comparison to HF mortality in European nations, this is a little higher. The Heart Failure Long-Term Registry (ESC-HF-LT) of the European Society of Cardiology enrolled 12440 HF patients from 211 clinics in 21 countries. According to this study, the 1-year all-cause mortality rate was 14.5% for chronic HF and 23.6% for acute HF [3].

It's critical to pay closer attention to high-risk individuals in order to identify the factors that predict mortality in HF patients and lower morbidity and mortality in this patient population. Numerous prognostic models have been suggested for this purpose [4-6]. However, there is a limited application of these models in clinical settings. 39372 individuals with HF from 30 cohort studies were included in one of these models, the MAGGIC meta-analysis [4]. Age, lower ejection fraction (EF), NYHA functional class, serum creatinine, diabetes, no prescription for a beta-blocker, lower systolic blood pressure, lower body mass, time since diagnosis, being a current smoker, chronic obstructive pulmonary disease, male gender, and no prescription for an angiotensin-converting enzyme inhibitors (ACEI) or angiotensin receptor blockers (ARB) were found to be independent predictors of mortality in HF in this analysis [4]. This meta-analysis's authors created an integer score to gauge the probability of HF mortality.

In the past 15 years, it has been discovered that red blood cell distribution width (RDW), an easily measured hemogram parameter representing the change in erythrocyte volume, is a prognostic marker in HF patients [7–10]. RDW demonstrated the strongest association with morbidity and mortality among the laboratory indicators of the Candesartan in Heart Failure: Assessment of Reduction in Mortality and Morbidity (CHARM) study program, according to the researchers [7]. Several more studies followed this one in showing the predictive importance of RDW in HF patients [8–11]. This study looked into how RDW affected prognosis in a group of Turkish patients with acute decompensated HF (ADHF).

Main Points;

- Heart failure represents a significant public health issue in Turkey with high morbidity and mortality.
- Identifying factors influencing morbidity and mortality is important to lower the risk in high-risk patients.
- Red blood cell distribution width is a prognostic marker in Turkish patients with acute decompensated HF.
- One percent increase in RDW is associated with a 44% increase in 1-year mortality in this patient population.

MATERIALS AND METHODS

One hundred and one individuals hospitalized at the cardiology clinic with ADHF were included in the study. Patients above 18 years of age with a diagnosis of acute HF met the inclusion criteria. Patients with all varieties of HF were included. Only those under the age of 18 were excluded. In order to participate in the study, the patients gave their informed consent. The study was approved by the regional clinical studies ethics committee (Kırıkkale University, date: 2012/12/17, approval number: 12/14-01). The 1964 Helsinki Declaration and its following amendments, as well as other related ethical norms, guided every procedure carried out for this study. A single-center prospective cohort kind of observational study was intended for the investigation.

Both the heart rate and blood pressure were taken upon admission. At the time of discharge, the patients' pulse rates were also noted. Additionally, the patients' HF Killip status was noted during the physical examination. When an electrocardiogram (ECG) was obtained, conditions such as left bundle branch block and atrial fibrillation were identified. All patients had venous blood collected at the time of admission for biochemistry and a full blood count. RDW and other hemogram parameters were measured using an automated haematology analyzer (Abbott Cell-Dyn 2700; Abbott Laboratory, Abbott Park, Illinois, USA). A Vivid 3 Pro Echocardiography device (General Electric Corp, Milwaukee, WI, USA) was used for transthoracic echocardiography. HF with reduced ejection fraction (HFrEF) was defined as HF with EF $\leq$ 40%, HF with mildly reduced EF (HFmrEF) was defined as HF with EF between 41-49%, and HF with preserved EF (HFpEF) was defined as HF with EF $\geq$ 50%. We kept track of cardiac issues such as arrhythmias (atrial fibrillation, ventricular arrhythmias, etc.), cardiogenic shock, and the requirement for inotropic assistance while in the hospital.

One-year all-cause mortality was the study's primary outcome. After a year of follow-up, the patients' survival status was ascertained either directly during cardiology outpatient appointments, over the phone with their relatives, or by consulting their medical files.

Statistical Analysis

The study's statistical analysis was conducted using the statistical package for the social sciences (SPSS) 17.0 software from SPSS Inc. in Chicago, Illinois, USA. The distributional characteristics of the continuous variables were determined using the

Kolmogorov-Smirnov test. In contrast to categorical variables, which were compared between groups using chi-square and Fischer's exact tests, continuous variables were compared using the Student-t test and the Mann-Whitney U test (for normally distributed and non-normally distributed variables, respectively). The chi-square test was once more used to compare categorical variables among the three RDW groups, and the Kruskal-Wallis test was used to compare continuous variables among the three RDW groups.

To determine the factors influencing one-year mortality, we conducted a univariate logistic regression analysis. After that, multivariate logistic regression analysis was used to identify the independent determinants of acute decompensated HF mortality at one year. Based on their statistical significance as a single variable, we included variables in the multivariable logistic regression analysis, considering a p-value less than 0.05 as significant.

RESULTS

One hundred and one ADHF patients were enrolled in the study. 56 (55%) of the patients were men, and 45 (45%) were women. The range of patient ages was 24-90, with a mean age of 72 and the median age was 74. The length of hospitalization varied from 1 to 19 days, with 4 days serving as the median. 49 (48.5%) individuals had HFrEF, 8 (8%) had HFmrEF, and 44 (43.5%) had HFpEF out of the total. Regarding the patients' Killip classification, 77 (76%) had Killip Class 2, 18 (18%) had Killip Class 3, and 6 (6%) had Killip Class 4 HF. 46 (45%) of the patients were on sinus rhythm, while 55 (55%) had atrial fibrillation. On the ECG, 15 (15%) patients showed left bundle branch block. At the time of hospital admission, the median heart rate of the patients was 84 beats per minute (bpm). The patients' median heart rate at discharge was 78 bpm. According to the patients' hemogram data, the mean RDW value was 16.1% (13.0-27.2%) whereas the median value was 15.9%. Seventy individuals (69%) reported RDW values that were high (RDW ≥ 15%). Table 1 lists the fundamental characteristics of the entire patient population.

During their stay in the hospital, 38 (38%) patients received positive inotropic treatment, 18 (18%) patients had cardiogenic shock, 2 (2%) patients developed acute renal insufficiency, and 1 (1%) patient developed new atrial fibrillation. Nine (9%) patients passed away while they were hospitalized, and 92 (91%) patients were discharged. At the end of the 1-year follow-up period, 87 patients had finished the follow-up term, 14 (14%) patients had lost follow-up, and 40 (39.6%) patients had passed away.

In this HF population, cardiogenic shock, usage of inotropic drugs, serum creatinine, RDW, and potassium values were found to be associated with 1-year mortality (Table 2). All of these variables were taken into account in the multivariate logistic regression analysis. According to the results of this analysis, serum RDW value and inotropic medication use were independent predictors of 1-year mortality in ADHF patients (Table 3). Additionally, this investigation showed that in decompensated HF patients, a 1% rise in RDW value corresponded to a 44% increase in 1-year all-cause death.

Table 1. Basal characteristics of the total patient population

Characteristic	
Heart failure type	
HFrEF (n,%)	49 (48.5%)
HFmrEF (n,%)	8 (8%)
HFpEF (n,%)	44 (43.5%)
Age (median, 25th-75th percentile)	74 (66-79)
Ejection fraction (%) (median, 25th-75th percentile)	45 (30-52.5)
Hospitalization days (median, 25th-75th percentile)	4 (3-6)
Systolic blood pressure (mmHg) (median, 25th-75th percentile)	120 (110-140)
Diastolic blood pressure (mmHg) (median, 25th-75th percentile)	70 (70-80)
Heart rate at hospital admission (beats per minute) (median, 25th-75th percentile)	84 (77-100)
Serum creatinine (mg/dl) (median, 25th-75th percentile)	1.0 (0.80-1.40)
Serum haemoglobin (g/dl) (mean±SD)	12.7 ± 2.3
Serum range of distribution width (RDW) (%) (median, 25th-75th percentile)	15.9 (14.6-16.9)
Killip classification	
2 (n,%)	77 (76%)
3 (n,%)	18 (18%)
4 (n,%)	6 (6%)
Mitral regurgitation	
None (n,%)	5 (5%)
Mild (n,%)	23 (23%)
Medium (n,%)	41 (41%)
Serious (n,%)	32 (31%)
Atrial fibrillation on ECG (n,%)	46 (45%)
Cardiogenic shock (n,%)	18 (18%)
Inotropic drug use (n,%)	38 (38%)

According to RDW values, we separated the entire group into three subgroups. RDW values in the first group were less than 15.0%, in the second group ranged from 15.0 to 16.9%, and in the third, RDW values were equal to or higher than 17%. When clinical and laboratory traits were evaluated between these three categories, it was found that the extremely high RDW group (RDW ≥ 17%) had serum hemoglobin values that were lower than those of the other two groups, but the other traits were comparable between the three subgroups (Table 4). Patients in the highest RDW group had a 2.5-fold higher one-year mortality rate than those in the normal RDW group. The first and second RDW groups' 1-year mortality rates did not significantly differ from one another.

Table 2. Univariate logistic regression analysis for 1-year mortality prediction in patients with decompensated heart failure

Variables	B value	OR value	95% CI for OR		p-value
			Lower	Upper	
Inotropic drug use	1.488	4.428	1.762	11.126	0.002
Cardiogenic shock	1.644	5.176	1.529	17.525	0.008
Serum creatinine	1.366	3.918	1.415	10.850	0.009
Serum potassium	0.818	2.266	1.110	4.628	0.025
Serum RDW	0.468	1.597	1.142	2.233	0.006

*Univariate logistic regression analysis found significant associations with inotropic drug use, cardiogenic shock, serum creatinine, serum potassium, serum rdw values and 1-year mortality in decompensated heart failure patients. †B: Logistic regression coefficient, OR: Odds ratio, CI: Confidence interval, RDW: Red cell distribution width

Table 3. Multivariate logistic regression analysis for 1-year mortality prediction in patients with decompensated heart failure

Variables	B value	OR value	95% CI for OR		p-value
			Lower	Upper	
Inotropic drug use	1.150	3.159	1.168	8.542	0.023
Serum RDW	0.365	1.440	1.023	2.027	0.036

*Cardiogenic shock, inotropic drug use, serum creatinine, rdw, and potassium were entered into the multivariate logistic regression analysis. †B: Logistic regression coefficient, CI: Confidence interval, OR: Odds ratio, RDW: Red cell distribution width

Table 4. The comparison of the clinical and laboratory characteristics among the subgroups with normal, high and very high rdw values

Characteristic	Normal rdw group (rdw<15%) n=31	High rdw group (rdw 15-16.9%) n=46	Very high rdw group (rdw≥17%) n=24	p-value
Heart failure type				
HFrEF (n,%)	18 (37%)	21 (43%)	10 (20%)	
HFmrEF (n,%)	0 (0%)	6 (75%)	2 (25%)	
HFpEF (n,%)	13 (30%)	19 (43%)	12 (27%)	0.118
Age (median, 25th-75th percentile)	77 (68-80)	74 (63-78)	72 (67.5-78)	0.511
Ejection fraction (%) (median, 25th-75th percentile)	40 (30-50)	45 (30-55)	48 (31.25-50)	0.861
Hospitalization days (median, 25th-75th percentile)	4 (3-6)	4 (3-6)	4 (3-5)	0.676
Systolic blood pressure (mmHg) (median, 25th-75th percentile)	130 (110-140)	120 (110-140)	125 (110-130)	0.936

Diastolic blood pressure (mmHg) (median, 25th-75th percentile)	70 (70-80)	75 (60-82.5)	80 (70-80)	0.985
Heart rate at hospital admission (beats per minute) (median, 25th-75th percentile)	84 (80-92)	86 (75.5-102.5)	81 (72-106)	0.668
Serum creatinine (mg/dl) (median, 25th-75th percentile)	0.9 (0.8-1.1)	1.0 (0.8-1.3)	1.1 (0.8-1.7)	0.267
Serum haemoglobin (g/dl) (mean±SD)	13.2±1.4	13.1±2.1	11.3±3.1	**0.002**
Killip classification				
2 (n,%)	25 (33%)	34 (44%)	18 (23%)	
3 (n,%)	4 (22%)	10 (56%)	4 (22%)	
4 (n,%)	2 (33%)	2 (33%)	2 (33%)	0.843
Mitral regurgitation				
None (n,%)	2 (40%)	1 (20%)	2 (40%)	
Mild (n,%)	6 (26%)	11 (48%)	6 (26%)	
Medium (n,%)	13 (32%)	17 (41%)	11 (27%)	
Serious (n,%)	10 (31%)	17 (53%)	5 (16%)	0.774
Atrial fibrillation on ECG (n,%)	15 (27%)	26 (47%)	14 (26%)	0.710
Cardiogenic shock (n,%)	4 (22%)	10 (56%)	4 (22%)	0.602
Inotropic drug use (n,%)	9 (24%)	17 (45%)	12 (31%)	0.279

* ECG: Electrocardiography, HFmrEF: Heart failure with mildly reduced ejection fraction, HFpEF: Heart failure with preserved ejection fraction, HFrEF: Heart failure with reduced ejection fraction, RDW: Red cell distribution

DISCUSSION

Nearly all HF patients have their red cell distribution width assessed using an easily accessible hemogram test. RDW was first identified as a prognostic factor in HF patients according to the CHARM trial [7]. In both acute and chronic HF patients, higher RDW values increase the risk of death [7–11]. In HF patients, elevated RDW values were also linked to a higher probability of the combined end-point of death or rehospitalization [12]. In HF patients, a 1% rise in RDW value was linked to a 10% increase in the chance of death in the future and a 9% increase in the probability of hospitalization, according to a meta-analysis on the topic [13]. Additionally, elevated RDW values raise the risk of death and the onset of HF in a group with baseline coronary artery disease but no HF [14]. Numerous studies also demonstrate the superior value of serial RDW monitoring in hospitalized HF patients compared to baseline RDW measurement. A higher risk of future death or rehospitalization is implied by an increase in RDW during hospitalization than by baseline RDW values [15, 16]. Another study in HF found that RDW has additive predictive value for B-type natriuretic peptide (BNP), a prognostic marker with a long history [17]. Furthermore, it has been demonstrated that the bio-width index, which is created by multiplying BNP by RDW and then dividing the result by 10, improves the accuracy of BNP and RDW in predicting mortality in HF patients with anemia [18].

Red blood cell size variation in peripheral blood is measured by RDW. A decline in RDW value is not clinically significant. Anisocytosis, another name for an elevated RDW value, has numerous critical consequences for cardiovascular outcomes in both the general population and HF patients [19]. Ageing, inflammation, oxidative stress, renal dysfunction, nutritional issues, beta-thalassemia, hemolytic anemia, hereditary spherocytosis, sickle cell anemia, after blood transfusion, chronic liver disease, folate deficiency anemia, vitamin B12 deficiency anemia, and myelodysplastic syndrome are just a few conditions that can cause an increase in RDW value [20]. The precise mechanism underlying the elevated risk of HF patients with anisocytosis is unknown. There are numerous proposed mechanisms, though. Anisocytosis and inflammation interact, according to one suggested mechanism. Anisocytosis may develop from abnormally early erythrocyte production, which is recognized to be a contributing factor to bone marrow dysfunction [21]. It is well known that inflammation has a role in the onset and development of HF [22].

Additionally, various forms of anaemia may present with anisocytosis. Anaemia is also a well-known prognostic marker in patients with heart failure [23]. Nevertheless, after accounting for haemoglobin values, certain studies [11, 24] discovered that

higher RDW values were predictors of death in HF patients. Another theory is that the prognostic effect of anisocytosis in HF is caused by oxidative stress, which can lead to defective erythropoiesis and unfavorable cardiac remodeling. Patients with anisocytosis may have abnormal erythrocytes that deoxygenate cardiomyocytes, leading to cardiac death or fibrosis [25]. Furthermore, lower heart rate variability was associated with higher RDW in HFrEF patients, which may have negative effects on these patients' prognoses [26]. Elevated RDW was discovered to be related to worse LV deformation, as measured by LV global longitudinal strain in speckle tracking echocardiography, in a different study conducted in HF patients with EF < 50%, which is another unfavorable prognostic marker in the HF population [27].

This study proved that RDW, in a Turkish group of ADHF patients (HFpEF, HFrEF, and HFmrEF patients), is a distinct predictive marker of 1-year mortality. The use of inotropic medications while hospitalized was the other additional predictive factor found in the study. In our investigation, serum RDW values and serum hemoglobin values were correlated. Serum hemoglobin levels were lower in the subgroup of patients with the highest RDW values (≥ 17%) than they were in the patients with lower RDW values (< 17%). This was seen in HF patients in numerous previous studies [24,28], and various types of anemia are also linked to elevated RDW values [20]. The three RDW subgroups did not differ in any other clinical or laboratory measures. The mortality in the subgroup with the highest RDW values was 2.5 times higher at 1 year compared to the cohort with normal RDW values. Patients' 1-year mortality in the usual RDW group (RDW< 15%) and the slightly enhanced RDW group (RDW between 15 and 16.9%) did not differ from one another. When RDW rose above 14.7% in the CHARM program, HF mortality began to rise; similarly, when RDW rose above 15.3% in the Duke Databank dataset [7]. RDW was linked to mortality in a subset of patients with RDW values ≥ 16.6% in a smaller AHF study, which is consistent with the findings of the current analysis [9]. Therefore, this disparity may be explained by the patient numbers included in the study and also in the RDW subgroups. In the current study, a 1% increase in serum RDW value led to a 44% increase in the 1-year mortality rate of these HF patients. This result is a little higher than the results of other research, which revealed that a 1% increase in RDW values was associated with a 10-12% increase in mortality [10, 13, 29]. A 39% all-cause mortality rate within a year indicated a substantial mortality risk for the study's patient cohort. The baseline RDW value for 69% of the cohort was high (≥ 15%). This may help to explain the present study's higher mortality risk associated with higher RDW values. We should point out that this is a modest, one-center study. The study's small sample size may have also overstated the impact of RDW on population's overall mortality. In Turkey, HF is still a serious health issue. Understanding the high-risk category of patients with HF requires the identification of mortality predictors. In ADHF patients, RDW is an easily obtainable hemogram measure. It might offer crucial prognostic data. Additionally, repeated measurements might even be more useful in identifying these patients' risk for mortality and morbidity.

Limitations

The present study had several important drawbacks, including a limited sample size and a single-center design. The high percentage of patients who were lost to follow-up may be another drawback, especially for a study of this small scale. Last but not least, the endpoint's inclusion of solely all-cause mortality and exclusion of cardiovascular mortality and HF rehospitalization may be considered as a restriction.

CONCLUSIONS

In conclusion, red cell distribution width is an independent prognostic marker for 1-year mortality in Turkish patients hospitalized for acute decompensated heart failure. The data from this study shows that a 1% increase in serum RDW value leads to a 44% increase in 1-year mortality among these patients.

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Informed Consent: The patients provided informed consent to participate in the study.

Conflict of interest: The author declares that he has no conflicts of interest.

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Ethical Approval: The local clinical studies ethics committee approved the study (Kırıkkale University, Date: 12/17/2012, Approval number: 12/14-01). All procedures performed in this study were in accordance with the ethical standards of the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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Original Research

Predictive Effects of Platelet Indices in Cirrhotic Patients with or without Portal Vein Thrombosis

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ABSTRACT

Objective: Portal vein thrombosis (PVT) is a common finding in liver cirrhosis. Besides low portal blood flow, thrombophilia, bacterial translocation and endotoxemia, platelets which are considered as important source of prothrombotic agents may play a role in thrombotic events in cirrhosis. Large platelets have been reported to have numerous granules that result in greater thrombotic and proinflammatory activity. We aimed to define the role of platelet indices in PVT among cirrhotic patients.

Method: Cirrhotic patients admitted to Gastroenterology Clinic and having a dynamic radiological examination were assessed retrospectively. Demographic and laboratory findings were recorded including platelet distribution width (PDW) and mean platelet volume (MPV). Severity of cirrhosis was assessed with MELD (Model for End Stage Liver Disease) and Child-Pugh-Turcotte (CPT) scores

Results: Study included 255 patients. Mean age was 60.6±10.2 years. 41.6% of patients were female. 50 (19.6%) patients had PVT. Patients with PVT did not differed from those without PVT in age, gender and presence of diabetes mellitus. Median platelet count was lower in patients with PVT (100 (22-370) vs 79.5 (22-573), p: 0.033). Mean MPV and PDW levels were similar between PVT and non-PVT groups (p >0.05). Although median MELD scores did not differ between groups, median CPT scores were significantly higher in PVT compared to non-PVT group (p:0.027).

Conclusion: Cirrhotic patients with PVT had more prominent thrombocytopenia, but similar MPV and PDW levels compared to those without PVT.

Keywords: Platelet indices; cirrhosis; portal vein thrombosis.

INTRODUCTION

Blockage of main portal vein (PV) or its tributaries by thrombus is named as portal vein thrombosis (PVT). It is a well-known complication of cirrhosis. PVT could also complicate many conditions such as myeloproliferative diseases, malignancies and infection [1]. Cirrhotic patients have different incidence and prevalence rates reported for PVT associated with different target population and disease severity [2]. PVT prevalence in cirrhosis varies between 1% and 25% [3]. 3-year and 5-year incidence of PVT for cirrhotic patients have been reported as 7.6% and 10.7%, respectively [3,4]. Low portal blood flow, inherited or acquired thrombophilia, bacterial translocation and endotoxemia may have a role in occurrence of PVT in cirrhosis [1,5].

Platelets are important source for prothrombotic agents [6]. Platelet activation is initial step of thrombosis. Upon activation, adhesion and aggregation of platelets, secretion of platelet granules and enhancement of thrombin generation take place. With activation, platelets become larger, aggregate, synthesize and produce active mediators [7,8]. Platelets secrete a variety of substances including ADP, histamine, serotonin, fibronectin, fibrinogen, thrombospondin, thromboxane A2, platelet-derived growth factor, von Villebrand factor and so on, from their granules [9]. Large platelets include more granules that result in greater thrombotic and proinflammatory activity [7,10]. The mean platelet volume (MPV) defines the average volume of circulating platelets. Platelet distribution width (PDW) is an index of variation between individual platelet size Thus, MPV and PDW are suggested as indicators of platelet function and activation [11].

Association between MPV and arterial thrombosis has been reported in cases of ischemic cerebrovascular or cardiac events [12-15]. Increased MPV was also reported to be associated with pulmonary thromboembolism [16]. The relationship between PVT and platelet indices remains uncertain. We aimed to define the role of platelet indices in cirrhotic patients with non-malignant PVT.

MATERIALS AND METHODS

Patients admitted to Gastroenterology Clinic in Başkent University Adana Dr. Turgut Noyan Training and Research Hospital with liver cirrhosis from 2015 to 2020 were analyzed retrospectively. This study was approved by the Institutional Review Board of Başkent University (KA 22/258-31.05.2022-E-94603339-604.01.02-131417).

Cirrhosis was diagnosed by histological examination or by radiology when compatible radiological findings, varices and/or splenomegaly were present. MELD (Model for End Stage Liver Disease) and Child-Pugh-Turcotte (CPT) scores were applied to assess the severity of cirrhosis. Cirrhotic patients having a dynamic examination of computed tomography or magnetic resonance imaging were included into the study. Patients with known malignancy, sepsis, thrombophilia, chronic inflammatory diseases, renal insufficiency, acute coronary or cerebrovascular event, on anticoagulation or anti-aggregation due to any condition and age lower than 18 years were excluded.

Main Points;

- It has been found that cirrhotic patients with PVT had higher median CPT score and lower median platelet count, but similar mean MPV and PDW levels compared to those without PVT.

Age, gender, comorbid diseases, etiology of cirrhosis, presence of portal vein thrombosis in dynamic radiological examination were recorded for all patients, Laboratory tests comprising liver function tests and complete blood count, including white blood cell (WBC), PDW, MPV and platelet count were recorded.

Statistical Analysis

Statistical analysis was performed using SPSS software (version 23.0, IBM Corp., Armonk, NY, USA). Normally distributed continuous variables were described as means ± standard deviations. Non-normally distributed continuous variables were described as medians. Comparison of categoric variables were performed using $\chi 2$ test and Ficher test. Comparison of groups for normally distributed data was made by using Student's t-test. The Mann–Whitney U test was used for non-normally distributed data. For Spearman's coefficient, $p < 0.05$ was accepted as significant.

RESULTS

Cirrhotic patients admitted to our clinic between 2015 and 2020 were screened. Initial screening revealed 450 patients. A total of 195 patient were excluded, due to sepsis (n:30), thrombophilia (n:5), acute coronary or cerebrovascular event (n:20), malignancy (n:105) and not having appropriate radiologic imaging of CT or MRI (n:45). Final analysis included 255 patients. Mean age of patients was 60.6±10.2 (20-83) years. 106 (41.6%) of all patients were female. Demographic characteristics and laboratory parameters of patients were shown in table 1.

Hepatitis B and hepatitis C were present in 60 (23.5%) and 42 (16.5%) patients, respectively. Alcoholic liver disease, PBC/Autoimmune hepatitis and NASH were present in 12, 12 and 20 patients, respectively. Hemochromatosis and hepatitis B/D were present in the remaining 6 patients. No underlying etiology was defined in 103 (40.4 %) patients.

Fifty (19.6%) patients with cirrhosis had PVT. Patients with PVT did not differ from those without PVT in regards to age, gender and presence of diabetes mellitus (Table 2).

Table 1. Demographic characteristics and laboratory parameters of patients

Characteristic		**All patients (n: 255)**
Male /Female (n)		149/106
Age *		60.6±10.2
Etiology n (%)	HBV	60 (23.5)
	HCV	42 (16.5)
	Alcohol	12 (4.7)
	Cryptogenic	103 (40.4)
	NASH	20 (7.8)
	Autoimmune /PBC	12 (4.7)
	Others	6 (2.4)
PVT n (%)	Present	50 (19.6)
	Absent	205 (80.4)
Radiology n (%)	CT	214 (83.9)
	MRI	41 (16.1)
DM n (%)		99 (38.8)
Hb*		11.8±2.3
WBC*		5406.6±2391.1
Neutrophil*		62.4±10.2
Lymphocyte*		24.1±8.9
Platelet*		109.8±65.1
MPV*		9.4±4.8
PDW*		39.1±17.1
Albumin*		3.37±0.7
Total bilirubin*		2.39±2.9
INR*		1.36±0.3
AST*		48.2±43.4
ALT*		45.5±63.5
Creatinine *		0.86±0.5
Na*		137.4±4.6
MELD*		13.1±5.97
CHILD*		7.32±2.1
Ascites n (%)	No	116 (45.5)
	Low-moderate	96 (37.6)
	Tense-refractory	43 (16.9)

* Mean ± Standard Deviation

Abbreviations: **HBV**: Hepatitis B Virus, **HCV**: Hepatitis C Virus, **NASH**: Non-alcoholic steatohepatitis, **PBC**: Primary biliary cholangitis, **PVT:** Portal vein thrombosis, **CT**: Computed tomography, **MRI**: Magnetic resonance imaging, **DM**: Diabetes Mellitus, **Hb**: Hemoglobin, **WBC**: White blood cell count, **MPV:** Mean platelet volume, **PDW**: Platelet distribution width, **INR**: International normalization ratio, **AST**: Aspartate aminotransferase, **ALT**: Alanine aminotransferase, **Na**: Sodium, **CPT**: Child-Pugh Turcotte score, **MELD**: The Model for End Stage Liver Disease

Table 2. Characteristics and laboratory parameters of patients according to presence of portal vein thrombosis

		No PVT (n:50)	PVT present (n:205)	p
Female n (%)		89 (43.4)	17 (34.0)	0.226
Age**		62 (25-83)	61,5 (20-83)	0.841
DM n (%)		77 (37.6)	22 (44.0)	0.402
Ascites n (%)	No	102 (49.8)	14 (28.0)	**0.022**
	Low-moderate	71 (34.6)	25 (50.0)	
	Tense-refractory	32 (15.6)	11 (22.0)	
Hb*		12.1±2.2	10.5±2.2	**<0.001**
WBC**		5050 (1580-16400)	4495 (1010-10100)	0.233
Neutrophil*		61.6±10.4	65.6±10.4	**0.022**
Lymphocyte**		24.6 (2.9-53.9)	20.8 (6.88-36.4)	**0.002**
Platelet**		100 (22-370)	79.5 (22-573)	**0.033**
MPV**		8.94 (6.09-19.2)	8.6 (6.07-81.0)	0.134
PDW**		42.5 (14.5-75.5)	46.5 (16.9-81.8)	0.259
Albumin**		3.5 (1.8-4.89)	3.09 (1.57-4.21)	**0.005**
Total bilirubin*		1.55 (0.4-23.5)	1.7 (0.46-12.5)	0.441
INR**		1.27 (1-4.18)	1.38 (1-2.63)	**0.050**
AST**		47 (13-474)	36.5 (14-175)	**0.001**
ALT**		33 (9-880)	23 (10-250)	**0.001**
Creatinine **		0.76 (0.3-5.89)	0.78 (0.49-2.92)	0.319
Na**		139 (122-145)	138 (114-144)	0.153
MELD**		11 (5-39)	12.5 (6-29)	0.121
CPT**		7 (5-15)	7 (5-16)	**0.027**

*****Mean ± Standard Deviation, ******Median (Upper Limit-Normal Limit).

Abbreviations: PVT: Portal vein thrombosis, **DM**: Diabetes Mellitus, **Hb**: Hemoglobin, **WBC**: White blood cell count, **MPV:** Mean platelet volume, **PDW**: Platelet distribution width, **INR**: International normalization ratio, **AST**: Aspartate aminotransferase, **ALT**: Alanine aminotransferase, **Na**: Sodium, **CPT**: Child-Pugh Turcotte score, **MELD**: The Model for End Stage Liver Disease

Ascites in any grade was observed more frequently in PVT group compared to non- PVT (p: 0.022). Patients with PVT had lower median platelet count (100 (22-370) vs 79.5 (22-573), p: 0.033), but mean MPV and PDW levels were similar between groups (p >0.05). Although mean WBC count was similar, mean neutrophil count was significantly higher among those with PVT (61.6±10.4 vs 65.6±10,4, p: 0.022). Mean albumin level was lower for those with PVT (p: 0.005). Although mean total bilirubin level was similar, INR was higher in those with PVT (p:0.050). Patients with PVT had higher median MELD score, but this trend was not statistically significant (p: 0.121). Median CPT score were significantly different and higher in PVT group (p:0.027).

DISCUSSION

PVT was present in 19.6% of our patients. Prevalence of PVT among patients with cirrhosis ranges between 0.6% and 22% [17]. Rate of PVT depends on both severity of cirrhosis and the method used for diagnosis. Similar with our results, Abdel-Razik et al. reported 17.9% of PVT rate in cirrhotic patients [11]. Prevalence of PVT assessed by ultrasound, was reported to be 8–25% in candidates for liver transplantation [18]. A 12-months prospective study of cirrhotic patients by Zocco et al reported a PVT incidence rate of 16% [19]. Ultrasound is sensitive method for diagnosis of PVT in cirrhosis. Ultrasound has 92% specificity and 89% sensitivity in detection of PVT [20]. Sensitivity further declines in the case of partial PVT. It is also

operator dependent and presence of ascites, gas and obesity may also affect the diagnosis. Dynamic BT that we used is a specific tool for diagnosis of PVT in cirrhosis [20,21].

Chen et al. reported age and sex as risk factors for PVT, however age and sex were similar between PVT and non-PVT patients in our study [22]. Diabetes mellitus was defined as an independent risk factor for PVT by Abdel-Razik et al [11]. Similarly, DM was reported as a risk factor for thromboembolic events for cirrhotic patients in a report by Gîrleanu et al. [23]. The rationale for association between diabetes mellitus and PVT comes from endothelial abnormalities that frequently occur among diabetic patients. Endothelial abnormalities may enhance platelet activation. In our cohort, we did not find any association between DM and PVT. We did not assess endothelial abnormalities in our study.

Patients with PVT had lower platelet levels in our study. Two studies by Ushitora et al. and Abdel-Razik et al. reported similar results [11,24]. Platelets are considered as an important source of prothrombotic agents associated with inflammatory markers [6]. Etiology of thrombocytopenia in chronic liver disease is multifactorial. Sequestration of platelets within spleen, suppression of bone marrow due to various causes, including antiviral therapy, alcohol, myelosuppressive effects of hepatitis-C (HCV) infection, or decreased activity of thrombopoietin (TPO), and platelet destruction through anti-platelet antibodies can cause or contribute to cirrhotic thrombocytopenia [25]. Gîrleanu et al. and Abdel-Razik et al. reported higher platelet indices of MPV and PDW for cirrhotic patients with PVT [11,23,26]. Larger platelets and increased platelet indices of MPV and PDW were related to pre-thrombotic state of patients with cirrhosis, despite thrombocytopenia observed in such patients [23]. Other studies reported that platelets with large size have more granules that result in greater thrombotic and proinflammatory activity [7,10]. Compared to controls, PDW, but not MPV, was higher and platelet count was lower in splanchnic vein thrombosis in non-cirrhotic patients in a study by Sharma et al. [27]. However, we did not find any significant difference for MPV and PDW between two groups in our study. Swelling of platelets in edetic acid (EDTA) may be an explanation for this result. Platelets swell in EDTA in a time dependent manner making the measurements with standard anticoagulant of hematology potentially unreliable [28]. Data is more stable when measurement is applied within two hours of blood sampling [29]. This situation should be taken into account during interpretation of MPV and PDW results.

PVT is reported to be a complication of decompensated liver disease and is associated with late-stage liver cirrhosis [20]. White blood cell count, albumin, bilirubin and prothrombin time were not risk factors for PVT in cirrhotic patients according to findings of Abdel-Razik et al [11]. An association between low albumin level and presence of PVT in cirrhotic patients was reported by another study [23]. In our results low mean albumin and higher INR levels were present in PVT group. Median CPT score was also significantly higher in PVT group, however an incrementally higher MELD score in PVT group did not reach statistical significance compared to patients who did not have PVT.

Mean neutrophil count was significantly higher among those with PVT in our study. Cirrhotic patients with portal hypertension are known to have high levels of bacterial translocation and increased serum levels of inflammatory mediators, such as tumor necrosis factor-α, interleukin-6 and interleukin-8 [30]. Platelets indirectly respond to pathogen invasion through interactions with leukocytes and the endothelium. Following antigen recognition, platelets often become activated. Activated platelets can directly kill pathogens, or facilitate pathogen clearance by activating macrophages and neutrophils, promoting neutrophil extracellular traps (NETs) formation, forming platelet aggregates and microthrombi. Platelet activation may also exacerbate inflammation and promote endothelial damage and thrombosis [31]. A recent study reported neutrophil to lymphocyte ratio (NLR) ≥3.14 was associated with 2.89 times increased risk of PVT [32]. In a study by Nery F et al. as markers of systemic inflammation, IL-6 and lymphopenia were predictive for PVT independently of markers of portal hypertension for cirrhotic patients [33].

A meta-analysis by Lin et al. concluded higher MPV to be associated with the presence of PVT. This meta-analysis of 7 studies which were all case control studies included high degree of heterogeneity. Predictive effect of MPV on PVT was less obvious in the presence of larger diameter of portal vein. Meta-analysis concluded that real effect of MPV was dubious and more studies were needed to explain a possible relationship with PVT [29].

Limitation

Our study is retrospective and data does not include portal vein measurements and could not document the relationship between portal vein diameter and platelet indices in the case of PVT. Also,

time period from sample collection to laboratory measurement is not exactly known which precludes the conclusion of EDTA effect precisely.

CONCLUSION

Cirrhotic patients with PVT had more prominent thrombocytopenia, but similar MPV and PDW levels compared to those without PVT. Further studies are needed to elucidate the effect of these parameters for PVT in cirrhosis.

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Original Research

Effects of Exposure to Radiofrequency at 2.45 GHz on Structural Changes Associated with Lipid Peroxidation in Prepubertal Rat Testicular Tissue

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ABSTRACT

Objective: The increasing use of electronic devices, accompanied by advancing technologies, has led to heightened exposure to non-ionizing electromagnetic radiation (EMR). This exposure instigates the accumulation of free radicals and oxidative damage in tissues, consequently impacting biological systems. Notably, the testis is among the tissues adversely affected by EMR. Numerous studies have highlighted the pivotal role of the testis in sperm production, emphasizing the potential implications of any damage on the reproductive system. This study aims to assess the levels of lipid peroxidation through histological evaluation in the testicular tissue of prepubertal male rats exposed to electromagnetic radiation at varying electric field intensities within the 2.45 GHz radiofrequency (RF) range.

Methods: The experimental group comprises six subdivisions, including a sham control group, as well as groups exposed to varying electric field strengths (EFS) of 0.6 V/m, 1.9 V/m, 5 V/m, 10 V/m, and 15 V/m, respectively. Excluding the sham control group, the remaining subgroups were subjected to a daily 2.45 GHz RF exposure for 1 hour starting immediately after fertilization. This exposure to different electric field intensities continued for 45 days post-birth.

Results: The samples obtained from the RF radiation-exposed rats exhibited elevated malondialdehyde (MDA) values and decreased glutathione (GSH) values in the testicular tissue. Furthermore, a comparative analysis between the microwave radiation-exposed group and the control group revealed distinct histological alterations in the testicular tissue.

Conclusion: In conclusion, our findings indicate that exposure to microwave radiation at an electric field intensity of 15 V/m can lead to significant histopathological and oxidative parameter changes in Wistar rats. These results underscore the potential effects of such exposure on human health.

Keywords: Testicular Injury, Prepubertal Rats, Malondialdehyde, Glutathione, Radiofrequency

INTRODUCTION

The widespread use of wireless communication devices, radar systems, communication gadgets, cell phone base stations, high-voltage power lines, radio and television transmitters, transformer substations, electrical appliances in homes and workplaces, and numerous electrical systems worldwide emit a broad spectrum

of electromagnetic radiation (EMR) [1, 2]. Particularly, today's mobile phone models (1800MHz - 2200MHz), laptop computers (1000 MHz - 3600MHz), and wireless networks operate at high frequencies, emitting RF (2.45 GHz) [1, 2]. Numerous studies encompassing both animal and human research have investigated the potential health effects of EMR, shedding light on the need to create and maintain a protected environment during the prenatal, neonatal, and postnatal periods for a healthy childhood. Given the ongoing uncertainties surrounding the impact of EMR, the Council of Europe recommends the implementation of restrictions on internet access and mobile phone usage in educational institutions, as a precautionary measure to safeguard the younger generation from the potential risks of radiation exposure [3]. Due to the increasing sensitivity among children, the World Health Organization (WHO) led a comprehensive study on electromagnetic fields (EMF) between 2006 and 2010, pioneering research on the effects of early exposure to microwave radiation. [4]. The WHO, declaring that the potential adverse effects of EMR on health are more significant in children compared to adults, organized a crucial workshop in 2004 focusing on examining children's sensitivity to electromagnetic fields and addressing related research topics. Scientists from 43 different countries discussed research on this topic and decided to increase the number of studies on the effects of EMF on children. [5]. Thus, international authorities have focused their attention on radiation exposure during childhood.

In assessing the potential environmental impacts on the development of childhood diseases, it is crucial to recognize not only that childhood exposures differ from those of adults, but also that age plays a significant role in sensitivity to these exposures [6]. Our literature reviews revealed that studies investigating the effects of prenatal EMR exposure are widespread, whereas studies examining the effects on infants and young children post-birth are scarce [7]. It has been reported that exposure to 2.45 GHz RF radiation, even at low doses, in the fetal period will lead to profound and definite consequences on health [8-11]. Zhang et al. have identified neurobehavioral disorders associated with exposure and gender-specific learning and memory deficiencies in their study on microwave radiation exposure during the fetal period in mice [12]. On the other hand, Othman et al. have determined that prenatal exposure to the radiofrequency of conventional WiFi devices leads to postnatal neurodevelopmental disorders and oxidative stress [13]. Additionally, various studies indicate that radiation exposure has adverse effects on brain or liver development, causing mutations in DNA and disturbances in the structure of membrane lipids [14-16]. Furthermore, our study's focus on testicular tissue is crucial due to its high sensitivity, as spermatogenesis is regulated by a complex and delicate regulatory mechanism [17]. Several studies have indicated that EMR leads to reductions in spermatogenesis and sperm motility by affecting Leydig cells that produce the testosterone hormone [18, 19]. There are limited studies evaluating the effects of 2.45 GHz RF on prepubertal rat testicular tissue. Hence, this study aims to determine the levels of lipid peroxidation and histologically evaluate the testicular tissue of male rats exposed to electromagnetic radiation at 2.45 GHz RF, starting from prenatal stages up to the prepubertal period, under different electric field intensities.

Main Points;

- Consistent with studies on lipid peroxidation, we observed an increase in MDA level and a decrease in GSH level, especially at 15 V/m electric field intensity. This suggests that EMR exposure causes oxidative stress and biological changes may occur.
- As a result of histological examinations, disruptions in the organization of spermatogenic cells and lack of spermatogonia were detected in seminiferous tubules at 10 and especially 15 V/m electromagnetic field values. These findings are similar to the pathological data reported in testicular tissue after radiation exposure. This disruption in spermatic cell organization will also affect spermatogenesis and sperm quality.

MATERIALS AND METHODS

Experimental Design, Animals and Groups

Male Wistar rats, 45 days old, were obtained from the Ondokuz Mayıs University Animal Experiments Theoretical Ethics Committee (OMÜ HAYDEK) (Samsun, Türkiye). The experimental phases of the study were conducted in accordance with the guidelines of the National Institutes of Health (NIH) for the care and use of laboratory animals. The study protocol was approved by HAYDEK under decision number 2019-23. All animals were kept under a constant 12:12-hour light-dark cycle and were provided ad libitum access to food.

The experimental groups and the procedures applied to these groups are explained below.

Group I (G1) (n=9): The sham group, which did not expose rats to any RF radiation.

Group II (G2) (n=8): The group was subjected to 1 hour/day of 0.6 V/m at 2.45 GHz RF radiation.

Group III (G3) (n=8): The group was subjected to 1 hour/day of 1.9 V/m at 2.45 GHz RF radiation.

Group IV (G4) (n=8): The group was subjected to 1 hour/day of 5 V/m at 2.45 GHz RF radiation.

Group V (G5) (n=8): The group was subjected to 1 hour/day of 10 V/m at 2.45 GHz RF radiation.

Group VI (G6) (n=8): The group was subjected to 1 hour/day of 15 V/m at 2.45 GHz RF radiation.

The 2.45 GHz RF has been utilized in this study due to its widespread use in industrial and medical applications, scientific research, military fields, and household devices in today's. In our study, although radiation exposure can occur for different durations in real-life situations, we adopted the exposure duration from Shokri et al.'s studies as a reference (one hour) [20]. Mantiply et al. measured the minimum EFS value at ground level from microwave towers as 0.6 V/m, and this value was determined as the minimum EFS applied in our study (G2) [21]. Joseph et al. determined that the most dominant Electric Field Strength (EFS) value detected in outdoor settings originated from GSM900, with an EFS magnitude of 1.9 V/m. This value was established as the applied EFS for our G3 group [22]. Additionally Nassiri et al. determined the electric field intensities of many schools and hospitals to be 5-15 V/m [23]. In our study, when determining the EFS values applied to groups G4, G5, and G6 we were inspired by the minimum and maximum values identified by Nassiri et al. Accordingly, we applied them systematically, increasing in order, as 5, 10, and 15 V/m.

The RF exposure was initiated on the first day of pregnancy for the rats in the exposure group (G2, G3, G4, G5, G6), and it continued for 45 days in male offspring rats after birth. The male rats in the sham control group were allowed to grow under normal conditions until reaching prepubescence. The day after the final exposure, prepubescent male rats from all groups were sacrificed under anesthesia, and testicular tissues were collected for lipid peroxidation analyses and histological examinations, which were stored under appropriate conditions. The schematic representation of the conducted applications is summarized in Figure1.

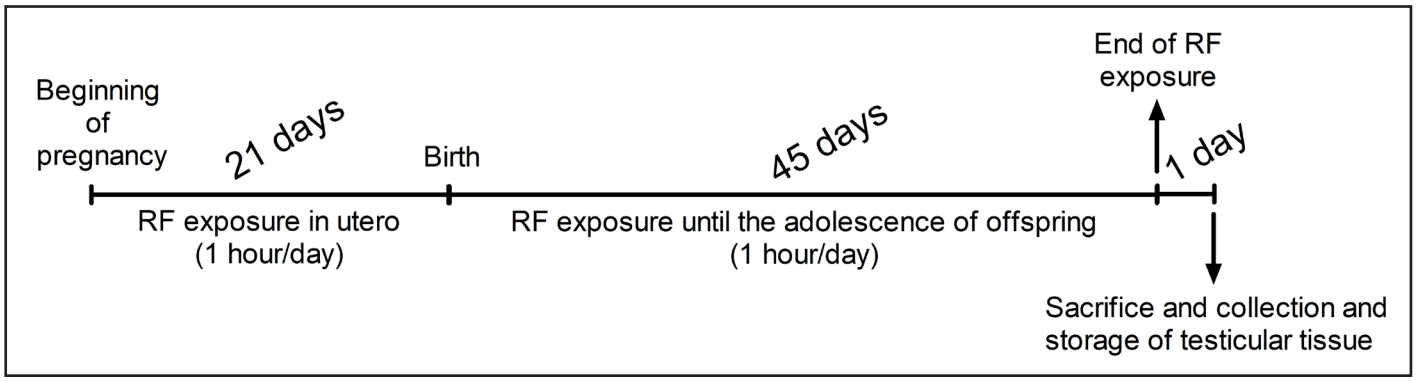

Figure 1. Schematic representation of radiofrequency (RF) exposure application processes.

SAR Evaluation

In this study, a 2004X-RF Wi-Fi system generator (2004X-RF Wi-Fi system generator, Everest Co., Adapazarı, Türkiye) with a Monopole antenna and a maximum output power of 0-1 Watt was utilized to produce 2.45 GHz RF at various electric field intensities. Before the study, Spectran NF-5035 spectrum analyzer (Spectran NF-5035, AARONIA AG, Germany) was used to identify frequencies in the range of 1 Hz to 1 MHz, and RF-Explorer 6G Combo (EMRSS,Germany) was employed for the range of 15 MHz to 6.1 GHz to determine the frequencies present in the environment. According to the Spectran NF-5035 measurements, the highest magnetic field strength level in the environment was found to be 286 μA/m, while the measurement using the RF-Explorer 6G Combo indicated the

highest measurement value as -70 dBm. The isotropic electric field probe for the Narda EMR-300 in the 100 kHz-3 GHz frequency band (EMR300 Probe model, Type 8C, 2244/90.21, Germany) and the Narda SRM-3006 isotropic Model 3501/03 probe in the 27 MHz-3 GHz frequency band range were utilized to measure the ambient electric field and magnetic field strengths before exposure. The lowest and highest ambient electric field measurements were determined to be 92 mV/m and 103 mV/m, respectively.

Different RF levels were applied daily to the five experimental exposure groups, with a plexiglass holder positioned in the center of a monopole antenna to ensure uniform distribution of electric field values. 66-day electric field strength (EFS) measurements were conducted on the head and back regions of each rat for 6 minutes in accordance with the regulations of the Information Technologies Authority. The EFS values measured using the Narda EMR-300 were used to calculate specific absorption rate (SAR) values in 10 g tissue using Computer Simulation Technology (CST) program (CST Studio Suite, version of 2018, USA), which helps the discretization of Maxwell equations by Finite Integration Technique (FIT). Subsequently, the SAR distributions for the whole body and testis were calculated with the EFS values and utilized for dosimetric evaluation.

Biochemical Analysis

The isolated testicular tissues for biochemical analyses were stored -80°C. On the day of the study, all samples were thawed at room temperature and homogenized to obtain the homogenate. Testicular tissue malondialdehyde (MDA) level measurements were conducted using the Mihara and Uchiyama method [24] on the SPECTROstar device (BMG Labtech, Germany), with the results reported in nmol/g protein. Testicular tissue glutathione (GSH) levels were determined using the Ellman method [25] on the SPECTROstar device (BMG Labtech, Germany), with the data presented as mg/g tissue.

Histological Analysis and Johnsen Criteria

For histological analysis, the tissues were fixed in 10% formaldehyde at +4ºC for 24 hours and embedded in paraffin blocks. Sections of 4 µm thickness were taken and stained with Hematoxylin & Eosin. These sections were examined using a BX51 microscope (Olympus) in a blind manner, with all images captured using the DP72 (Olympus) camera. The dimensions of seminiferous tubules, spermatogenic cells, their organization, as well as Sertoli and Leydig cells were evaluated. Furthermore, the testicular biopsy preparations were examined under a microscope, and the Johnsen scoring system was applied for each preparation based on the degree of germ cell maturation, with at least 50 tubules assessed per preparation [26]. The criteria formulated by Johnsen were used to evaluate testicular biopsies. According to these criteria, a ten-point scoring system was described as follows: 10- fully organized germinal epithelium with numerous sperm cells, 9- presence of numerous sperm cells with irregular germinal epithelium, 8- presence of a few sperm cells, 7- no sperm cells but numerous spermatids, 6- absence of sperm cells, but a few spermatids present, 5- no sperm cells or spermatids, but numerous spermatocytes present, 4- a few spermatocytes, 3- only spermatogonia, 2- only Sertoli cells, no germ cells, and 1- acellular seminiferous tubules [27].

Statistical Analysis

The data were analyzed using Prism software (GraphPad, version 6.07, USA) and Microsoft Excel (Microsoft 365 Apps, USA). The number of experimental animals for each group was determined using the Power Analysis method. The normality of the data distribution was evaluated with the Shapiro-Wilk normality test, and the homogeneity of variances was assessed with the Levene test. All data showing a normal distribution were presented as mean ± standard deviation (M ± SD). One-way analysis of variance and the Tukey post-hoc test were used for the statistical analysis data. p-values <0.05 were considered as statistically significant.

RESULTS

SAR Results

The whole-body SAR values of the exposure groups are 0.48 µW/kg, 0.53 mW/kg, 3.44 mW/kg, 15.1 mW/kg, and 34.9 mW/kg. The testicular tissue-specific SAR values for 10g of tissue are 0.0054 mW/kg, 0.0605 mW/kg, 0.4070 mW/kg, 1.7345 mW/kg, and 4.1091 mW/kg, respectively (Figure 2).

Biochemical Results

The MDA levels in Group 6 were significantly higher than the other groups (Group 1, Group 2, Group 3, Group 4, and Group 5) ($p < 0.05$). In comparison to the sham control group (Group 1), there was an increase in all exposure groups (Groups 2, 3, 4, and 5), although it was not statistically significant ($p > 0.05$) (Figure 3).

GSH levels in Group 6 were significantly lower than the other groups (Group 1, Group 2, Group 3, Group 4 and Group 5)

($p<0.05$). Compared to the sham control group (Group I), there was a decrease in all exposure groups (Groups 2, 3, 4 and 5), although not statistically significant ($p>0.05$)(Figure 4).

Histological Results and Johnsen Criteria

No significant morphological deterioration was observed in groups 1, 2, 3 and 4 in the H&E images examined. It was observed that all cell development stages were present, from spermatogonia, primary spermatocytes, spermatids and spermia. The organization of spermatogenic cells was smooth. In Groups 5 and 6, slight disruptions in the organization of spermatogenic cells and lack of spermatogonia were observed in some seminiferous tubules. No significant difference was observed in seminiferous tubule diameters in any of the groups. Leydig cells were present in the intertubular areas in all groups (Figure 5).

According to the Johnsen scoring results of seminiferous tubular sections, a statistically significant difference was detected

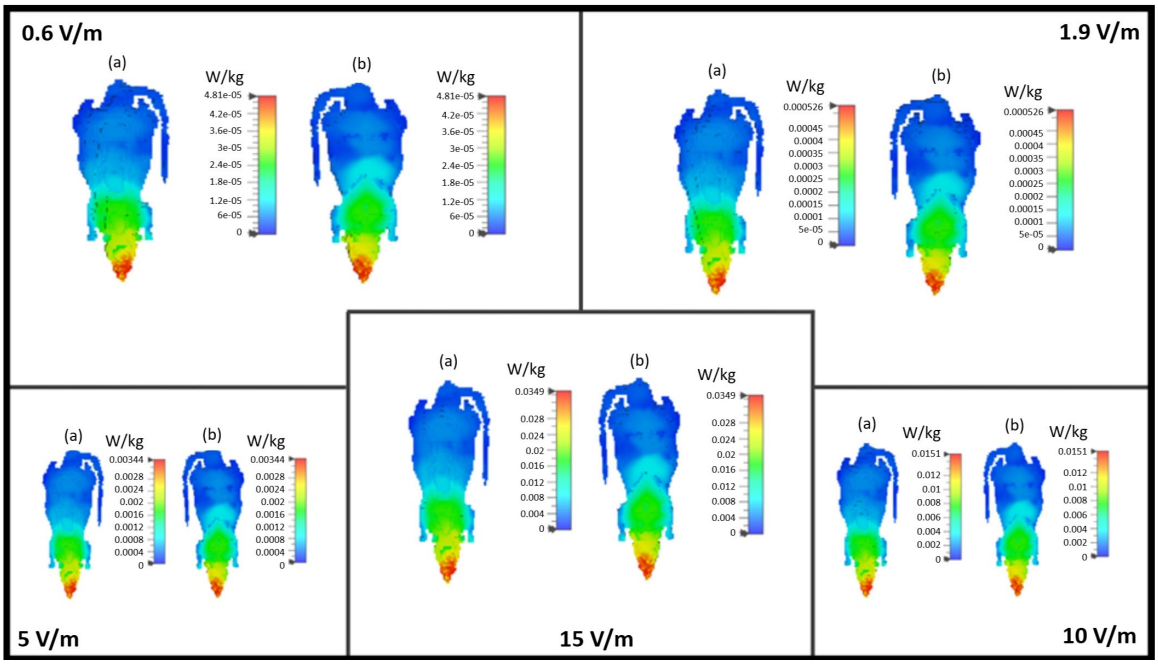

Figure 2. SAR 10g distribution in the rat model. a) Top view b) bottom view

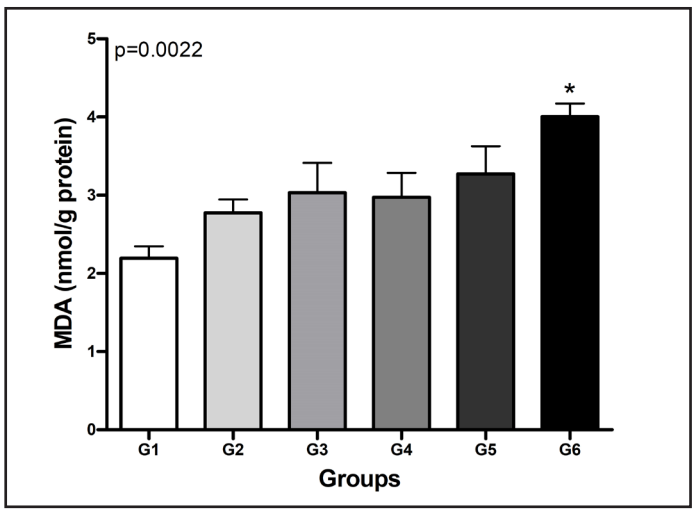

Figure 3. MDA (nmol/g protein) values of testicular tissues of all experimental groups. MDA (nmol/g protein) values of testicular tissues are shown as mean ± standard error. * indicates the degree of significance between the groups at the $p<0.05$ level.

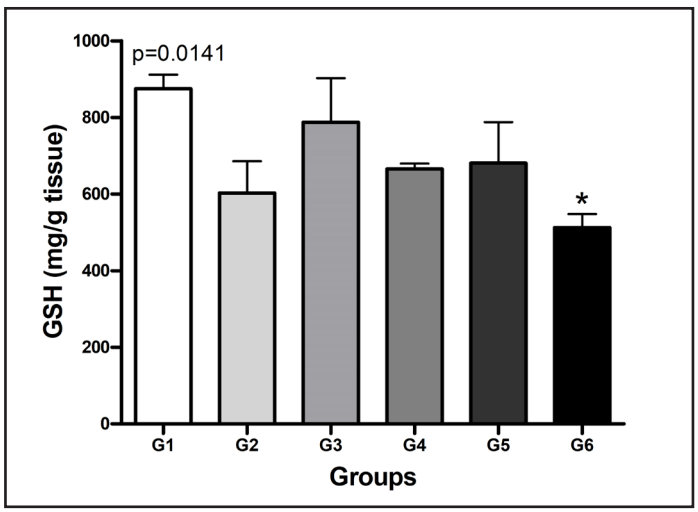

Figure 4. GSH (mg/gr tissue) values of testicular tissues of all experimental groups. GSH (mg/gr tissue) values of testicular tissues are shown as mean ± standard error. * indicates the degree of significance between the groups at the $p<0.05$ level.

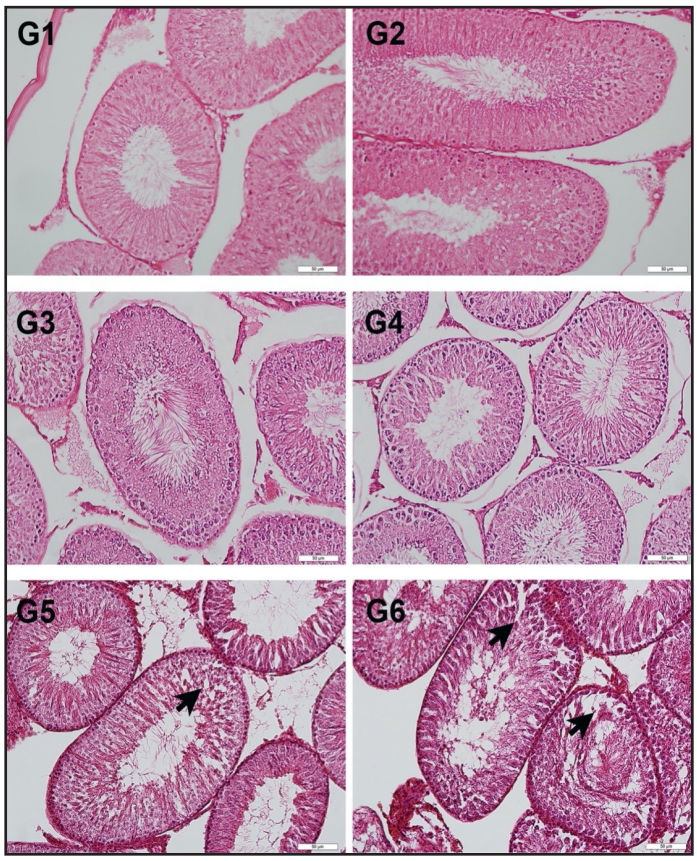

Figure 5. Testicular tissues of groups 1, 2, 3, 4, 5 and 6 are shown at 40x magnification. G1, G2, G3 and G4: Normal seminiferous tubule morphology is observed in Group1, 2, 3 and 4. G5, G6: Disorganization appears in some seminiferous tubules in Groups 5 and 6 (black arrow).

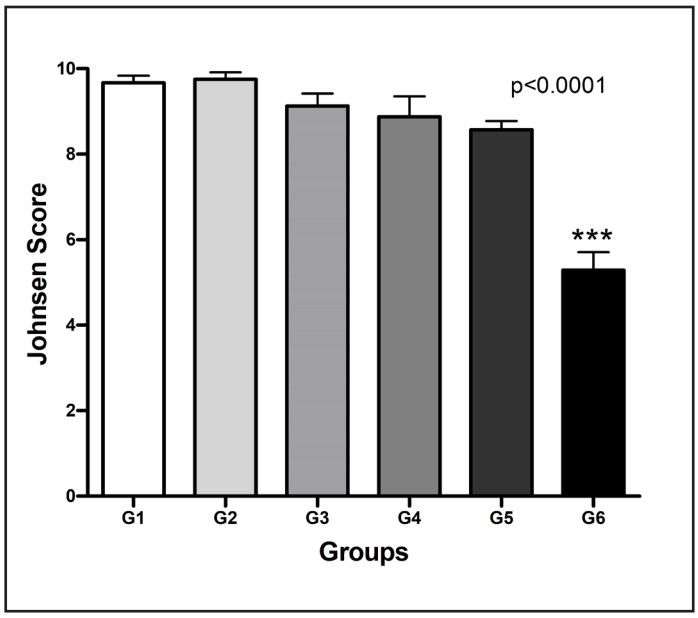

Figure 6. Johnsen score of seminiferous tubular sections. *** indicates significance at p<0.0001 level for G6 vs other groups.

between the G6 group and all other groups (Figure 6).

DISCUSSION

With the advancement of technology, electronic devices that hold a significant place in our daily lives and the associated technical infrastructures have made radiation exposure inevitable. Our study focused on the effects of 2.45 GHz RF exposure, particularly at an intensity of 15 V/m EFS during the prepubertal phase in 45-day-old male rats. Histological examination confirmed that this exposure led to lipid peroxidation. The findings indicate that EMR can significantly impact testicular tissue, particularly at an intensity of 15 V/m.

In recent years, the discussion surrounding the potential harmful effects of EMR exposure on children has gained significant attention. These concerns are particularly relevant during fetal development, as adverse pregnancy outcomes may result from such exposure. The absorption of EMR is influenced by various factors, including the individual's size, the dielectric properties of organs, as well as the frequency and polarization of the radiation [28, 29]. Moreover, the level of EMR exposure is known to impact the cell membrane's penetrative ability, leading to alterations in cellular and metabolic activities. Studies have indicated that EMR exposure induces oxidative stress by producing an excess of reactive oxygen species (ROS), thereby contributing to tissue damage [30-33]. Several researchers have reported a correlation between EMR exposure and the generation of free radicals, which exhibit strong oxidizing capabilities, ultimately interfering with normal cellular functions. These findings align with previous studies that have highlighted the oxidative damage caused by EMR, leading to reduced sperm count, motility, and daily sperm production, alongside significant testicular histopathological changes [14, 34, 35].

Some studies on microwave radiation have indicated that exposure can lead to a reduction in the size of testicular organs and the diameter of seminiferous tubules, potentially resulting in significant impacts on testosterone hormone levels [36-40]. The effects of exposure to 900, 1800, and 2100 MHz RF radiation on DNA breaks and oxidative changes in rat testicular tissue have been investigated. As a result, it has been demonstrated that particularly at high frequencies (1800 and 2100 MHz), there is an increase in the MDA (malondialdehyde) levels, indicating a potential induction of oxidative stress in rat testicular tissue [40]. EMR has the potential to alter biological lipid membranes and these changes are seen in the structural and functional properties of the cell [41]. In another study using 1 gigahertz

electromagnetic field on rats, oxidative stress parameters were investigated and as a result, it was found that MDA level increased and GSH concentration decreased significantly [42]. In a study investigating the effect of 2.45 GHz electromagnetic radiation (EMR) on oxidant and antioxidant status in rats, glutathione peroxidase (GSH-Px) decreased and the concentration of MDA levels increased. It has shown that the role of oxidative mechanisms in EMR-induced tissue damage can improve oxidative damage through antioxidant substances [43]. Studies have shown that EMR can increase lipid peroxidation and thus increase MDA level [44, 45]. In this study, we observed an increase in MDA level (Figure 3) and a decrease in GSH level (Figure 4), especially at 15 V/m electric field intensity, in line with the studies conducted with lipid peroxidation.

Our study evaluated the histopathological effects of exposure to various levels of electromagnetic fields on prepubertal rats by examining the presence of pathological lesions in the testis. Upon examination of the histological structure of the testicular tissue, no significant histological deterioration was observed at electromagnetic field values of 0.6 V/m, 1.9 V/m, and 5 V/m. However, at electromagnetic field values of 10 and 15 V/m, disruptions in the organization of spermatogenic cells in certain tubular structures, along with a lack of spermatogonia, were detected (Figure 5). These findings are consistent with the pathological data reported by Hasan et al., which indicated similar changes in testicular tissue following exposure to 4G mobile phone radiation in mice [39]. The observed disruptions in the organization of spermatic cells are likely to affect spermatogenesis and sperm quality. As a matter of fact, previous studies have demonstrated impaired sperm motility and quality [13, 14], suggesting that exposure to electromagnetic radiation triggers oxidative stress and significant biological changes.

Limitations

In this study, only male rats at 45 days of age (prepubertal) were utilized. Although different electric field intensities were applied, the RF effects were examined only at a single frequency (2.45 GHz), and frequency-dependent effects were not evaluated. While the testicular damage in our data was associated with oxidative stress, ultrastructural evaluations regarding mitochondria, which play a key role in oxidative damage, were not conducted due to our study constraints. It is believed that these limitations, which will be elucidated in future studies, will lead to a better understanding of the damage induced in the testicular tissue by RF exposure.

CONCLUSION

In this study, the evaluation of lipid peroxidation levels and histology in prepubertal male rat testicular tissue exposed to electromagnetic radiation at different electric field intensities at 2.45 GHz RF revealed a dose-dependent negative effect. These findings have the potential to lead to significant changes in the quality of life for individuals exposed to EMR over extended periods. Notably, this exposure may result in infertility due to disruptions in the reproductive systems of individuals or potentially transmit adverse effects to the next generations.

Conflict of interest: The authors declare that they have no potential conficts of interest to disclose.

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Ethical Approval: This study was conducted in accordance with the Declaration of Helsinki. The study protocol was approved by the Experimental Animals Ethics Board of Ondokuz Mayıs University's Experimental Medicine Research and Application Center (Approval number: 2019-23). This research was performed on the animals (rat).

Author Contributions: Conception: AK, NAU – Design: NAU – Supervision: NAU, AK, EGM, BKE – Funding: None – Materials: AK, NAU, EGM, BKE, AA – Data Collection and/or Processing: NAU, AA – Analysis and/or Interpretation: NAU, SV, AA – Literature: AK, NAU – Review: AK, NAU, EGM, AA – Writing: NAU, AK, AA – Critical Review: NAU.

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Original Research

Plasma Leptin, Nesfatin 1, NPY, and Zinc Levels in Obese and Metabolic Syndrome Children

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This study was presented as a oral presentation at the International Congress of Turkish Neuroendocrinology Society [Abstract Book OC-025. 29 June-1 July 2018-Malatya, Turkey].

ABSTRACT

Objective: The aim of this study is to investigate the relationship between leptin, nesfatin 1 and NPY hormones and zinc in boys and girls diagnosed with metabolic syndrome and obesity.

Methods: This study included a total of 6 groups. Group 1 Boy Control, Group 2 Girl Control, Group 3 Obese Boys, Group 4 Obese Girls, Group 5 Boys with Metabolic Syndrome, Group 2 with Girls with Metabolic Syndrome. Plasma leptin, nesfatin-1, NPY (by ELISA method) and serum zinc (by AA method) levels were determined in blood samples obtained from the subjects.

Results: Leptin and zinc levels were significantly higher both in boy and girl patients with metabolic syndrome than in obese and control children. Nesfatin-1 and NPY levels were significantly lower both in girl and boy obese and metabolic syndrome children compared to their control groups.

Conclusion: In the current study a significant increase in plasma leptin and serum zinc levels and a significant decrease of plasma nesfatin-1 and NPY levels were observed in boys and girls with metabolic syndrome. The findings of our study show that leptin, nesfatin-1 and NPY levels may be important biomarkers in the assessment of metabolic syndrome risk in both girls and boys.

Keywords: Children, metabolic syndrome, obesity, leptin, nesfatin 1, NPY, zinc.

INTRODUCTION

Obesity is a disease which causes many diseases such as diabetes mellitus, heart disease, stroke, joint disorders, sleep disturbance, cancer, and hypertension, as well as increases the risk of mortality, and must be definitely treated [1]. Metabolic syndrome is a disease characterized by hyperinsulinemia, dyslipidemia, low high-density lipoprotein (HDL), and atherosclerosis in addition to obesity. Some patients are prediabetics, while the others have Type-2 diabetes [2]. Leptin is a protein hormone secreted mainly from white adipose tissue. Research on leptin, which is involved in the regulation of food intake, has proven the importance of this hormone in the regulation of body weight [3]. Nesfatin-1 is a hormone secreted from the hypothalamus and suppresses the hunger center. The suppression of food intake by nesfatin-1 occurs through a leptin-independent mechanism. [4]. Neuropeptide Y (NPY) is a hormone released by the hypothalamus which increases food intake. Whereas NPY injection to rats causes hyperphagia,

administration of NPY with repeating doses longer than 10 days leads to the development of obesity [5]. Zinc, which plays important roles in growth, development and immune systems, is also closely related to metabolism and the endocrine system. The roles of zinc in the regulation of fat and carbohydrate metabolism, insulin resistance and body weight are critical. Zinc is also involved in the regulation of nutrition. Symptoms of zinc deficiency are the same as those of anorexia nervosa. Therefore, zinc is considered to be involved in the pathogenesis of anorexia nervosa. Obese persons have low zinc and high leptin levels, indicating an important association between zinc and nutrition, thus between zinc and leptin [6]. There is also an important relationship between zinc and NPY regulation during anorexia which occurs in zinc insufficiency [6].

This decrease in nutrition, despite raised NPY levels in zinc insufficiency is defined as NPY resistance [7].

The aim of this study is to determine the relationship between leptin, nesfatin 1 and NPY hormones and zinc, which are effective in regulating food intake in boys and girls diagnosed with metabolic syndrome and obesity.

MATERIALS AND METHODS

Sixty children, 30 boys and 30 girls, who applied to the Pediatric Endocrinology outpatient clinic of Konya Training and Research Hospital were included in this study. Study protocol was approved by the Selcuk University Medical Faculty, Non-interventional Clinical Research ethic committee. The subjects included in the study were divided into 6 equal groups:

Groups for boys:

Group 1 Boy Control: This groups included boys aged 10-15 years and had no any health problem.

Main Points;

- The findings obtained in this study show that plasma leptin, nesfatin1 and NPY levels can be important biomarkers in the assessment of metabolic syndrome risk in both girls and boys.
- A second important finding of the present study is that the high serum zinc obtained in both girls and boys with metabolic syndrome suggests that zinc may play a role in the pathogenesis of the metabolic syndrome.

Group 2 Obese Boys: This groups included boys aged 10-15 years and diagnosed with obesity.

Group 3 Boys with Metabolic Syndrome: This group included boys aged 9-15 years and diagnosed with metabolic syndrome.

Groups for girls:

Group 1 Girl Control: This groups included girls aged 10-15 years and had no any health problem.

Group 2 Obese Girls: This groups included girls aged 10-15 years and diagnosed with obesity.

Group 3 Girls with Metabolic Syndrome: This group included girls aged 10-15 years and diagnosed with metabolic syndrome.

Inclusion and Exclusion Criteria, and Criteria of Exclusion After Beginning of the Study

International Diabetes Federation (IDF) criteria were used as metabolic syndrome criteria. Accordingly, a percentile of body mass index higher than 95th percentile was considered as obesity [8], and a waist circumference measuring higher than 95th according to age and gender was accepted as metabolic syndrome, considering reference values defined for Turkish children [9]. The exclusion criteria included chronic systemic diseases, syndromic obesity, hypercortisolism, hypothyroidism, gonadal dysfunction and drug usage. Any of the subjects in metabolic syndrome, obesity and control groups who rejected to participate was planned to be excluded. None of the subjects requested to be excluded during the study.

Collection of Blood Samples

Plasma and serum samples obtained from the blood samples that were collected from the subjects for routine laboratory investigations following at least 10-12 hours of fasting, were kept at -80°C. NPY (ng/mL), leptin (pg/mL) and nesfatin-1 (ng/mL) levels were determined with ELISA method, and serum zinc levels (µg/dL) with Atomic absorption method in the plasma samples.

Biochemical Analysis

Plasma leptin (Catalog No: ELH-Leptin-1), nesfatin 1 (Catalog No: EIH-NESF) and NPY (Catalog No: EIA-NPY) analyzes were determined as ng/ml by ELISA method.

Serum Zinc Analysis: The zinc levels were determined with Atomic absorption spectrophotometer. The values were

calculated as µg/dL.

Statistical Analysis

Statistical interpretation of the obtained data was done with a computer package program (SPSS 22.0). The arithmetic means and standard deviations (SD) of all parameters were calculated. By applying the "Shapiro-Wilk" test, it was determined that the data showed normal distribution. One-way analysis of variance (ANOVA) was used to determine the difference between the groups, while the Bonferroni test was used to find the group that caused the difference. P<0.05 values were considered significant.

RESULTS

Zinc, Leptin, Nesfatin-1 and NPY Results of Study Groups (girls and boys)

In our study, the highest zinc and leptin levels were obtained in boys and girls with metabolic syndrome (p<0.05, Tables 1 and 2). There was no significant difference between the zinc levels of obese children (girls and boys) and the control group. However, leptin levels of obese boys and girls were higher than the control groups (p<0.05, Tables 1 and 2). Nesfatin-1 levels of boys and girls with metabolic syndrome were significantly lower than all other study groups (p<0.01, Tables 1 and 2).

Nesfatin-1 levels of the obese (girls and boys) groups were lower than the control group (p<0.05) and higher than the metabolic syndrome groups (girls and boys) (p<0.05). The highest plasma NPY levels were obtained in the control groups (p<0.05, Tables 1 and 2).

Correlations of Male Groups Participating in the Study

The correlations between zinc, leptin, nesfatin-1 and NPY levels of the male groups were also examined in the study. Accordingly, it was determined that there was a high level of positive correlation between leptin and nesfatin 1 (r= 0.684 and p=0.029) and leptin and NPY (r= 0.877 and p= 0.001) only in the control group (p<0.05, Table 3). Again in the same group, a high level of positive correlation was observed between NPY and nesfatin 1 (r= 0.805 and p= 0.005) parameters. However, no significant correlation was found in any parameter in the other groups (p>0.05, Table 3).

Correlations of Female Groups Participating in the Study

In the study carried out, the correlations between Zinc, Leptin, Nesfatin-1 and NPY levels of the female groups were examined. Accordingly, there was a high level of positive correlation between NPY and nesfatin 1 (r= 0.730 and p= 0.016) parameters only in the control group (P<0.05, Table 4). In the other groups, no significant correlation was found in any parameter (p>0.05, Table 4).

Table 1. Comparison of Leptin. Nesfatin-1. NPY and Zinc Levels Between the Boys Included in the Study

Groups	Leptin (ng/ml)	Nesfatin-1 (ng/ml)	NPY (ng/ml)	Zinc (µg/dl)
Control	2.77±0.73^{c}	3.92±1.16^{a}	6.71±1.05^{a}	170.17±3.20^{b}
Obese Boys	18.71±5.89^{b}	2.19±0.77^{b}	2.96±0.42^{b}	170.69±3.86^{b}
Boys with Metabolic Syndrome	26.85±12.90^{a}	1.78±0.50^{c}	3.24±0.16^{b}	210.38±4.80^{a}

a.b.c: *Means with different superscripted letters in the same column are statistically significant (P<0.05).

Table 2. Comparison of Zinc. Leptin. Nesfatin-1 and NPY Levels Between the Girls Included in the Study

Groups	Leptin (ng/ml)	Nesfatin-1 (ng/ml)	NPY (ng/ml)	Zinc (µg/dl)
Control	2.78±1.48^{c}	4.16±0.71^{a}	8.78±3.70^{a}	140.24±2.75^{b}
Obese Girls	25.73±7.80^{b}	3.16±0.41^{b}	4.32±1.17^{b}	130.33±5.99^{b}
Girls with Metabolic Syndrome	35.90±12.30^{a}	2.25±0.41^{c}	4.62±1.54^{b}	200.92±6.27^{a}

a.b.c: *Means with different superscripted letters in the same column are statistically significant (P<0.05).

Table 3. Correlations of Male Groups Participating in the Study

Correlations						
Groups			**Zinc**	**Leptin**	**Nesfatin1**	**NPY**
1	Zinc	Pearson Correlation	1	0.358	0.029	0.313
		Sig. (2-tailed)		0.310	0.936	0.379
		N	10	10	10	10
	Leptin	Pearson Correlation	0.358	1	0.684*	0.877**
		Sig. (2-tailed)	0.310		0.029	0.001
		N	10	10	10	10
	Nesfatin1	Pearson Correlation	0.029	0.684*	1	0.805**
		Sig. (2-tailed)	0.936	0.029		0.005
		N	10	10	10	10
	NPY	Pearson Correlation	0.313	0.877**	0.805**	1
		Sig. (2-tailed)	0.379	0.001	0.005	
		N	10	10	10	10
2	Zinc	Pearson Correlation	1	-0.285	-0.095	0.376
		Sig. (2-tailed)		0.425	0.794	0.284
		N	10	10	10	10
	Leptin	Pearson Correlation	-0.285	1	0.434	-0.089
		Sig. (2-tailed)	0.425		0.210	0.808
		N	10	10	10	10
	Nesfatin1	Pearson Correlation	-0.095	0.434	1	-0.066
		Sig. (2-tailed)	0.794	0.210		0.857
		N	10	10	10	10
	NPY	Pearson Correlation	0.376	-0.089	-0.066	1
		Sig. (2-tailed)	0.284	0.808	0.857	
		N	10	10	10	10
3	Zinc	Pearson Correlation	1	0.381	0.345	0.473
		Sig. (2-tailed)		0.277	0.329	0.167
		N	10	10	10	10
	Leptin	Pearson Correlation	0.381	1	0.107	-0.134
		Sig. (2-tailed)	0.277		0.769	0.713
		N	10	10	10	10
	Nesfatin1	Pearson Correlation	0.345	0.107	1	0.497
		Sig. (2-tailed)	0.329	0.769		0.144
		N	10	10	10	10
	NPY	Pearson Correlation	0.473	-0.134	0.497	1
		Sig. (2-tailed)	0.167	0.713	0.144	
		N	10	10	10	10

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Table 4. Correlations of Female Groups Participating in the Study

Correlations						
Groups			**Zinc**	**Leptin**	**Nesfatin1**	**NPY**
1	Zinc	Pearson Correlation	1	-0.033	-0.205	0.010
		Sig. (2-tailed)		0.927	0.569	0.979
		N	10	10	10	10
	Leptin	Pearson Correlation	-0.033	1	-0.151	0.085
		Sig. (2-tailed)	0.927		0.677	0.815
		N	10	10	10	10
	Nesfatin1	Pearson Correlation	-0.205	-0.151	1	0.730*
		Sig. (2-tailed)	0.569	0.677		0.016
		N	10	10	10	10
	NPY	Pearson Correlation	0.010	0.085	0.730*	1
		Sig. (2-tailed)	0.979	0.815	0.016	
		N	10	10	10	10
2	Zinc	Pearson Correlation	1	-0.544	-0.405	-0.202
		Sig. (2-tailed)		0.104	0.246	0.576
		N	10	10	10	10
	Leptin	Pearson Correlation	-0.544	1	0.364	-0.203
		Sig. (2-tailed)	0.104		0.302	0.574
		N	10	10	10	10
	Nesfatin1	Pearson Correlation	-0.405	0.364	1	0.020
		Sig. (2-tailed)	0.246	0.302		0.957
		N	10	10	10	10
	NPY	Pearson Correlation	-0.202	-0.203	0.020	1
		Sig. (2-tailed)	0.576	0.574	0.957	
		N	10	10	10	10
3	Zinc	Pearson Correlation	1	0.210	0.247	-0.298
		Sig. (2-tailed)		0.561	0.492	0.403
		N	10	10	10	10
	Leptin	Pearson Correlation	0.210	1	-0.319	0.086
		Sig. (2-tailed)	0.561		0.368	0.814
		N	10	10	10	10
	Nesfatin1	Pearson Correlation	0.247	-0.319	1	0.259
		Sig. (2-tailed)	0.492	0.368		0.470
		N	10	10	10	10
	NPY	Pearson Correlation	-0.298	0.086	0.259	1
		Sig. (2-tailed)	0.403	0.814	0.470	
		N	10	10	10	10
*. Correlation is significant at the 0.05 level (2-tailed).						

DISCUSSION

Discusssion of Leptin Results

In the current study, leptin levels in the metabolic syndrome groups (both girls and boys) were significantly higher than in all other study groups. Plasma leptin levels of the obesity group were lower than the metabolic syndrome group but higher than the control group.

High leptin concentration in obese children is defined as correlated with various variables of metabolic syndrome. Therefore, it is accepted that higher leptin levels may play an important role in the etiopathogenesis of metabolic syndrome [10]. Therefore, it is suggested that leptin levels may be a potential biomarker in determination of the risk for both cardiovascular diseases and metabolic syndrome in advance [11]. In a study performed for this purpose, leptin levels were determined in 65 healthy, 46 overweight, and 164 obese children (160 boys, 115 girls). At the end of the study, critical leptin level for metabolic syndrome was found as 13.4 ng/dL. It has been proposed that, each 1 ng/dL increase in leptin level may increase the risk of metabolic syndrome by about 3%. It was concluded in the mentioned study that leptin may be a biomarker in determination of the risk for development of metabolic syndrome in prepubertal children [11]. Similarly, it was demonstrated in a study by Obeidat et al. [12] on 630 persons (308 male and 322 female) that serum leptin levels may be an important biomarker in assessment of the risk for metabolic syndrome both in women and men, independently from obesity. In the present study, plasma leptin levels were higher in both boys and girls with metabolic syndrome than the other groups, without gender differences. In our study, the mean plasma leptin level was 35.90 ng/dL in girls with metabolic syndrome, and 26.85 ng/dL in boys with metabolic syndrome. Again in our study, plasma leptin levels of obesity group were lower than the metabolic syndrome, and higher than the controls both in girls and boys. Especially based on the critical leptin level of 13.4 ng/dL recommended by Madeira et al. [11], plasma leptin level of 25.83 ng/dL obtained in obese girls and 18.71 ng/dL obtained in obese boys indicate that both groups are potential candidates for metabolic syndrome. High plasma leptin levels we obtained both in girls and boys with metabolic syndrome are consistent with the results of above mentioned studies [10-13].

Discussion of Nesfatin-1 Results

In the present study, the lowest nesfatin-1 levels were obtained in the metabolic syndrome group. Nesfatin-1 levels of obesity group were higher than the metabolic syndrome group and lower than the control group.

Recently described Nesfatin-1 is a hormone synthesized in the hypothalamus and is effective on the regulation of nutritional behaviour [13]. It has been suggested that nesfatin-1 level found significantly lower in polycystic ovary syndrome (PCOS) patients compared to the control group may be associated with other metabolic markers including metabolic syndrome [13]. It has been reported that nesfatin-1 levels are significantly lower in patients with diabetes and metabolic syndrome compared to healthy persons, decreased level of nesfatin-1 is associated with insulin resistance which is seen in patients with metabolic syndrome [14]. Aksu et al. [15] found that Nesfatin-1 levels were found to be significantly lower in patients with metabolic syndrome compared to healthy controls. It has been suggested that Nesfatin-1, which is known to play a role in the pathophysiological mechanisms of insulin resistance, may be a useful factor for the development of new therapeutic targets in the treatment of obesity in the future [15]. In fact, Nakata et al. [16] proposed that regulatory processes of Nesfatin-1 and its precursor nucleobindin 2 (NUCB2) may provide new targets in the treatment of metabolic syndrome diseases. In this study, decreased Nesfatin-1 levels that we obtained both in the metabolic syndrome groups and obesity groups are consistent with results of above mentioned studies.

Discussion of NPY Results

In the present study, NPY levels were significantly lower both in the metabolic syndrome and obesity groups compared to the control subjects.

Polymorphism functions in the human neuropeptide Y gene (rs16139) is associated with metabolic disorders including metabolic syndrome and early-onset Type 2 diabetes (T2D) [17]. Rabaglino et al. [18] reported that oral administration of antibacterial Triclosan (TCS) to pregnant rats alters food intake in cub rats by increased NPY release, predisposing to metabolic syndrome in adolescence. In conclusion, disruptions in NPY release in metabolic syndrome and/or metabolic syndrome like events are critical [19]. Pathological increase in NPY release promotes energy storage via central and peripheral mechanisms, predisposing to metabolic syndrome [20]. In a study with both obese and non-obese PCOS patients, a significant increase was found compared to the healthy persons. It was concluded that insulin resistance observed in PCOS patients may be related to the differences in NPY release and effects of these differences on

metabolic pathways [21].

In our study, we found decreased NPY levels both in girl and boy metabolic syndrome and obesity groups compared to the control groups. Pathologic increase in NPY is known to promote energy storage via central and peripheral mechanisms, predisposing to metabolic syndrome [20]. Participants of our study consisted of girls and boys diagnosed with metabolic syndrome and obesity. Decreased NPY levels we obtained in these groups are likely to be resulted from increased leptin levels. Because the most prominent effect of leptin hormone is seen by suppressing NPY release at hypothalamus level [22].

Discussion of Zinc Results

There is a proven relationship between zinc and leptin. Zinc can either directly affect leptin gene expression or indirectly increase glucose utilization by adipose tissue, resulting in leptin production [23]. Studies on the relationship between zinc and nesfatin-1 are scarcely any. It has been reported that zinc supplementation has an increasing effect on decreased Nesfatin-1 levels in a high-fat diet-induced obese rat model. As a result, it was concluded in the mentioned study that oral zinc can prevent obesity-related metabolic diseases by improving energy balance [24]. Zinc interferes with the activity of NPY, a potent stimulant of nutrition. Zinc is required for the synthesis of Galanin, a molecule critical for NPY's receptor activity. When zinc is deficient, galanin cannot be synthesized, and in this case, NPY resistance occurs [23]. As a result, there is a critical and complex relationship between zinc, an important trace element, and the hormones leptin, nesfatin-1 and NPY.

In the present study, the highest serum zinc levels were obtained in the metabolic syndrome group. It was questioned that zinc which is an important trace element may play a critical role in metabolic syndrome disease which incidence is rapidly increasing worldwide [25]. Based on this point, in a study conducted on South Korean adult persons, serum zinc levels were determined in 1926 participants. Serum zinc levels were tended to decrease especially among female participants. In the same study, an association between zinc levels and metabolic syndrome disease was underlined, and it was reported that zinc levels should be examined also in terms of gender [25]. There are data indicating that gene variations of ZNT8 gene which is one of the zinc carrier proteins play a role in metabolic syndrome disease [26]. There are also evidence of that zinc also plays a role in insulin resistance seen in patients with polycystic ovary syndrome (PCOS) [25]. PCOS patients have lower zinc levels compared to healthy controls [27]. Torkanlou et al. [28] reported significantly lower serum zinc levels in 706 obese subjects compared to the controls.

Results of the above mentioned authors indicate decreased zinc levels in metabolic syndrome and/or related metabolic disorders. Whereas in the present study we obtained higher zinc levels in the metabolic syndrome group compared to the control subjects. From this aspect, our result is not in parallel with the above reports. Here, the critical issue may be that; all pediatric metabolic syndrome patients are under an increased risk for diabetes [29]. Therefore, attention is drawn to the need for a pediatric Mets definition in children with metabolic syndrome [29]. In the present study, high serum zinc levels we obtained in children with metabolic syndrome may be important for diabetes risk. In diabetes, serum zinc levels vary depending on the type of Diabetes (30). Whereas Type 2 diabetes is in general associated with decreased plasma or serum zinc levels, these levels mostly increase in Type 1 diabetes [30]. Increase of zinc levels in Type 1 diabetes is caused by rapid breakdown of pancreatic beta cells, and passage of zinc stored in beta cells to the extracellular fluid [30, 31]. This event occurs in the beginning of Type 1 diabetes, and later increased urinary excretion zinc results in a decrease in serum zinc levels [30, 31]. In our study, we did not plan follow-up of the girls and boys with metabolic syndrome after the study in terms of the development of diabetes. However, high serum zinc levels can be said to be a marker for diabetes risk, and may be important fro this aspect.

In a study from China, serum zinc levels were determined in 52 patients with metabolic syndrome and 149 healthy control subjects. Patients with metabolic syndrome were found to have higher zinc levels compared to the controls [32]. Higher zinc levels we obtained in girls and boys with metabolic syndrome are consistent with the results of Yu et al. [32].

When results of this study were evaluated as a whole; significant increases were found in plasma leptin and zinc levels of girls and boys with metabolic syndrome, and significant suppression in plasma nesfatin-1 and NPY levels. Whereas leptin levels of the obese girls and boys were lower than the metabolic syndrome groups and higher than the control groups, nesfatin-1 and NPY levels of the obese groups were higher than the metabolic syndrome groups and lower than the control groups.

Based on the results of this study, it can be said that determination of plasma leptin levels may be an important biomarker in evaluation of the risk for metabolic syndrome both in girls and boys. Again, high plasma leptin zinc levels obtained both in girls and boys with metabolic syndrome support the opinion that zinc may play a role in the pathogenesis of metabolic syndrome.

In conclusion; it can be said that high serum zinc levels obtained in girls and boys with metabolic syndrome may be a marker for the risk of diabetes. When results of this study were evaluated as a whole; metabolic syndrome and obesity lead yo change in the levels of leptin, nesfatin-1 and NPY hormones that are effective in the regulation of food intake. These changes may be associated especially with the increased zinc levels in metabolic syndrome.

Limitations

The limiting factor in the current study is the inability to molecularly examine the relationship between metabolic syndrome and zinc.

In future studies, revealing the possible roles of zinc transport proteins in metabolic syndrome may provide important information.

CONCLUSION

When the results of our study are evaluated as a whole;

1. A significant increase in plasma leptin and zinc levels, and a significant reduction of plasma nesfatin-1 and NPY levels were observed in boys and girls with metabolic syndrome.

2.The findings of our study show that leptin, Nesfatin-1 and NPY levels may be important biomarkers in the assessment of metabolic syndrome risk in both girls and boys.

3. High serum zinc obtained in both girls and boys with metabolic syndrome supports the idea that zinc may play a role in the pathogenesis of metabolic syndrome.

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Conflict of Interest: The authors declare that they have no potential conficts of interest to disclose.

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Ethical Approval: This study was conducted in accordance with the Declaration of Helsinki. The study protocol was approved by the Experimental Animals Ethics Board of Selcuk University Experimental Medicine Research and Application Center (2015-1). This research was performed on the animals (rat).

Author Contributions: Conception: Condept:RM;AKB – Design:RM; AKB - Supervision:RM; AKB- Fundings: -Materials:AA, OU, SBB, EM, SSE, MB - Data Collection and/or Processing: AA, OU, SBB, EM, SSE, MB - Analysis and/or Interpretation: AA, OU, SBB, EM, - Literature: AA, OU, SBB, EM, - Writing:OU, SBB- Critical Review:RM;AKB.

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Original Research

Finding the Most Effective Method in Anatomy Lesson in Nursing Education: A Comparison of Classical Lecture and Flipped Classroom

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ABSTRACT

Objective: The objective of this research is to determine the comparative superiority between classical face-to-face education and flipped classroom models from the students' perspective.

Methods: This educational intervention study involved 109 first-term students from Akdeniz University Faculty of Nursing who participated in all the discussed flipped training and classical lecture courses. The study included the administration of feedback forms and an exam.

Results: The averages of the total student feedback scores for the classical lecture and flipped classroom were 45.9± 11.7 and 46.0± 8.5, respectively, and the difference between them was not statistically significant (student t-test, p=0.986). The mean of the knowledge acquisition test total scores were found to be 4.79 ± 1.62 and 4.82 ± 1.65, respectively, and the difference between them was not statistically significant (student t-test, p=0.872)

Conclusion: In conclusion, the results suggest that while the flipped classroom approach does not negatively impact knowledge acquisition or student satisfaction compared to traditional lectures, it does not offer a significant overall advantage. Further research and exploration may be needed to fully understand the potential benefits and limitations of the flipped classroom model in enhancing critical thinking skills and knowledge absorption.

Keywords: Flipped Classroom, Face-to-face Education, Nursing Education, Anatomy

INTRODUCTION

Anatomy courses hold indisputable importance in nursing education, both during faculty education and in professional practice. They are typically included as one of the foundational courses in nursing education, particularly in the first semester, providing a fundamental understanding of nursing core education [1]. While traditional face-to-face lectures have been the long-standing approach for anatomy education in nursing faculties worldwide, there is a growing interest in exploring more effective and innovative educational models [2]. Online education, in particular, has gained widespread use globally [3]. The influence of technology on medical and educational sciences has brought forth a multifaceted impact, encompassing both positive enhancements and potential drawbacks. Counterfeit and replicated journals may lead researchers astray; in order to safeguard valuable discoveries, scientists need to remain watchful, meticulously assess publications, and opt for esteemed journals known for their meticulous peer-review procedures and significant influence ratings [4]. Despite this potential drawback, technology has made significant contributions to the

field of anatomy education, introducing methods such as three-dimensional (3D) anatomy atlases, virtual anatomical images, and digital reminder cards [5]. In parallel, the flipped classroom model has been increasingly adopted in our country and globally, benefiting from technological advancements [6]. Additionally, it has been discovered that delivering lectures in the form of video recordings in higher education is advantageous for students, as they can set their own study pace, engage in question-answering, and interact more with the content [7].

In our study, we hypothesize that the flipped classroom method does not compromise the level of student satisfaction and knowledge acquisition achieved through traditional face-to-face education, and may even yield better results. Accordingly, the aim of this study is to compare the flipped classroom method with the classical education method in terms of student satisfaction and knowledge acquisition levels.

MATERIALS AND METHODS

Study Setting

The undergraduate education program at Akdeniz University Faculty of Nursing is four years, and theoretical and applied Anatomy courses are offered in the education program during the first semester of the first year. Theoretical courses are mainly taught in the form of classical lecture courses, and as of the 2022-23 academic year, some theoretical courses have started to be delivered using the flipped classroom model.

Study Group

The study group consists of 109 students who are first-term students at Akdeniz University Faculty of Nursing and have attended all of the flipped classroom and classical lectures discussed in the study. Students who did not attend any of the flipped classroom and classical lecture courses were excluded from the study.

Main Points;

- The study aimed to compare traditional in-person classes with the flipped classroom model in human anatomy education, focusing on their impact on critical thinking skills and learning.
- Results showed that the flipped classroom didn't hinder learning or satisfaction but didn't significantly outperform traditional lectures in terms of learning outcomes.
- The study didn't establish a clear advantage or disadvantage of the flipped classroom over traditional lectures.

Study Design and Flow

In the study, which was organized as an educational intervention study, flipped classroom activities constitute the educational intervention. Comparisons were made with classical lecture in terms of these two parameters to investigate whether this intervention compromise students' satisfaction and knowledge acquisition. In Akdeniz University Faculty of Nursing, 1A and 1B classes of the first term, randomly divided into two groups, the subject titled 'Anatomy of Peripheral Arteries' was taught using two different methods. One group received instruction through the flipped classroom, while the other group received traditional face-to-face lectures. For the flipped classroom group, two video recordings were prepared: 'Anatomy of Upper Extremity Arteries,' lasting 39 minutes, and 'Arteries of Lower Extremities,' lasting 26 minutes. These videos were uploaded into the section reserved for the lesson in Microsoft Teams (Microsoft Corp, Redmond, WA). Following this, a 50-minute discussion lesson was held with the students who came to the class after watching the video recordings, using interactive methods in the lecture hall.

The group that received traditional face-to-face training attended a 50-minute lecture on 'Peripheral Artery Anatomy,' presented by the trainer using slides. During this session, students were allowed to ask questions or express their opinions.

The same trainer conducted the presentations in the video recordings prepared for the flipped classroom group, managed the discussion session in the flipped classroom group, and delivered the classic face-to-face lecture. The trainer is a faculty member with high skills and experience in education. A questionnaire including a feedback form was administered to all students at the end of the discussion session of the flipped classroom and classical lecture course through Google Forms (Google LLC, Mountain View, CA). In addition, a knowledge acquisition exam consisting of multiple-choice questions was applied to both groups at the end of the classses via Google Forms.

Data Collection Tools Used in the Study

Student feedback form: A 12-item form, prepared by experts in the field of medical education, was used to determine the satisfaction levels of students with both the classical lecture and the lesson taught with the flipped classroom model. The feedback form is presented in Table 1. Students were asked to read each item and evaluate the extent to which they agree with the statement by giving a score between 1 and 5 (1: Strongly

disagree, 2: Disagree, 3: Undecided, 4: Agree, 5: Strongly agree). The total score for each form could range from the lowest 12 to the highest 60 points.

Knowledge acquisition exam: To measure the knowledge acquisition levels of students at the end of the lecture and flipped classroom, a test containing ten multiple-choice questions with five options and a single correct answer was administered. The questions used in the exam are presented in Table 2.

Analysis of the Data

All statistical analyzes were performed using IBM SPSS version 23. The averages and standard deviations of the total scores obtained from the feedback form and the post-lesson knowledge acquisition exam were calculated using descriptive statistics. Student t-test was used to compare the mean values of both groups. Analysis results with a P value below 0.05 were considered significant

Table 1. Student Feedback Form

1. The information provided about the method's process before the training was sufficient for me to understand the flow of the class correctly.
2. The lecture was well-organized, including the allotted time, the timing of breaks, the conduct of discussions, etc.
3. The physical environment in which the training took place was comfortable
4. The educator helped us understand different aspects of the subject through feedback, explanations, and discussions.
5. I believe that the instructor can contribute best to our learning with this method.
6. The instructor successfully managed the entire training process.
7. This instruction method increased my interest in the subject.
8. This instruction method helped me gain a good understanding of the subject.
9. I was able to maintain focus on the subject covered using this method for an extended period of time.
10. I actively participated in the learning process in this method.
11. I believe that the information acquired in this method will be more permanent.
12. Overall, I am satisfied with the implementation of this method.

Table 2. Knowledge Acquisition Exam

What is the name of the second part of the aorta?
What is the name of the first part of A. subclavia?
Which is the first and thickest branch of A. subclavia?
What name does A. subclavia get after it passes under the clavicle?
After which level does A. axillaris continue as A. brachialis?
Which of the following is the normal range of heart rate (heart rate/rate) in an adult when resting?
The pulse groove is located between the tendons of which of the following two muscles?
Which artery pulsation can be felt between spina iliaca anterior superior and tuberculum pubicum on the lig. inguinale?
Which arter can be pulsated on the dorsum of the foot?
Which of the following arteries' pulsation can be felt on fossa malleolus medialis?

RESULTS

This study took place in the context of the 4-year undergraduate nursing program at Akdeniz University Faculty of Nursing in the 2022-23 academic year. The research involved 109 first-term students who participated in both flipped classroom and classical lecture courses Those who did not attend any of these courses were excluded. The study aimed to assess whether flipped classroom activities compromised student satisfaction and knowledge acquisition compared to traditional lectures in human anatomy education.

In the study, two groups of first-term students were randomly assigned to either the flipped classroom or the traditional lecture approach for the subject “Anatomy of Peripheral Arteries.” The flipped classroom group watched two pre-recorded video lectures and participated in a 50-minute discussion session. The traditional lecture group attended a 50-minute in-person lecture on the same topic. Both groups received instruction from the same experienced faculty member.

Data collection involved a feedback form to measure student satisfaction and a knowledge acquisition exam with multiple-choice questions. Statistical analysis was conducted using IBM SPSS, calculating average scores and standard deviations. The student t-test was used to compare group mean values, with significance set at a P value below 0.05.

Table 3. Results of Other Studies in the Literature

Author and Year	Aim	Program	population	Data collecting	Analysis Method	Results
Geist et al. (2015) [8]	Evaluating the place of flipped classroom in knowledge acquisition	Pharmacology education in nursing education	Face to face training n = 40 Flipped training n = 46	Both groups took 3 unit tests and one final test during the semester.	The pre-test and post-test results were evaluated.	In all three unit tests, the flipped training group showed significantly more success than the other group ($p<0.05$). No significant difference was found in the final exam.
Harrington et al. (2015) [10]	Objectively comparing the learning outcomes of both groups	Pre-graduate medical and surgical nursing course	Face to face training n = 41 Flipped training n = 41	The performance of both groups was measured with 3 exams, 24 quizzes and 1 written essay.	explanatory and inferential statistics (t-tests, confidence interval, equivalence intervals MANKOVA)	No significant difference was found between the two groups.
Chu et al. (2019) [9]	Examining the effectiveness of flipped classroom	Evidence-Based Nursing Practice Course	Face to face training n = 75 Flipped training n = 76	An exam was administered to both groups before the course, after the course, and 1 month after the end of the course.	The results before and after the course and 1 month later were evaluated.	The knowledge of both groups increased after the course compared to before the course. The knowledge of the group trained with flipped classroomincreased more and was found to be significant. However, 1 month after the course, the rate of remembering information decreased in both groups.
Holman et al. (2016) [11]	Comparison of flipped classroom with traditional lecture	Pharmacology and psychiatric nursing courses in pre-graduate nursing education	Face to face training n = 119 Flipped training n = 117	A Likert scale questionnaire was applied to both groups at the end of the lesson. In addition, the final exam results were compared.	The results were evaluated with ANOVA one-way variant analysis.	The classroom with face-to-face education in the pharmacology course had higher results than the flipped classroom in the psychiatric nursing course. In the survey results, the overall satisfaction score in both courses was found to be higher in face-to-face education, but it was not considered significant. ($p > 0.05$)

The averages of the total student feedback scores for the classical lecture and flipped classroom were 45.9± 11.7 and 46.0± 8.5, respectively, and the difference between them was not statistically significant (student t-test, p=0.986). The mean of the knowledge acquisition test total scores were found to be 4.79 ± 1.62 and 4.82 ± 1.65, respectively, and the difference between them was not statistically significant (student t-test, p=0.872).

DISCUSSION

In this study, which was carried out to compare the flipped classroom and the classical lecture in terms of student satisfaction and knowledge acquisition levels, no difference was found between the two methods in terms of the parameters compared. Studies in the literature, a summary of which are presented in Table 2, generally found similar results.

In the study by Geist et al., in which both methods were compared in terms of knowledge gain in nursing pharmacology education, students in the flipped classroom group had higher short-term knowledge gains, while no difference was found between the two groups in a long-term comparison [8]. A similar result was obtained by Chu et al. in the Evidence-Based Nursing Practice Course, comparing the two methods [9]. In our study, however, no difference was found between short-term knowledge gains. A similar result was obtained in the study of Harrington et al. [10] There may be various reasons why there was no difference in short-term knowledge acquisition between classical lecture and flipped classroom in our study. The first of these may be related to the subject area, and the flipped classroom applied in an area such as anatomy that contains precise knowledge, producing thoughts on the underlying logic and mechanisms and learning activities by in-depth discussion may not have led to further knowledge gain. Or conversely, considering the characteristics of the field, it is possible for the students to have achieved the same knowledge gained with the classical lecture. The characteristics of the trainer can be considered as another factor. An experienced educator, who gives the classical lectures very effectively and uses interactive methods that can appeal to students with different learning styles during the class, may be adequately transferring the same information in both methods. The possibility that we focus on the most is the inadequacy of the students in fulfilling the requirements of the flipped classroom. If students do not watch the course material prepared for them in advance, the expected efficiency may not be obtained from the in-class meeting time, where no information is given directly and discussions are conducted instead.

Holman et al. compared classical and flipped classroom in pharmacology and psychiatric nursing courses in pre-graduate nursing education and they reported that students recognized multiple benefits of the flipped model, such as heightened engagement between instructors and students, better readiness, and an augmented learning experience [11]. In our study, there was no difference between the two methods in terms of student satisfaction. There are studies showing that students are more satisfied with learner-centered education methods where they actively participate in the lesson, share their opinions and questions with their peers and instructors, and have self-learning opportunities [12]. Flipped education is becoming more and more common all over the world [13], and there are various studies in the literature on this subject. Related to this, our expectation was that students would be more satisfied with flipped classroom, but our findings did not support this. We think there may be two reasons for this. The first one is about student behaviors. We think that students with different learning tendencies may benefit variously from flipped training. Green et al. showed that kinesthetic learners reported positive feelings about the flipped classroom model and visual learners reported negative feelings [14]. The students who attend the discussion session without preliminary preparation cannot participate in the discussions sufficiently, and the trainer does not give any direct information, only contributes to the discussions and puts the final point, so the students who come without preliminary preparation may perceive the process as chaotic. A similar outcome was found in a case study conducted by Herreid et al., according to the study students who are new to this approach might initially show reluctance since it entails them to complete tasks at home instead of being introduced to the subject matter during school hours. As a result, they might arrive unprepared for class, affecting their participation in the interactive learning segment of the course [15]. We think that as students' experience with flipped classroom increases, their satisfaction level will increase as they become more familiar with the method. The second reason is related to the characteristics of the trainer, and if the trainer has ensured the active participation of the students and kept their interest and motivation high by using interactive methods in the classical lecture course, then the satisfaction levels of the students with the classical lecture may have been as high as their satisfaction levels with the flipped classroom. Chen et al. found supporting findings as variations were likely to exist in the knowledge and skills of teachers who developed and conducted the FC activity, as well as in the nature and characteristics of the learning materials used before the class

[16]. Since we did not evaluate the engagement of the students with the class in this study, we cannot reach a definite judgment on this issue.

Lelean et al. also suggested that the studies they reviewed primarily focused on immediate post-implementation outcomes of the flipped classroom method, indicating a need for more longitudinal research to comprehend longer-term performance and knowledge retention effects. Many studies revealed that students lacked a comprehensive understanding of the flipped classroom's rationale and benefits due to insufficient explanations, suggesting a need for improved communication for future implementations [17].

Limitations

The first limitation of the study is related to generalizability, and the generalizability of the results obtained from a study conducted on a single subject with a limited number of students studying in one semester of a school is low. There is a need for studies on this subject with larger working groups and different subjects in different institutions.

The second limitation is related to the study plan, and the active participation of the students in the lesson was not evaluated in this study. If we were able to make this assessment, we would be able to understand whether there was indeed higher student participation in flipped classroom as expected.

The final limitation is related to the study plan, and the long-term knowledge retention was not evaluated in this study. It is thought that such an evaluation will provide important data in evaluating the effectiveness of any training method used.

CONCLUSIONS

Considering our findings, flipped classroom does not seem to compromise knowledge acquisition and student satisfaction, but it is not superior classical lectures. In order to obtain more reliable results, it is recommended to continue using the method and to plan and perform new studies considering the limitations of this study.

Informed Consent: Informed consent was obtained from the students participating in the research.

Conflict of Interest: The authors have no financial or other conflicts of interest to declare.

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Original Research

The Use of Herbal Products/Dietary Supplements and Affecting Factors in Patients Applying to a Pediatric Neurology Outpatient Clinic: A Descriptive Questionnaire Study

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ABSTRACT

Objective: The use of herbal products/dietary supplements (HP/DS) in the pediatric population is increasing day by day. The interaction of HP/DSs with drugs with a narrow therapeutic index such as phenytoin, phenobarbital, and valproic acid, may cause problems in treatment. In this respect, it is very important to determine the use of HP/DS in children with neurological diseases and/or complaints. In this study, it was aimed to determine the use of HP/DS and the factors affecting the use of these products in individuals with a neurological complaint and/or disease who applied to the pediatric neurology outpatient clinic.

Methods: Parents were questioned face-to-face as part of the descriptive questionnaire research. 174 questionnaires with appropriate data quality were included in the study. The statistical software tool SPSS 23.0 was used to analyze the data.

Results: 44.6% of the parents stated that they gave HP/DS to their children. The findings of the analysis showed that kids whose parents use HP/DS are more likely to utilize these items themselves ($p<0.001$). The most commonly used products are linden (70.1%), bee products (26.0%), carob (18.2%), chamomile (13.0%), and lemon (13.0%). It has been determined that the reasons for parents to have their children use HP/DS are to strengthen the immune system (51.9%), improve general health status (40.3%), and supplement normal nutrition (27.3%), respectively.

Conclusion: This study revealed a high frequency of HP/DS use in children with pediatric neurological diseases/complaints in Türkiye. The frequency of HP/DS use was higher in children whose parents tended to consume HP/DS. Considering the high use of these products, healthcare professionals need to inform parents to prevent adverse effects caused by HP/DS.

Keywords: epilepsy, herbal products, dietary supplements, herb-drug interaction, pediatric neurology

INTRODUCTION

The use of herbal products for various purposes such as curing diseases, preventing diseases, or improving general health is as old as human history [1]. According to World Health Organization (WHO), the term "herbal product" includes raw drugs, teas, and pharmaceutical-formulated products obtained from plants [2]. Similar to the European Union regulations, herbal products on the market in Türkiye are classified as food

supplements or traditional herbal medicinal products. If the herbal product is presented with therapeutic properties, the registration application, evaluation, and approval procedures are carried out by the Republic of Türkiye Ministry of Health [3]. On the other hand, dietary supplements that have an important place in the market are defined as "the products for which the daily intake dose is determined by being prepared alone or in mixtures, in capsules, tablets, drops, disposable powder packs, liquid ampoules, dropper bottles, and other similar liquid or powder forms consist of nutrients such as vitamins, minerals, proteins, carbohydrates, fibers, fatty acids, amino acids or concentrates or extracts of the plants, plant, or animal originated substances, bioactive substances, and similar substances that have nutritional or physiological effects to supplement the normal diet" [4].

Herbal products/dietary supplements (HP/DS) are widely used in children and the frequency of use is increasing day by day. HP/DS use rate in children is 85% in Germany, 23% in Scotland, 15% in Denmark, 5% in England, 4.8% in Italy, and 3.9% in the United States [5]. In a study conducted by the United States Food and Drug Administration, Center for Disease Control and Prevention, with 2653 healthy mothers, it was revealed that 9% of parents use HP/DS for their babies in the first year of life [6]. In a study, it was discovered that in Türkiye, the frequency of use of herbs and herbal products in pediatric patients was 74.4% [7].

Main Points;

- There is a high frequency of HP/DS (44.3%) use in children with pediatric neurological diseases/complaints in Türkiye.
- The frequency of HP/DS use is higher in children whose parents tended to consume HP/DS.
- There is a positive relationship between the usage of HP/DS in children and the experience of parents using these products.
- No relationship is found between the duration of diagnosis of the disease and the use of HP/DS.
- There is no correlation between parental sociodemographic and personal characteristics and HP/DS usage in children.

It has been reported that different traditional treatment methods are used in the treatment of various neurological diseases including epilepsy, headaches, traumatic brain injury, neuromuscular disorders, developmental delay, and degenerative brain diseases in children [8-12]. The main purpose of these studies, which were mainly carried out under the umbrella of complementary medicine, was to determine the use of traditional treatment methods such as massage, acupuncture, chiropractic, prayer, and yoga. Even though the use of HP/DS included in traditional treatment methods has been questioned, no detailed evaluation has been made about these products. There is only one study in the literature that determines the frequency of HP/DS use and the factors affecting its use in children with neurological diseases. This study, which interviewed the families of 378 children with epilepsy, showed that the frequency of use of herbal products was 17.2% [13]. This study aims to determine the frequency of HP/DS use, the products used, and the factors affecting the use of these products in patients who applied to the pediatric neurology outpatient clinic.

MATERIALS AND METHODS

Research Population

The population of this descriptive study consists of children and their families who applied to Karadeniz Technical University (KTU) Farabi Hospital Pediatric Neurology Outpatient Clinic between 10.05.2021 and 10.06.2021. It was seen in previous hospital data that the average number of patients admitted to the pediatric neurology clinic was approximately 750 patients per month. Lee et al. reported that the prevalence of herbal product use in patients with epilepsy was 17.2% [13]. As a result of the power analysis using the OpenEpi Version 3.01 program according to the prevalence of herbal product use and the number of patients who applied to the clinic monthly, the minimum number of patients planned to be reached with a 5% error level and 95% confidence interval was determined as 170 people [14]. Children who were at least 3 months after the diagnosis of their neurological disease and/or complaint, who were citizens of the Republic of Türkiye, and who agreed to participate in the study and/or their families, were included in the study. Newly diagnosed and/or patients who refused to participate in the study were excluded. All patients who met the inclusion criteria between the specified dates were tried to be reached without sampling. During this period, a total of 205 people participated in the research. Researchers checked the obtained data, and 174 questionnaires with sufficient data quality were assessed.

QUESTIONNAIRE FORM

The questionnaire forms were created with the data obtained from the current literature review and applied using the face-to-face interview method. Prior to data collection, a pre-test was carried out by conducting the questionnaire to fifteen people. The input was taken into account, the questionnaire was finished, and the unclear expressions and inconsistencies were corrected. All of the patients or parents of the patients were informed about the content and purpose of the study. Only participants above 15 years were interviewed themselves and their responses were supported by their parents. For the other participants, information was obtained from their parents as their ages were not suitable to answer. Informed consent was obtained from each participant and each interview took approximately 10 minutes.

The questionnaire form which contains a total of 12 questions, was divided into three sections: "sociodemographic and personal characteristics", "characteristics of the participants regarding their diseases and treatments" and "features related to the use of herbal products/dietary supplements". The question types of the questionnaire consisted of two-choice, multiple-choice, and open-ended questions. In the sociodemographic and personal characteristics section, parents who participated in the study were questioned on parents' educational status, the gender of the child, and the child's age. In the section about the diseases and treatment characteristics of the participants, the disease diagnosis, presence of additional diseases, long-term/regular drug use associated with the disease, and drugs used were asked. In the section where information on the use of herbal products is researched, the use of these products, the types of products used, the reasons for using HP/DS, and the use of HP/DS by parents were investigated.

Patient statements formed the foundation for this study. No plant samples were obtained from the participants and no botanical description was made.

Statistical Analysis

The statistical package program IBM SPSS 23.0 was used to analyze the data. In descriptive statistics, mean, standard deviation, minimum and maximum are provided for numerical variables, whereas numbers and percentages are provided for categorical variables. To compare categorical variables in independent groups, the Chi-square test family ($\chi 2$ test, Yates Continuity Correction, Fisher's Exact test) was utilized. The statistical significance threshold of $p<0.05$ was approved.

RESULTS

140 (80.5%) of the parents participating in the study were mothers, and 95 (54.6%) of the parents were primary school graduates or did not graduate from a school. While the mean age of the patients was 9.1±4.7 years (min: 6 months, max: 17.5 years), 102 (58.6%) of them were male. 108 (62.1%) of the children were followed up with the diagnosis of epilepsy. The mean duration of diagnosis was calculated as 4.4±3.4 years (min: 3 months, max: 15 years). The sociodemographic and main characteristics of the participants were given in Table 1.

It was demonstrated that 77 (44.3%) of the patients were using HP/DS. In addition, the HP/DS usage habits of the parents of the patients were questioned. 58 (75.3%) of parents who let their children use HP/DS also used these products themselves. The use of HP/DS according to the basic characteristics of the participants is presented in Table 2. Accordingly, no correlation was found between the use of HP/DS and the parent, the parent's educational status, the gender of the child, the child's age, long-term/regular drug use associated with the disease, and the presence of additional disease (each $p>0.05$). A statistically significant relationship was shown between the use of HP/DS and the use of these products by the family ($p<0.001$). It has been determined that children whose families use herbal products use these products more.

The most used products were linden (n=54, 70.1%), bee products (n=20, 26.0%), carob (n=14, 18.2%), chamomile (n=10, 13.0%), and lemon (n=10, 13.0%). The HP/DS variety used by the participants is presented in Table 3.

Parents or patients were asked about the reasons for using HP/DS. It has been determined that the most common reasons for using HP/DS were strengthening the immune system (n=40, 51.9%), improving general health status (n=31, 40.3%), and supplementing normal nutrition (n=21, 27%). The reasons for the participants to use HP/DS are presented in Table 4.

DISCUSSION

The use of HP/DS in children is growing day by day, just as that is in adults. The results of the studies show that the usage of traditional therapy procedures in children has grown due to some chronic diseases and attention deficit hyperactivity disorder [8-10,15]. In these studies, the use of HP/DS was discussed together with the application of traditional treatment techniques. The number of studies examining the use of HP/

Table 1. Main characteristics of the participants (N=174)

	N	%
Parent		
Mother	140	80.5
Father	30	17.2
Other*	4	2.3
Parent's educational status		
Primary school and below	95	54.6
Middle school	18	10.3
High school	41	23.6
College, faculty and above	20	11.5
Gender of the child		
Girl	72	41.4
Boy	102	58.6
Child's age (years, mean ± sd)	9.1±4.7	
<10	91	52.3
≥10	83	47.7
Disease diagnosis[a]		
Epilepsy	108	62.1
Non-epileptic seizure	14	8
Cerebral palsy	7	4
Headache/migraine	17	9.8
Genetic diseases	8	4.6
Developmental delay-autism	22	12.6
Other[b]	31	17.8
Duration of diagnosis (years, mean ± sd)	4.4±3.4	
<5	125	71.8
≥5	49	28.2
Long-term/regular drug use associated with the disease		
Yes	116	66.7
No	58	33.3
Medicines used[a]		
Levetiracetam	48	41.4
Valproic acid	42	36.2
Carbamazepine	17	14.7
Phenobarbital	9	7.8
Clobazam	7	6.0
Oxcarbazepine	6	5.2
Lamotrigine	6	5.2
Other[c]	29	25

Presence of additional disease		
Yes	33	19.1
No	140	80.9

*4 participants (>15 years) were interviewed themselves and their responses were supported by their parents. For the other participants, information was obtained from their parents as their ages were not suitable to answer. [a]More than one option is marked. [b]hydrocephalus, Post-traumatic seizure (n=5), vertigo, trauma (n=3), intracranial mass, ocular deviation (n=2), medulloblastoma, post-infectious ataxia, craniostenosis, brachial plexus, corpus callosum adenosis, SVO, tic disorders, microcephaly (n=1) [c]methylphenidate (n=5), topiramate, clonazepam, diazomid (n=4), baclofen, sertraline, risperidone (n=3), sultiam, aripiprazole, piracetam, zonisamide (n=2), haloperidol, phenytoin, ethosuximide, amitriptyline, atomoxetine (n=1)

Table 2. Herbal products/dietary supplements use according to the main characteristic of the participants (N=174)

	Using (77, 44.3%)		**Not Using (97, 55.7%)**		**p**
	N	**%**	**N**	**%**	
Parent					
Mother	65	46.4	75	53.6	0.536[a]
Father	11	36.7	19	63.3	
Other	1	25.0	3	75.0	
Parent's educational status					
Middle school and below	46	40.7	67	59.3	0.200[b]
High school and above	31	50.8	30	49.2	
Gender of the child					
Girl	35	48.6	37	51.4	0.331[b]
Boy	42	41.2	60	58.8	
Child's age					
<10	41	45.1	50	54.9	0.824[b]
≥10	36	43.4	47	56.6	
Duration of diagnosis					
<5	55	44.0	70	56.0	1.000[c]
≥5	22	44.9	27	55.1	
Long-term/regular drug use associated with the disease					
Yes	52	44.8	64	55.2	0.829[b]
No	25	43.1	33	56.9	
Presence of additional disease					
Yes	15	45.5	18	54.5	1.000[c]
No	62	44.3	78	55.7	
Family HP/DS use					
Yes	58	92.1	5	7.9	**0.000**[a]
No	19	17.1	92	82.9	

$p < 0.05$; [a]: Fisher's Exact Test, [b]: $\chi 2$ test, [c]: Yates Continuity Correction

Table 3. Herbal products/dietary supplements used by the participants (N=77)

Herbal products/dietary supplements	N	%
Linden	54	70.1
Bee products	20	26.0
Carob	14	18.2
Daisy	10	13.0
Lemon	10	13.0
Sage	7	9.1
Rosehip	6	7.8
Ginger	6	7.8
Turmeric	6	7.8
Mint	6	7.8
Fish oil	5	6.5
Mulberry/grape molasses	4	5.2
Oregano	4	5.2
Unknown product	4	5.2
Other[a]	24	31.2

[a]Fennel (n=3), olive oil, coenzyme Q10, anise, lemon balm, walnut, cinnamon, apple cider vinegar, mallow (n=2), St. John's Wort, black pepper, udihindi, blueberry, date extract, cloves, cranberry, broccoli sprouts, cannabinoids, β-glucan, inositol choline, melatonin, menthol, uridine, cytidine, zinc, vitamin B12, pyridoxal phosphate (n=1)

Table 4. Reasons for participants to use herbal products/dietary supplements (N=77)

Reason for use of herbal products/dietary supplements	N	%
Strengthening the immune system	40	51.9
Improvement of general health	31	40.3
To supplement normal nutrition	21	27.3
Sedative	9	11.7
Relieving symptoms associated with the disease	5	6.5
Other[a]	12	15.6

[a]: In diseases other than pediatric neurological diseases (as antipyretic, and abdominal pain, gas pain, anemia, menstrual pain, and stomach disorders) (n=9), For healthy brain development (n=2), To eliminate the side effects of epilepsy drugs (n=1)

DS in children with neurological diseases and/or complaints is limited. In our study, we evaluated the use of HP/DS in children with pediatric neurological disease without the advice of a healthcare professional and factors affecting the use of these products.

A questionnaire study was conducted in Canada in which the use of traditional treatment methods was evaluated in children (n=206) with neurological disorders in two separate centers. In this study, the frequency of herbal product use was found as 25.2% [16]. In a survey study conducted with 352 parents in Saudi Arabia, it was determined that the participants applied various traditional treatment methods to their children with neurological disease, and the frequency of herbal product used among these methods was 30% [17]. Lee et al. reported that the frequency of use of medicinal plants in children with epilepsy was 17.2% in Korea [13]. In a survey study conducted with

parents (n=832) of children with chronic neurological disease in Türkiye, it was revealed that parents resorted to traditional methods for their children's disease. Among these methods, the frequency of using herbal products has been reported as 25% [18]. We found a higher rate of HP/DS use (44.3%) than in previous studies in the literature.

At the beginning of this study, we thought that the use of these products may be higher in children whose parents tend to use HP/DS. Previous studies have revealed that parents' experience of using traditional treatment methods affects their children's use of these practices [16,19-21]. Similar to the hypothesis we established at the beginning and the literature findings, the result of our study showed that there is a positive relationship between the use of HP/DS and the experience of parents using these products. Parents who have positive experiences with these products may also be inclined to use them for their children.

Previous studies have shown different results for the relationship between parents' educational status, child's gender and child's age, and HP/DS use. In some studies, these parameters were found to be associated with the use of HP/DS, while in others, the relationship could not be established [15,18,22-28]. Our results suggest that there was no relationship between sociodemographic and personal characteristics and HP/DS use.

In this study, we evaluated the relationship between participants' disease and treatment characteristics and HP/DS use. Jeong et al. reported that as the duration of diagnosis of the disease increases, the frequency of use of traditional treatment methods increases [20]. Hanson et al. did not find a significant relationship between the duration of diagnosis of the disease and the use of traditional treatment methods [26]. We found no relationship between the duration of diagnosis of the disease and the use of HP/DS. The rate of using traditional treatment methods was high in children with comorbidities [29]. Contrary to this finding, our study showed that the presence of the additional disease did not affect HP/DS use. In studies, no significant relationship was demonstrated between antiepileptic drug use and HP/DS use [19,21]. Our study supported this finding.

Plants contain many phytochemicals that show various pharmacological effects in their composition. The type and amount of these compounds vary according to the plant's growing conditions, geographical conditions, harvest time, and processing method [2]. Herbal medicinal products whose standardization and quality-control processes have been completed should be prepared. These products should be offered for sale through pharmacies. Herbal products are used in society by collecting them from nature or by purchasing them from other platforms such as herbalists, uncontrolled media channels, and the internet. This leads to some problems, including the collection of the wrong plants, adulteration, risk of contamination, and errors in product labels [2]. In the clinic, undesirable effects are encountered due to the effects of contaminants, heavy metals, substances added as a result of adulteration, toxic effects of plants, and allergic reactions [30]. Another problem that may arise due to the use of herbal products is herb-drug interactions. It has been proven in clinical studies that herbs *Andrographis paniculata* (Burm.f.), *Cimicifuga racemosa* (L.), *Citrus paradisi* Macfad., *Echinacea purpurea* (L.) Moench, *Hypericum perforatum* L., *Paeonia lactiflora* Pall., *Silybum marianum* (L.) Gaertn. and *Vaccinium macrocarpon* Aiton interact with midazolam, diazepam, carbamazepine, valproic acid and phenytoin [31-35]. There are case reports in the literature showing that the simultaneous use of herbal products with conventional drugs causes toxic effects in individuals with neurological diseases. In one of these cases, it was stated that the phenytoin and valproic acid levels of a patient who used an unknown dose of nutritional supplements containing Ginkgo and Saw palmetto extracts for about 1 year, decreased to the subtherapeutic level and consequently had a fatal seizure [36]. A male patient with a diagnosis of epilepsy and a history of 200 mg/day carbamazepine use simultaneously used ten grams of carbamazepine and one liter of pure grapefruit juice. Two hours after the use, he applied to the emergency service with unconsciousness and coma. The serum carbamazepine level of the patient was determined as 41.5 mg/L. Despite the treatment applied, it was observed that the carbamazepine level was still high (31.8 mg/L) in the measurements taken after 12 hours. High plasma concentration of carbamazepine despite the treatment applied in this case report was associated with the use of the drug together with grapefruit juice [37]. In a case series, four patients with a history of sertraline use were reported to develop serotonin syndrome within 2 to 4 days following the use of 300 mg St. John's wort two or three times a day [38]. In the literature, there are cases of severe sedation, mania, and serotonin syndrome resulting from the simultaneous use of St. John's Wort with other antidepressants such as fluoxetine and paroxetine [39]. According to our research, the five products that participants used most frequently were linden, bee products, carob, chamomile, and lemon. In this study, the interaction

between the HP/DS most commonly used by the patients and their conventional drugs was evaluated based on the literature data. We could not find sufficient data revealing the interaction between the products that patients prefer to use frequently and conventional drugs. It's conceivable that the use of these products has not been associated with any side effects or drug interactions that have not been documented. Determining the diversity of use of HP/DS is important in terms of detecting possible adverse effects and/or herb-drug interactions. Even if the effects and risks of using dietary supplements or herbal products have not been established, it is essential that you keep in mind that they could have a negative impact on your health. Depending on the herb-drug interactions, the increase in the pharmacological effects of the drugs may result in toxic effects or the desired response may not be obtained in the treatment due to the decrease in the effect. This is clinically important, especially for individuals in the pediatric population. If the blood concentration levels of drugs such as phenytoin, phenobarbital, carbamazepine, valproic acid, ethosuximide, and lamotrigine whose therapeutic levels are followed in the clinic, are at unexplained subtherapeutic or supratherapeutic levels, the use of HP/DS should be considered. HP/DS usage status should be questioned while taking anamnesis from the patients.

As with other studies measuring the tendency to use herbal products, this study has limitations. Our research is susceptible to selection and recalls biases due to the use of questionnaires. The study was single-center therefore, the results cannot be generalized to all pediatric neurology patients in Türkiye. It is impossible to draw conclusions about a particular plant or a product since botanical identifications of the plant species used are not made. However, our study is the first study on the use of herbal products in pediatric neurology patients in our country and its results support the results of studies conducted in other countries. However, our study contributes to the limited literature in terms of directly evaluating the use of HP/DS in pediatric neurology patients.

CONCLUSION

In conclusion, this study revealed that the prevalence of HP/DS use is common in children with pediatric neurological disease/complaints in Türkiye. It has been determined that the children of parents using HP/DS are more likely to use HP/DS. For this reason, health personnel, especially doctors and pharmacists, should take a role in creating social awareness on this issue and inform parents about this issue. In order to prevent cases that may arise from non-medical use, herbal medicinal products should be prepared after standardization and necessary quality control processes and presented to the market through pharmacies. Herbal medicinal products should be used under the control of health personnel.

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Conflict of Interest: The authors declare that there is no conflict of interest.

Ethical Approval: Ethical approval was obtained from the obtained from the Scientific Research Ethics Committee of Karadeniz Technic University, Faculty of Medicine to conduct the study (Date: 05.05.2021 No: 2021/110).

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Original Research

Evaluation of Changes in Facial Attractiveness and Estimated Facial Age After Blepharoplasty with an Artificial Intelligence Algorithm

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ABSTRACT

Objective: The aim of this study is to evaluate the effects of blepharoplasty operation on facial attractiveness and estimated facial age with an artificial intelligence-based algorithm over pre- and post-treatment facial photographs. In addition, it is aimed to make a comparison by reviewing the observable changes according to gender and operation type (upper, lower, combined).

Methods: Preoperative and postoperative photos of patients who underwent open access and copyright-free blepharoplasty operation on social media platforms (instagram and youtube) were collected. The photos were evaluated by an artificial intelligence algorithm trained to estimate facial age and evaluate facial attractiveness.

Results: A total of 541 patients, of which 454 (83.92%) were female and 87 (16.08%) were male. When all patients were evaluated without subgrouping, there was a -1.91±3.35 years younger face age and 0.43±0.64 point increase in facial attractiveness ($p<0.005$).

Conclusion: In this study, the effects of blepharoplasty on facial attractiveness and apparent age are presented with quantitative data. In addition, it has been concluded that artificial intelligence can be used in scoring the apparent age and facial attractiveness after blepharoplasty.

Keywords: Blepharoplasty; Artificial intelligence; Facial age; Facial attractiveness

INTRODUCTION

Blepharoplasty is a surgical procedure that aims to regulate the eyelids aesthetically and functionally. This procedure is usually performed to eliminate aesthetic problems such as puffiness, wrinkles and under-eye bags [1]. Blepharoplasty can also help to eliminate functional disorders of the eyelids [2], and in general, the primary purpose of individuals who have this procedure is to improve facial aesthetics [3,4].

Studies that made various anthropometric measurements of the structures of the eye were previously available in the literature[5,6], and numerous studies[3,4,7,8] have been conducted to evaluate the aesthetic results of blepharoplasty. The main evaluation criteria in these studies were related to the evaluation of professional observers or patient satisfaction. Aesthetic evaluations performed by doctors/surgeons are based on predetermined ideal facial features and rules such as the golden ratio [9]. However, aesthetic evaluations made by professionals based on these rules cannot fully reflect the aesthetic perception of the society [10,11]. Generally, in the aesthetic evaluation made in the society, how attractive the appearance of the person is measured. However, the factor of subjectivity also plays an important role in aesthetic evaluation. Subjectivity can change according to one's own views, preferences, and values.

Therefore, one person's aesthetic evaluation result may differ from another person's aesthetic evaluation result. Subjectivity can emerge from various aspects in aesthetic evaluation [12]. For example, there may be features that one person may find beautiful, and features that another person may not find beautiful. Subjectivity is also affected by factors such as gender, ethnicity, age and cultural values [13]. Therefore, the management of the subjectivity factor is important in aesthetic evaluation. In this way, it can be ensured that the person makes an aesthetic evaluation according to his own views and preferences and is open to the opinions of other people.

Advances in artificial intelligence (AI) have enabled these algorithms to be used in more and more fields [9]. Estimation of facial age and measurement of facial attractiveness are among these areas. In these algorithms, artificial intelligence uses the datasets it has learned by scanning various faces and makes an attractiveness assessment in line with these data [14–16]. Quantitative data such as ideal face ratios can be used to train datasets, while their combination with data from human evaluators can also be used. In this way, in the evaluations to be made, the beauty rules taught by dictation in certain proportions and the subjectivity factor can be used together. In addition, interpretation-free and reproducible results can be obtained.

The aim of this study is to evaluate the effects of blepharoplasty operation on facial attractiveness and estimated facial age with an artificial intelligence-based algorithm over pre- and post-treatment facial photographs. In addition, it is aimed to make a comparison by reviewing the observable changes according to gender and operation type (upper, lower, combined). The hypothesis of this study is that blepharoplasty provides rejuvenation and an increase in attractiveness in patients. In addition, it is thought that these parameters are affected differently according to the type of operation and gender.

Main Points;

- Rejuvenation was observed in 80.4% of the patients.
- There was an increase in attractiveness in 80.5% of the patients.
- There was a higher increase in attractiveness in women than in men.
- There was no statistically significant difference in attractiveness change between the types of surgery

MATERIALS AND METHODS

Obtaining the Sample

In this cross sectional cohort study, it was aimed to obtain a large and multinational sample size. The patient photos, which were open access and copyright-free on social media platforms (instagram and youtube), were the most appropriate source for this purpose. The obtained photographic data has been used only for artificial intelligence evaluation and has not been used or reproduced for any other purpose.

Search settings for Instagram: #blepharoplasty keyword (hashtag), for Youtube: 'blepharoplasty before and after' words were used and the search was carried out on 20.10.2022. All posts in Instagram posts and the first 1000 posts according to relevance in Youtube posts were evaluated.

Inclusion Criteria: (I) Patients undergoing blepharoplasty, (II) Patients with preoperative and postoperative full-face photos shared, (III) Specifying which type of blepharoplasty was performed, upper, lower or combined (upper+lower).
Exclusion Criteria: (I) Shipments that do not specify the type of surgery (II). Covering the patient's face by any method (III). Photographs without a full face photograph and showing only the eye area (IV). Presence of make-up on the patient's face (V). Smile on the patient's face emotional expression such as irritability (VI). Redness, bruising, swelling on the face (VII). Combination of another procedure (dermal filler, face lift, etc.) with blepharoplasty. The photographs were evaluated by an oral and maxillofacial surgeon. After applying the inclusion and exclusion criteria, appropriate photographs were used for the study.

Artificial Intelligence Algorithm

Built a deep Convolutional Neural Network (CNN) for multi-class age classification and attractiveness determination.

For age estimation, the model used a combination of three different datasets as training data and was trained from scratch. These datasets are the APPA-REAL dataset [9], the UTKFace dataset [17], and the IMDB-WIKI dataset [18]. Images were preprocessed by the researcher using proprietary facial recognition software and the model was evaluated in both an extended test set and Adience benchmark. In the test set, the model achieved a categorical accuracy of 51%. Age assessment gave a score between 0 and 100.

For the attractiveness score, the model was further fine-tuned using the Chicago Face Dataset [19] trained on a custom dataset from the BLINQ dating app [20]. The attractiveness rating gave scores from 1 (least attractive) to 10 (most attractive).

In order to prevent the artificial intelligence algorithm from being affected by the background, the background in the photographs of the patients was blackened using Adobe® Photoshop® CS6 software. Then, preoperative and postoperative photographs were evaluated by artificial intelligence in terms of apparent age and facial attractiveness, and the results were recorded.

Calculating Sample Size

In order to determine the sample size for the study, a pilot study was conducted with 30 patients. The objective of the pilot study was to evaluate the change in patients' apparent age as assessed by AI after blepharoplasty. The mean apparent age of the patients before surgery was 40.23±8.52, and the mean postoperative age was 38.45±8.95. Using these data, the effect size was calculated as 0.2035930. With an alpha margin of error of 0.05 and a power of 95%, the minimum required sample size was determined to be 263. However, in order to enhance the statistical power of the study, the researcher aimed to obtain as many samples as possible. The calculations were performed using G*Power software (latest ver. 3.1.9.7; Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany).

Statistical Analysis

Descriptive analyzes were made to give information about the general characteristics of the study groups. In the representation of variables, continuous variables are in the form of mean ± standard deviation; categorical variables are indicated as n (%). In examining the normality of the variables, Kurtosis and Skewness values were examined and values between -1.5 and +1.5 were accepted as having a normal distribution [21]. Paired Samples T-Test was used to examine the change of quantitative variables before and after surgery. Repeated measures one-way analysis of variance (ANOVA) tests were used while evaluating the relationships between the data of quantitative variables and the study groups (gender-operation type). Linear regression analysis was performed to measure the relationship between two quantitative variables. In determining the degree of statistical significance, if the p value is less than 0.05, it was considered significant. IBM SPSS Version 26.0 package software (IBM Statistical Package for the Social Sciences (SPSS) Version 26, SPSS inc., IBM Co., Somers, NY) was used for statistical calculations.

RESULTS

A total of 33,000 posts were evaluated on Instagram, while the first 1,000 posts were evaluated on Youtube, and a total of 34,000 posts were evaluated. A total of 541 patients, of which 454 (83.92%) were female and 87 (16.08%) were male, who met the inclusion and exclusion criteria from these posts were included in the study. The descriptive values obtained as a result of the evaluations made by the artificial intelligence algorithm are listed in Table 1.

A statistically significant rejuvenation and increase in attractiveness were observed after blepharoplasty operation ($p<0.05$) (Table 2). Rejuvenation was observed in 80.4% of the patients and an increase in attractiveness in 80.5%. In subgroup comparisons: Women compared to men, Combined blepharoplasty compared to upper and lower blepharoplasty, lower blepharoplasty compared to upper blepharoplasty provided more rejuvenation ($p<0.05$). There was a higher increase in attractiveness in women than in men ($p<0.05$). There was no statistically significant difference in attractiveness change between the types of surgery ($p=0.169$).

Linear regression analysis was used to identify two possible relationships. The first model (Figure 1) shows that age is related to post-operative attractiveness change (Regression coefficient: 0.135 [95% CI: −0.015; -0.004]; F:9.931; p = 0.02). According to the results of this model, it can be expected that there will be more aesthetic increase after surgery in younger patients. In the second model (Figure 2) shows the preoperative aesthetic score was not associated with the change in attractiveness after surgery (Regression coefficient: 0.059 [95% CI: −0.079; 0.014]; F:1.901; p = 0.169).

DISCUSSION

The evaluation to be made after any cosmetic surgery on the face shows how successful the results of the procedure are and whether the procedure has achieved its goals. Most of the blepharoplasty patients expect an increase in facial aesthetics [22]. The two most important parameters of facial aesthetics are facial attractiveness and facial age. To the best of our knowledge, this is the first study to evaluate the effect of blepharoplasty on facial attractiveness and facial age with artificial intelligence algorithms.

Due to the natural features of a person's face, it is difficult to accurately predict age based on an image. Facial aging variation

is complex and unique to a particular individual and many external factors such as lifestyle and climate [23]. Therefore, two individuals of the same chronological age may appear at different ages. In addition, there is an ordinal relationship and correlation between age tags. Age 40 is closer to 10 than 35, making age estimation more difficult compared to a problem where there is no correlation between grades [24]. Although face perception is controlled by a special region of the brain by the human visual system, age estimation through facial aesthetics can be influenced by individual, cultural and social experiences, but there is no personal information-based intervention with the interpretation of computer and artificial intelligence software applications, because only special algorithms are used [25,26].

As a result of the analysis, it was seen that blepharoplasty provided a rejuvenation in women (Mean: 2.07; p<0.001) and men (Mean: 1.03; p=0.005). The effect of blepharoplasty on facial age has been evaluated in a limited number of studies. One of these studies, Bater et al [4]., evaluated the results of blepharoplasty with a questionnaire study and reported that blepharoplasty provided 1.04 years of rejuvenation, similar to our present study. From this point of view, it can be said that blepharoplasty in the appropriate indication is promising for individuals who want a younger appearance.

Table 1. The gender and type of surgery of the patients who underwent blepharoplasty, and the apparent facial age and facial attractiveness scores obtained as a result of the evaluation of the photographs by artificial intelligence.

		Before surgery (T0)		After Surgery (T1)	
	N	Apparent age (years) Mean±SD	Attractiveness Mean±SD	Apparent age (years) Mean±SD	Attractiveness Mean±SD
All	541 (100.0%)	39.78±9.23	4.91±1.17	37.87±9.08	5.34±1.30
Females	454 (83.92%)	38.87±9.04	5.04±1.06	36.79±8.68	5.51±1.15
Males	87 (16.08%)	44.52±8.75	4.22±1.46	43.48±9.10	4.45±1.65
Subgroups by type of surgery					
Upper	116 (21.44%)	39.24±9.27	4.93±1.19	38.19±9.30	5.47±1.33
Lower	184 (34.01%)	36.57±8.56	4.91±1.23	34.70±8.73	5.33±1.34
Combined (Upper+Lower)	241 (44.54%)	42.49±8.89	4.89±1.12	40.13±8.54	5.34±1.25

Table 2. The change in facial attractiveness scores and facial appearance perceived by artificial intelligence after surgery, and comparison between and within groups.

	Apparent Age				Attractiveness			
	Mean Difference (T1-T0) Mean±SD	Impact of therapy		Difference between groups †p	Mean Difference (T1-T0) Mean±SD	Impact of therapy		Difference between groups †p
		*p	95% CI			*p	95% CI	
All	-1.91±3.35	**0.000**	-2.19;-1.62		0.43±0.64	**0.000**	0.37;0.48	
Subgroups by gender								
Females	-2.07±3.33	**0.000**	-2.38;-1.77	**0.008**	0.47±0.64	**0.000**	0.41;0.53	**0.001**
Males	-1.03±3.36	**0.005**	-1.75;-0.31		0.22±0.58	**0.001**	0.10;0.35	
Subgroups by type of surgery								
Upper	-1.05±3.48	**0.002**	-1.69;-0.41		0.53±0.66	**0.000**	0.40;0.65	
Lower	-1.87±2.96	**0.000**	-2.30;-1.43	**0.003**	0.41±0.62	**0.000**	0.32;0.50	0.169
Combined (Upper+Lower)	-2.35±3.50	**0.000**	-2.80;-1.91		0.39±0.64	**0.000**	0.31;0.47	

* Paired Samples Test

† Repeated Measures ANOVA (Greenhouse-Geisser)

T0: Before surgery ***T1:***After Surgery

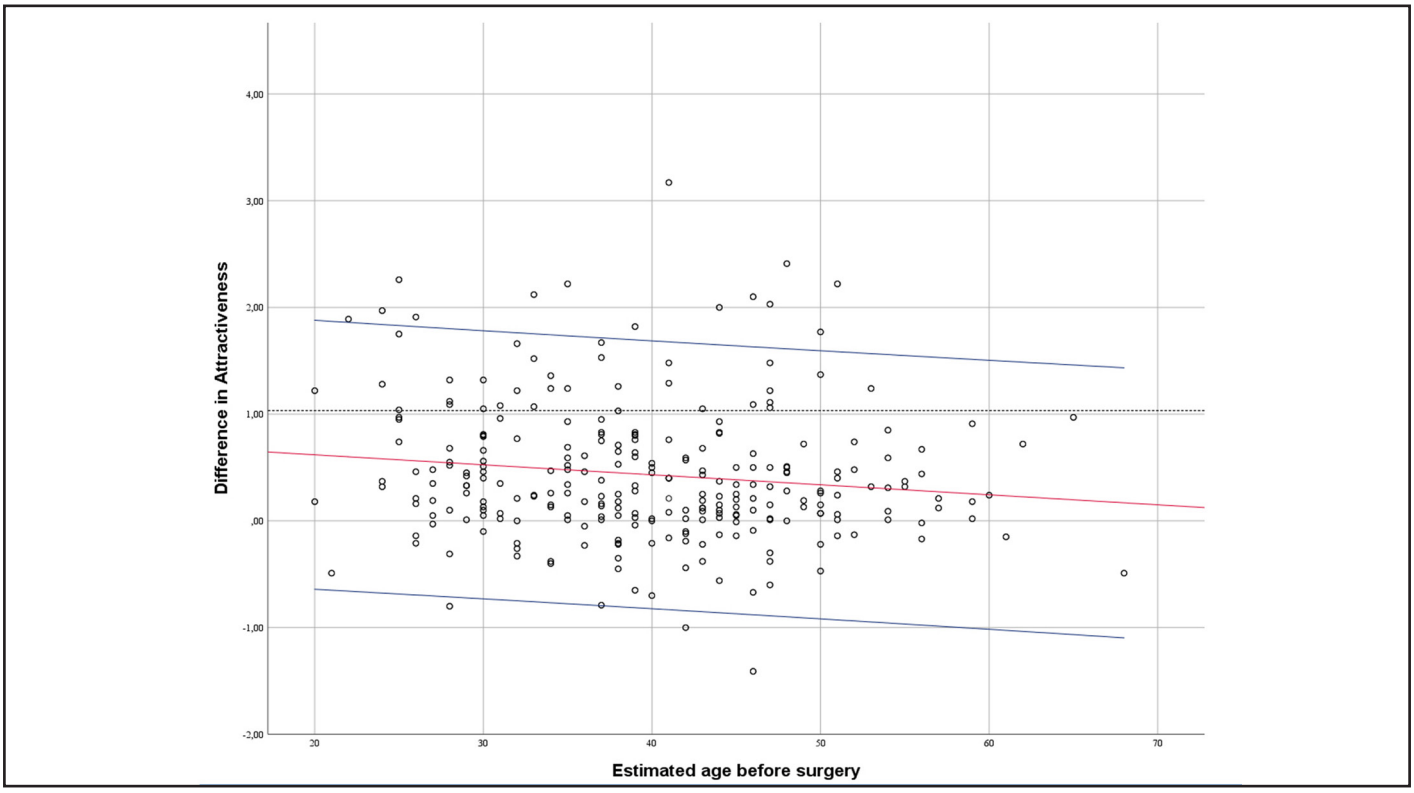

Figure 1. Scatter diagram with changes in attractiveness caused by the blepharoplasty plotted against preoperative age. The black dotted line represents the mean improvement of attractiveness, the red line represents linear regression, and the blue lines represent the 95% confidence interval.

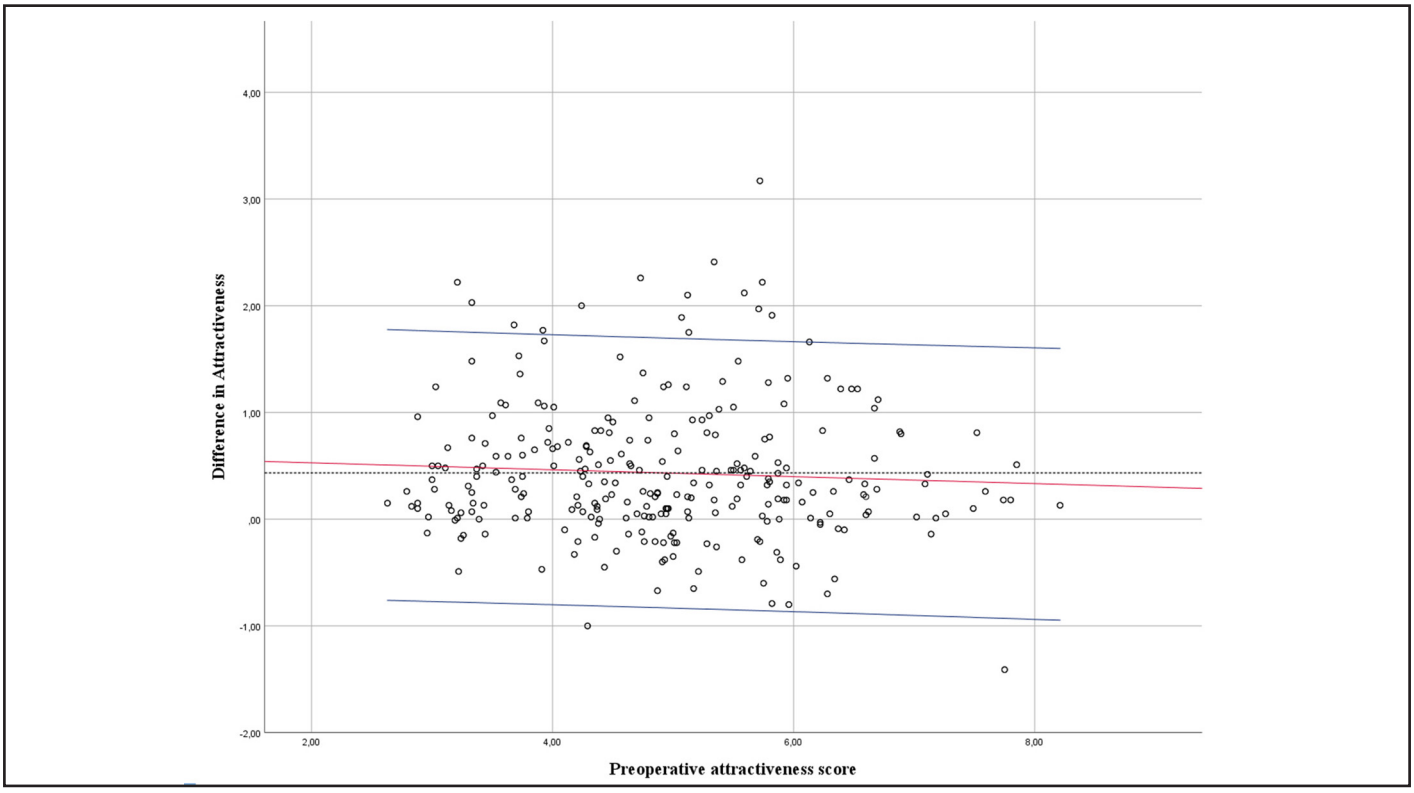

Figure 2. Scatter diagram with changes in attractiveness caused by the blepharoplasty plotted against preoperative facial attractiveness. The black dotted line represents the mean improvement of attractiveness, the red line represents linear regression, and the blue lines represent the 95% confidence interval.

Evaluation of facial attractiveness with quantitative parameters consisting of the ratio of several facial distances to each other may not always give accurate results [27,28]. This is a complex assessment influenced by many factors that depend on the evaluator and the evaluator . The artificial intelligence algorithm used in this study included a combination of certain face proportions along with the dataset from the BLINQ dating app [20], which included more than 17 million evaluations of more than 13,000 face images. Also, Chicago Face Dataset [19] was used to increase accuracy. Thanks to this artificial intelligence algorithm, human and computer comments are combined and the results of the surgery are evaluated in an objective and reproducible way. When the results of present study were evaluated in the facial attractiveness parameter, there was an increase in attractiveness after blepharoplasty in women (Mean: 0.47; p=0.000) and men (Mean: 0.22; p=0.001). The compatibility of the results of the present study with the results of previous studies evaluating the effect of blepharoplasty on facial attractiveness highlights the appropriateness and usefulness of artificial intelligence-based scoring.

By evaluating the results of different operation types, whether upper, lower or combined blepharoplasty was applied, rejuvenation in the perceived age in all three types of surgery also increased the attractiveness. Although combined blepharoplasty provided more rejuvenation than other types of surgery, the increase in attractiveness was less than that of other types of surgery. The model given in Figure 1 may be useful to explain this situation. Although there is no significant difference in preoperative age score between the operation type groups, patients who need combined blepharoplasty are generally older than patients who need only upper or only lower blepharoplasty. The model in Figure 1 emphasizes that the increase in attractiveness after surgery is greater in younger patients. However, it is controversial to what extent the clinical reflection of the small differences between the increases in attractiveness score after upper, lower and combined blepharoplasty can be discerned by the human eye.

Patcas et al. [9] evaluated the changes in apparent age and facial attractiveness after orthognathic surgery with an algorithm similar to the artificial intelligence algorithm in the presented study. In this study, they reported that changes in attractiveness were associated with baseline attractiveness, not age at baseline. The findings of the presented study contradict the findings of the study of Patcas et al.[9] while the increase in attractiveness score in the blepharoplasty patient population was not affected by baseline attractiveness, more attractiveness increased in younger patients. This situation may have arisen for two reasons. First, the blepharoplasty patient population is older than the orthognathic surgery patient population. Secondly, the changes made in the jaws may be perceived differently than the changes made around the eyes. New studies are needed to determine this situation clearly.

The use of artificial intelligence to evaluate clinical outcomes is becoming more and more common nowadays. Obtaining objective and reproducible results and continuous self-education of the algorithm by learning new information helps clinicians and patients. Creating simulation photographs by estimating the postoperative patient image and evaluating these photographs by artificial intelligence will help the clinician to provide realistic information to the patient and to ensure that the patient has realistic expectations. It will also reduce the gap between the patient's level of aesthetic expectation and the level of aesthetics the clinician can offer.

Limitations

Despite all these advantages, there are also situations where artificial intelligence is disadvantageous. While the patient who applies for an increase in facial aesthetics may think that the surgical correction of the area that he thinks is the problem area is very important and very valuable, it is unlikely that this situation can be fully represented by artificial intelligence algorithms. In addition, the attractiveness evaluation based on the artificial intelligence algorithm used in this study was carried out by training the data obtained from a dating platform. However, although it is an algorithm obtained by analyzing more than 17 million attractiveness assessments [20], training these algorithms with much more data input will provide much more inclusive results.

The source of the data evaluated in this study is social media. This poses a potential risk of bias. Each surgeon uses different techniques in operations and our patient population was multinational. In addition, although images with make-up and photoshop were excluded from the study during selection, it is still not possible to guarantee that this potential risk of bias is eliminated.

CONCLUSIONS

In conclusion, with the artificial intelligence evaluation made

in this study, it was concluded that blepharoplasty provides an increase in facial attractiveness and rejuvenation at the estimated facial age. Combined blepharoplasty provides more rejuvenation than only upper and only lower blepharoplasty. Blepharoplasties applied in younger patients provide more aesthetic increase than those applied in older patients. In addition, artificial intelligence has shown promising performance in the evaluation of blepharoplasty results and this article is expected to guide future studies.

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Informed Consent: In this study, which was carried out in line with the Declaration of Helsinki, the data were obtained from open access and copyright-free social media posts.

Conflict of Interest: No conflict of interest.

Authors' Contributions: Conception; Design; Supervision; Fundings; Materials; Data Collection and/or Processing; Analysis and/or lnterpretation; Literature Review; Writing; Critical Review: YB.

Ethical Approval: This study, which was evaluated by the Clinical Research Ethics Committee at Tokat Gaziosmanpaşa University with registration number 2023-KAEK-036, was determined to be exempt from requiring ethics committee approval and was documented as such.

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Original Research

Review of the Renal Artery Anatomy: In Chronic Kidney Disease and Healthy Individuals

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Part of this study was presented as an oral presentation at 2nd International Medical Congress of Izmir Democracy University (IMCIDU 2020).

ABSTRACT

Objective: The purpose of this study was to evaluate renal arteries and aorta anatomy in patients with chronic kidney disease (CKD) and compare them with a control group.

Methods: Computed tomography images of 800 individuals (551 of that were with CKD and 249 of them were completely healthy in terms of urinary system) were evaluated retrospectively. Age range of the individuals 19 - 91 years (mean 61.15 ± 12.58). The differences between the renal arteries diameters, diameters of aorta, courses of the renal arteries, their separation levels from the aorta was investigated between the control group and patients with CKD.

Results: Diameters of aorta and renal arteries are larger in CKD patients than healthy individuals and there was a negative correlation between the diameters of aorta with glomerular filtration rate. No statistically significant difference was observed between CKD patients and healthy individuals in terms of renal artery course. No statistically significant difference was detected between CKD patients and healthy individuals in terms of the branching level of the renal arteries from the aorta.

Conclusion: Our study is one of the first studies which these measurements were made in individuals with CKD, according to the available literature. This study is important in that it reveals that the diameters of the renal arteries are statistically significantly larger in patients with CKD than in healthy individuals. These data may be important for the surgeons in interventional applications.

Keywords: Anatomy, aorta, renal artery, renal insufficiency, tomography.

INTRODUCTION

Chronic kidney disease (CKD) is a pathophysiological process with a decrease in the number of functional nephrons and urinary abnormalities [1]. Accumulation of uremic toxins, electrolyte imbalance, excessive volum load and metabolic acidosis are components of this process [2].

Death in CKD most often occurs due to cardiovascular causes, and CKD is generally accepted as an important risk factor for cardiovascular diseases [2-5]. The risk of death and cardiovascular disease are increased even in the early stages [2, 3, 6]. Both atherosclerosis and arteriosclerosis are important predisposing factors for this cardiovascular death [7]. Cardiovascular death

can occur many causes for atherosclerosis to heart failure, consisting by different mechanisms [2]. Mortality rates from causes such as pulmonary embolism, cerebrovascular disease and myocardial infarction were remarkably higher in dialysis patients than in the general population [8]. All these data make it necessary to understand the changes in the vascular system of CKD patients.

With the progression of kidney failure, premature vascular aging and arterial stiffening occur. This stiffness is more prominented in the aorta than peripheric vessels. With premature vascular aging, an widening in arterial diameter develops and arterial wall hypertrophy can not compensate this [9]. Increase in arterial diameter and arterial stiffness are involved with arterial remodelling in patients with end stage renal disease [5]. Arterial remodeling, stiffness and expansion may occur even in the early stages in patients with a decrease in glomerular filtration rate (GFR) [9].

Renal arteries are two arteries branching from the aorta to the right and left sides, just below the superior mesenteric artery. The right kidney is positioned lower than the left kidney. However, the right renal artery seperated from the aorta at a higher level than the left renal artery [10].

We planned the current study to evaluate whether any changes in renal artery and aortic anatomy play a role in explaining the increased cardiovascular mortality rate and to see how the arterial wall hypertrophy and remodeling in the cardiovascular system of CKD patients reflects on the diameter of renal artery and aorta.

Main Points;

- Chronic kidney disease is a pathological process that has serious effects on the cardiovascular system.
- In this study, we aimed to investigate the effect of chronic kidney disease on renal arterial anatomy.
- Diameters of renal arteries were larger in patients with chronic kidney disease, than healthy individuals.
- Chronic kidney disease patients and healthy individuals did not exhibit a statistically significant difference in whether the renal artery followed a tortuous or straight course.
- There was no statistically significant difference between patients with chronic kidney disease and healthy individuals in terms of the vertebral level at which the renal arteries seperated from the aorta.

MATERIALS AND METHODS

In this retrospective study, approved by the local ethics committee (2019/238 approval number) we evaluated the computed tomography (CT) images of 800 individuals (551 of that were with CKD and 249 of them were completely healthy in terms of urinary system).

Right renal artery of 24 CKD patients were excluded in the study because of right renal agenesis in 4 patients, malignant neoplasm in the right kidney in 1 patient, right nephrectomy in 17 patients, and severe atrophic right kidney in 2 patients. The left renal artery of 16 CKD patients were also excluded because 1 patient had hematoma in the left kidney, left otonefrectomy in 1 patient, left nephrectomy in 11 patients and severe atrophy in the left kidney in 3 patients. The contralateral kidneys of these patients were included in the study.

Examinations were done using 256 – sections double tube CT device (Siemens, Somatom, Definition Flash, Germany) and using 16 – sections CT device (Siemens, Scope, Germany) at routine 3 mm section thickness. CT images of the patients with a cross – section thickness of 3 mm were transferred to the PACS (Picture Archiving Communication Systems). In patients with both contrast and non - contrast tomography, the examinations were performed on contrast - enhanced tomography. Renal arteries were evaluated morphologically and morphometrically on the coronal, axial and sagittal plane images.

The diameters of the renal arteries and the aorta in the level where the renal arteries depart from were measured by computed tomography (Figure 1 and Figure 2). Measurements were made by selecting the artery with the largest diameter that seperates from the aorta and enters the hilum of the kidneys in patients with more than one renal artery feeding one kidney. The difference between the renal arteries diameters, diameters of aorta, the course and shape of the renal artery, its separation level from the aorta was investigated between the healthy individuals and CKD patients. Curved and angular arteries were considered to be tortuous (Figure 3).

Statistical Analysis

IBM SPSS 21.0 package program was used to analyze the data. Before statistical analysis, the normality of the data was controlled with the Shapiro-Wilk normality test. Variables are presented as mean ± standard deviation and/or median (min-

max). Independent sample t test or Mann Whitney U test were used in the comparison of the two groups. In comparison of categorical data, Pearson chi-square and Fischer's Exact test were used according to the expected frequency and size of the crosstab. Statistical significance level was taken as 5%. Relationships between numerical parameters were examined with Spearman's rho correlation coefficient.

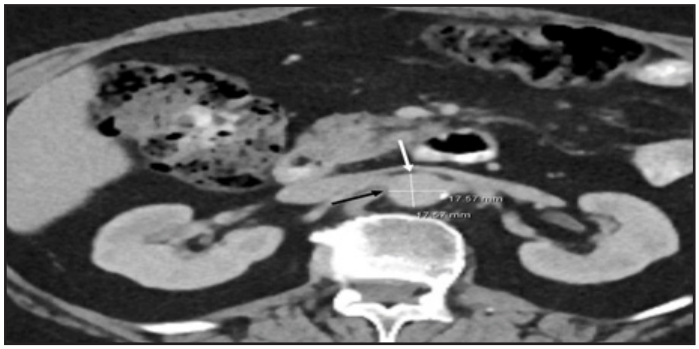

Figure 1. The morphometric measurements of the transverse (black arrow) and anteroposterior (white arrow) diameters of the aorta

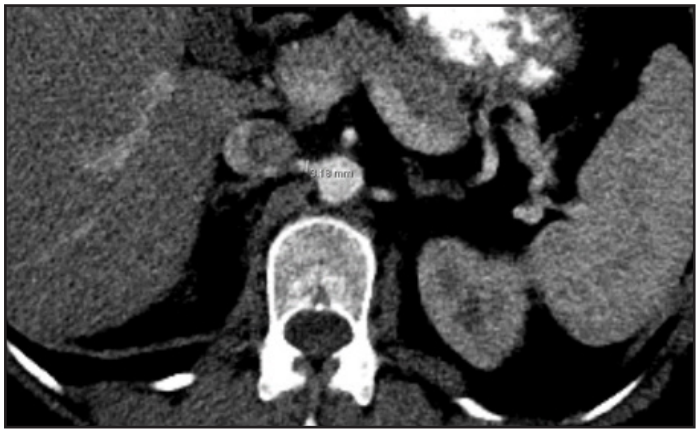

Figure 2. Measurement of right renal artery diameter

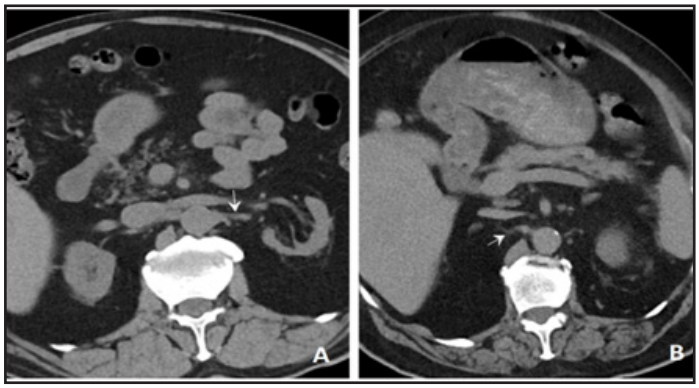

Figure 3. Course of the renal arteries **A**: a straight left renal artery, **B**: a tortuous right renal artery

RESULTS

Age range of all individuals included in study was 19 – 91 years. The average age of CKD patients is 62.01 ± 14.52, the average age of healthy individuals is 59.25 ± 6.06; and the average age of all individuals included in the study was 61.15 ± 12.58.

292 of the patients with CKD were male, 259 were female and 137 of the healthy individuals were male and 112 were female. 371 of all patients were female and 429 were male. GFR values of 439 of 551 patients with a diagnosis of CKD could be reached. The mean GFR value of patients with CKD is 44.86 ± 35.02 ml/min per 1.73 m². Only 44 of the healthy individual's GFR value (mean 101.68 ± 9.37 ml/min per 1.73 m²) were available on the records.

There was a negative correlation between the diameters of aorta with GFR in individiuals. There was a positive correlation between age and aortic diameter, there was a positive correlation between age and renal artery diameter (Table 1).

Arterial diameters of healthy individuals and CKD patients are shown in Table 2 and Table 3. All arterial diameters are larger in patients with CKD than healthy individuals. The diameter of left renal artery was statistically significantly higher ($p < 0.001$) in patients with than healthy individuals. The diameter of right renal artery was statistically significantly higher ($p < 0.001$) in patients with CKD than healthy individuals. Aortic transverse and antero-posterior diameters in the section where the right renal artery seperates from the aorta were significantly larger in CKD patients than healthy individuals ($p = 0.007$ and $p = 0.013$; respectively). The transverse diameter of the aorta, at the level where the left renal artery seperates from the aorta, was found to be statistically significantly higher ($p = 0.004$) in CKD patients compared to healthy individuals. The antero-posterior diameter of the aorta, at the level where the left renal artery seperates from the aorta, was found to be larger in CKD patients compared to healthy individuals, no statistically significant difference was observed ($p = 0.056$).

All renal arteries and aortic diameters are statistically significantly larger in males than females (Table 4 and Table 5).

Left renal artery was tortuous in 65% of CKD patients and 67.1% of healthy individuals. Right renal artery was tortuous in 70.2% of CKD patients and 74.3% of healthy individuals. There was no statistically significant difference between the healthy

control group and CKD patients in terms of the course of the left and right renal artery (p = 0.579 and p = 0.239, respectively [Pearson chi-square]).

Left renal artery was most commonly separated from the aorta at the L1 corpus level in patients with CKD; in healthy individuals, it was most often separated from the L1 - L2 intervertebral disc level. Right renal artery was seperated from most frequently the L1 corpus level in patients with CKD and healthy individuals. Level of renal ostium in CKD patients and healthy individuals are shown in Table 6. No statistically significant difference was detected between healthy individuals and CKD patients in terms of the level of both the left and right renal ostium with respect to the vertebral level (p = 0.150 [Pearson chi-square], p = 0.889 [Fisher's exact test]; respectively).

Table 1. Correlation of aorta and renal artery diameters with age and GFR

	Transvers diameter of the aorta at the level of the left renal ostium	**Antero – posterior diameter of the aorta at the level of the left renal ostium**	**Diameter of the left renal artery**	**Transvers diameter of the aorta at the level of the right renal ostium**	**Antero – posterior diameter of the aorta at the level of the right renal ostium**	**Diameter of the right renal artery**
Age						
r	0.404	0.396	0.170	0.389	0.393	0.136
p	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**
N	*784*	*784*	*784*	*776*	*776*	*776*
GFR						
r	-0.264	-0.263	-0.008	-0.230	-0.224	-0.084
p	0.000**	0.000**	0.854	0.000**	0.000**	0.071
N	*475*	*475*	*475*	*466*	*466*	*466*

**p < 0.001, r = Correlation coefficient, N= Number of cases, GFR= Glomerular filtration rate
Spearman's rho correlation test

Table 2. Measurements of aorta and renal artery diameters according to the groups

	All individuals N = 784	**Healty individuals N = 249**	**Patients with CKD N = 535**	**p**
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	
Antero-posterior diameter of the aorta at the level of the left renal ostium	18.81 ± 2.95 mm	18.54 ± 2.55 mm	18.94 ± 3.11 mm	0.056
Diameter of left renal artery	5.58 ± 1.55 mm	5.13 ± 1.15 mm	5.79 ± 1.66 mm	0.000**
	All individuals N = 776	**Healty individuals N = 249**	**Patients with CKD N = 527**	**p**
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	
Transvers diameter of the aorta at the level of the right renal ostium	19.26 ± 3.03 mm	18.87 ± 2.54 mm	19.45 ± 3.23 mm	0.007*
Antero-posterior diameter of the aorta at the level of the right renal ostium	19.25 ± 2.93 mm	18.90 ± 2.47 mm	19.42 ± 3.11 mm	0.013*
Diameter of right renal artery	5.49 ± 1.61 mm	5.05 ± 1.15 mm	5.70 ± 1.74 mm	0.000**

*p < 0.05, **p<0.001, N= Number of cases, SD= Standart Deviation, CKD= Chronic kidney disease
Independent sample t test

Table 3. Transvers diameter of the aorta at the level of the left renal ostium according the groups

	All individuals N = 784	**Healty individuals N = 249**	**Patients with CKD N = 535**	**p**
Median	18.19 mm	17.4 mm	18.45 mm	0.004*
Minimum	10 mm	12 mm	10 mm	
Maximum	40 mm	26 mm	40 mm	
*p < 0.05, N= Number of cases, CKD= Chronic kidney disease Mann Whitney U test				

Table 4. Diameters of aorta and renal arteries according to the sex.

	All individuals N = 784	**Female N = 365**	**Male N = 419**	**p**
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	
Antero-posterior diameter of the aorta at the level of the left renal ostium	18.81 ± 2.95 mm	17.67 ± 2.69 mm	19.80 ± 2.80 mm	0.000**
Diameter of left renal artery	5.58 ± 1.55 mm	5.36 ± 1.46 mm	5.78 ± 1.60 mm	0.000**
	All individuals N = 776	**Female N = 360**	**Male N = 416**	**p**
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	
Transvers diameter of the aorta at the level of the right renal ostium	19.26 ± 3.03 mm	18.20 ± 2.84 mm	20.18 ± 2.90 mm	0.000**
Antero-posterior diameter of the aorta at the level of the right renal ostium	19.25 ± 2.93 mm	18.21 ± 2.69 mm	20.15 ± 2.83 mm	0.000**
Diameter of right renal artery	5.49 ± 1.61 mm	5.20 ± 1.58 mm	5.75 ± 1.59 mm	0.000**
**p<0.001, N= Number of cases, SD= Standart Deviation, CKD= Chronic kidney disease Independent sample t test				

Table 5. Transvers diameter of the aorta at the level of the left renal ostium according sex

	All individuals N = 784	**Female N = 365**	**Male N = 419**	**p**
Median	18.19 mm	17 mm	19.09 mm	0.000**
Minimum	10 mm	10 mm	13 mm	
Maximum	40 mm	28 mm	40 mm	
**p<0.001, N= Number of cases Mann Whitney U test				

Table 6. Renal ostium levels respect to the vertebraes.

Right renal ostium level	**All individuals N = 776**	**Healthy individuals N = 249**	**Patients with CKD N = 527**
T12	16 (2.1%)	5 (2%)	11 (2.1%)
T12 – L1	58 (7.5%)	19 (7.6%)	39 (7.4%)
L1	330 (42.5%)	100 (40.2%)	230 (43.6%)
L1 – L2	282 (36.3%)	97 (39%)	185 (35.1%)
L2	78 (10.1%)	26 (10.4%)	52 (9.9%)
L2 – L3	11 (1.4%)	2 (0.8%)	9 (1.7%)
L3 – L4	1 (0.1%)	0 (0%)	1 (0.2%)
Left renal ostium level	**All individuals** *N = 784*	**Healthy individuals** *N = 249*	**Patients with CKD** *N = 535*
T12	11 (1.4%)	3 (1.2%)	8 (1.5%)
T12 – L1	44 (5.6%)	17 (6.8%)	27 (5%)
L1	301 (38.4%)	82 (32.9%)	219 (40.9%)
L1 – L2	303 (38.6%)	111 (44.6%)	192 (35.9%)
L2	115 (14.7%)	34 (13.7%)	81 (15.1%)
L2 – L3	10 (1.3%)	2 (0.8%)	8 (1.5%)
N: Number of cases, CKD= Chronic kidney disease			

DISCUSSION

Recognition of the normal anatomy of the renal arteries is of great importance for surgeons and radiologists performing diagnostic or therapeutic renal angioplasty. Given the impact of CKD on the cardiovascular system, it is inevitable that the artery that supplies the kidney will receive its share of this effect. Our study is one of the first studies to evaluate the renal artery anatomy in terms of arterial diameter, arterial course and renal ostium level in CKD patients, according to the available literature.

We found that all renal arteries and aorta diameters were larger in individuals with CKD than in healthy individuals. Exposure of the arterial wall to high pressure triggers an increase in vessel diameter [11]. Kidney patients, with difficult blood pressure control, are expected to have larger arterial diameters than healthy individuals. The fact that the individuals included in our study are generally composed of the elderly population and hypertension is seen quite frequently in the advanced age suggests that impaired blood pressure control is not the only responsible for the larger artery diameters in patients with CKD. Briet et al.[4] in their study, they found that the internal diameter of the common carotid artery was significantly larger in CKD patients than in hypertensive and normotensive individuals. They reported that the lumen diameter, which increases as the GFR decreases, coincides with the arterial accelerated aging in CKD patients [4]. Our results are consistent with previously reported information in the literature that arterial diameter increase and arterial enlargement are observed in CKD patients [4, 9].

Although all aortic and renal artery diameters were found to be larger in individuals with CKD compared to healthy individuals, it was interesting that GFR correlated with aortic diameter but not with renal artery diameter (Table 1). This can be explained by the close association of arterial enlargement in patients with CKD, with arterial remodeling and arterial stiffening. Because accelerated aging, which affects the cardiovascular system of CKD patients, is more evident in the aorta than peripheric vessels [9].

Mazzacaro et al. [12] in their study, examined computed

tomography angiograms of individuals with thoracoabdominal aneurysm, non-dilation of thoracoabdominal aorta and infrarenal aneurysm; they found that the mean diameter of the right renal artery in all individuals was 5.4 mm and the mean diameter of the left renal artery was 5.2 mm. Hazırolan et al. [13] reported that the diameter of the renal artery was 5 - 6 mm. As reported by Merklin and Michels [14] the right and left renal artery diameters were generally similar and the mean value was 5.5 mm. The values of 5.49 mm for the right renal arteries and 5.58 mm for the left renal arteries, which we found by taking the mean value of the diameters of the right and left renal arteries of all individuals, were compatible with the literature.

Arterial tortuosity has been associated with hypertension and some vascular diseases in various articles [11, 15, 16]. In addition, knowing whether the renal arteries are tortuous is important in the management of complications after renal transplantation [17]. Hegedüs [18] found that 61.2% of the arteries were tortuous and 38.8% were straight. In our study, we found a similar curl rate for both groups, slightly higher than Hegedüs reported. Because arterial curvature has previously been associated with causes that disrupt organ blood supply, such as transient ischemic attacks and myocardial ischemia [11, 16], it was interesting that we found a higher rate of renal artery tortuosity in healthy individuals (67.1%) than in patients with CKD (65%), although there was no statistically significant difference. This situation can be clarified by the fact that increased blood pressure, which is one of the important factors affecting artery tortuosity, could not be evaluated and patients data were not included in the study, considering the increased incidence of cardiovascular hypertension in the elderly population.

There have been many studies investigating the localization of the renal ostium respect to the vertebral level [19-22]. Çiçekcibaşı et al. [21] found that in a study conducted on 90 fetuses, the right renal artery was separated at the 92.2% L1 corpus level and the 3.8% at the L2 corpus level; these rates were 94.1% and 3.8%, respectively, for the left renal artery. Özkan et al. [20] in their study by evaluating the angiographies of 855 patients, showed that the right renal artery was separated from 43% L1, 23% L1 - L2 and 32% L2; reported that these rates were 37%, 22% and 38% for the left renal artery, respectively. Fataftah et al. [22] found that the right renal artery is separated at the level of L1 41%, L1 - L2 36.6%, L2 17.2%; they reported that these rates were 40.5%, 34.4% and 22% for the left renal artery, respectively. We detected that the right renal artery was separated from the aorta in 42.5% of the individuals at the L1 corpus level, in 36.3% at the L1 - L2 intervertebral disc level and in 10.1% at the L2 corpus level; these rates were 38.4%, 38.6.1% and 14.7% for the left renal artery, respectively. These rates we found were compatible with other radiological studies in the literature.

A study conducted in patients with suspected cardiovascular hypertension showed that the right renal artery was separated at the level of 31% L1, 17% L1 - L2, 49% L2 and reported that these rates were 22%, 22%, 50% for the left renal artery, respectively [19]. We found it appropriate to compare the data of this study with the results of patients with CKD in our study. We showed that the right renal artery was separated from the level of 43.6% L1 corpus level, 35.1% L1 - L2, and 9.9% L2 in patients with CKD, and these rates were 40.9%, 35.9% and 15.1% for the left renal artery, respectively. Differences in the data presented by two studies with similar patient populations, and in the current study, there was no statistically significant difference between the CKD patients and healthy individuals in terms of the level at which renal arteries leave the aorta may indicate that there is no relationship between kidney disease and level of the renal ostium with respect to the vertebral level.

Limitations

Our study had some limitations as in all retrospective studies. Since our study was a retrospective study, the blood pressure values of our study group patients diagnosed with CKD by the nephrology clinic and control group patients could not be listed. Because the effects of blood pressure control on the cardiovascular system cannot be ignored, it is important to standardize patients according to their blood pressure values. In addition, since the study group included chronic kidney disease patients, computed tomography of a small number of patients could be obtained with contrast. Since it is very difficult to evaluate the arterial structures in non-contrast tomography, we were able to include fewer individuals with clear images than we planned.

CONCLUSIONS

In conclusion this study shows that, diameters of aorta and renal arteries are larger in CKD patients than healthy individuals. There was a negative correlation between the diameters of aorta with GFR. There was a positive correlation between age and aortic diameter. There was a positive correlation between age and renal artery diameter. There was no statistically significant

difference in renal artery course between patients with CKD and healthy individuals. There was no statistically significant difference between patients with CKD and healthy individuals in terms of the level at which the renal arteries branched from the aorta.

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Conflict of interest: The authors declare that they have no conflicts of interest.

Informed Consent: Not available.

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Ethical Approval: All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This study approved by the local ethics committee (2019/238 approval number).

Author Contributions: Conception: NÜD - Design: NÜD, GÇ - Supervision: NÜD - Fundings: none -Materials: GÇ, MK, İA - Data Collection and/or Processing: GDA, GÇ, MK, İA - Analysis and/or Interpretation: GDA, NÜD, GÇ, MK, İA - Literature: GDA, NÜD, ZF - Review: ZF, AKK - Writing: GDA, ZF, AKK - Critical Review: ZF, AKK

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Original Research

Tubuloside A Induces DNA Damage and Apoptosis in Human Ovarian Cancer A2780 Cells

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ABSTRACT

Objective: Ovarian carcinoma is one of the most lethal gynecological cancers, as it responds later to diagnostic methods and therapeutic responses in advanced stages. Many phytochemical compounds have been shown to be protective against cancer. Tubuloside A (TbA) is the main compound extracted from the plant *Cistanche tubulosa*, and its pharmacological effects have been studied broadly. Until now, the role of TbA in human ovarian carcinoma is unknown. The goal of this study was to evaluate the effects of TbA on DNA damage and apoptosis in A2780 cell lines.

Methods: Different concentrations of TbA (1, 5, 25, 50, and 100 µM) and 5- Fluorouracil (1, 5, 25, 50, and 100 µM) treated to the human ovarian cancer cell (A2780) line for 24 h. After incubation, cell viability (MTT), genotoxicity (Comet analyses), and mRNA expression analyses of apoptotic markers (*Caspase-3, Bax, Bcl-2,* and *p53*) were determined.

Results: Applied doses of 50 and 100 µM of TbA and 5- Fluorouracil significantly reduced cell viability. Also, TbA increased DNA damage in A2780 cells. Additionally, TbA up-regulated the mRNA expressions of *caspase-3, Bax,* and *p53*, which are apoptosis-inducing factors, and down-regulated the expression of *Bcl-2*.

Conclusion: These results show that the *p53* and *caspase-3* signaling pathways may exhibit a key role in TbA-associated effects on A2780 cells and TbA may be a potential drug aspirant for ovarian cancer therapy.

Keywords: Tubuloside A, cell viability, genotoxicity, apoptosis, ovarian cancer

INTRODUCTION

Ovarian cancer is one of the lethal among gynecological tumors and comes after uterine cancer in terms of incidence [1]. Ovarian cancer cases are hidden and it is very difficult to determine whether it is benign or malignant. However, diagnosing ovarian cancer at an advanced stage limits its treatment options [2]. Developing diagnostic methods and effective treatments for this disease group are promising. Statistical results report that the survival rate in ovarian cancer is 70-90% and it is increasing rapidly day by day [3].

Resistance to chemotherapeutic drugs is a frequently encountered problem in cancer treatment. One of the main reasons for this problem is the drug resistance that develops in cancer cells. This process has a very complex infrastructure and is an important cause of tumor heterogeneity. One of the

important consequences of drug resistance is that cancer cells become insensitive to therapeutics and escape from apoptosis [4]. Changes in apoptotic genes (such as *p53*, *Bcl-2*, and *Bax*) and signal transduction pathways have been reported in drug-resistant cells [5]. Studies report that *Bcl-2*, together with *Bcl-Xl* and *Mcl-1*, modulates resistance to chemotherapy and reduces survival in ovarian cancer patients [6,7]. There are different cancer subgroups in which at least one family member of these anti-apoptotic proteins is overexpressed, and these are more common in inherently resistant cancers [8]. Currently, available anticancer therapies contain treatments based on targeting cancer cell DNA integrity and/or replication, which indirectly triggers apoptosis in tumor cells [9,10]. The discovery of new compounds and the determination of their potential cytotoxic and apoptotic effects are important for increasing the success rate of treatment.

Tubuloside A (TbA) is a phenylethanoid glycoside obtained from *Cistanche tubulosa* plant and is widely used in the purpose of memory-augmentative, impotency and constipation [11]. In *in vivo* studies demonstrated that *C. tubulosa* extract exhibited hepatoprotective effects against liver damage-induced d-galactosamine (d-GalN)/lipopolysaccharide in mice [12] and hypoglycemic and hypolipidemic effects in diabetic rodents [13,14]. However, *in vitro* studies, it was reported that Tubuloside B (TbB) obtained from *C. salsa* plant antagonized TNF-α-induced apoptosis in SH-SY5Y cells and exhibited neuroprotective effect [15]. Furthermore, it was reported that acteoside (81%), echinacoside (75%), cistantubuloside A (83%), 2'-acetylacteoside (93%), and cistanoside A (33%), which were isolated from *C. tubulosa* plant, showed antitumor activity on mouse skin melonama cells [16].

In the current studies, there has been no study on the role of TbA on ovarian cancer. This study reports for the first time whether TbA has possible cytotoxic potential in ovarian cancer cells. In this regard, the effect of TbA on A2780 ovarian cancer cells; cell viability test was determined by changes in mRNA expression of DNA damage and apoptotic markers.

Main Points;

- Ovarian cancers are one of the most common cancer types in the reproductive system.
- Tubuloside A (TbA), a phenylethanoid glycoside, significantly reduced ovarian cancer cell viability
- TbA increased DNA damage and apoptosis in ovarian cancer cell line
- TbA may be a potential drug aspirant for ovarian cancer therapy

MATERIALS AND METHODS

Cell Culture

The A2780 ovarian cancer cells were provided by Professor Süleyman Sandal from the İnönü University Faculty of Medicine. The cells were first removed from the nitrogen tank and inoculated in flasks (25 cm^2). A2780 cells were nourished with 1640-RPMI medium (prepared by adding 100 U/mL penicillin, 10% FBS, and 0.1 mg/mL streptomycin). The medium was changed (in a twice a week) and the cells were cultured in 5% CO_2 in an incubator medium at 37°C (Thermo Forma, USA). Cells that were approximately 80% confluent in the flask, were removed by trypsin-EDTA solution, stained with trypan blue (0.4%), and counted under an inverted microscope. To determine the effect of the compounds on cell viability, seeding was done in 96-well plates with approximately $15x10^3$ cells in each well.

Cell Viability Analyzes

1, 5, 25, 50 and 100 µM doses of TbA (ChemFaces, Wuhan, Hubei, China) and 5- Fluorouracil (5-FU, Sigma-Aldrich, MO, USA) were prepared in dimethylsulfoxide (DMSO; the final concentration of DMSO was maintained at 0.1%) and applied for 24 hours. After incubation, the medium in the well was removed and 50 µL of the prepared MTT solution (0.5 mg/ml) was transferred to each well. After 3 hours of incubation, the MTT solution in the wells was removed and DMSO (100 µL) was transferred to well. The optical densities were measured on an ELISA (Thermo, USA) at a wavelength of 570 nm [17]. Control wells (wells containing only medium) were read and values were accepted as 100% viable cells and percent viability values were calculated.

Genotoxicity Analyses

For genotoxicity analyses, cells were seeded in 6-well plates and 50 and 100 µM doses of TbA were applied for 24 hours. The level of damage to cell DNA was determined using the alkaline Comet assay technique according to Singh et al. with minor modifications [18]. First, the slides were coated with 1% normal melting agarose (NMA) prepared in phosphate buffer. 24 hours after application of the compounds, cells were removed from the plates and counted under a microscope. 10 µL of the suspension (approximately 10000 cells) was transferred to an

tube and 80 µL of low melting agarose was added. This cell mixture was placed on slides coated with NMA and a coverslip was covered. The preparations were left at +4 °C and in the dark for 15 minutes. The coverslips were then carefully stripped and the slides were kept in lysis solution at +4 °C for 1 hour. Cold electrophoresis buffer was placed on the slides placed in the same plane on the horizontal electrophoresis tank and electrophoresis was performed at 25 volts (300 mA) for 25 minutes. After electrophoresis, the slides were washed with neutralization buffer three times for 5 minutes at +4 °C. Finally, the slides were stained with ethidium bromide and images were taken under a fluorescence microscope (Zeiss Axio1, Germany). Images were processed using Tritek Comet Score software. One hundred cells were randomly counted from slides and tail DNA (%) parameters were determined and data were given as median (10-90 percentiles). This value was considered an indicator of DNA damage [19].

mRNA Expression Analyses

For mRNA analyses, cells were propagated in 100 mm culture disks and applications were carried out. The cell medium was then aspirated and the cells were washed with cold PBS. Cells were collected into tubes with the help of a scraper. RNA was isolated from cells using the RNA Purification Kit (GeneJet, Thermo Fisher Scientific). The quantity and/or quality of the obtained RNAs were measured at A260/280 UV wavelengths using the Nanodrop (Maestrogen/MN-913) device. The amounts of RNA isolated from each group of cells were calculated to yield 1µg RNA in total for cDNA extraction. DNase I (Thermo Fisher Scientific, USA) was then applied to remove DNA from RNA and cDNA was obtained from the RNA using cDNA Synthesis Kit (RevertAid, Thermo Fisher Scientific, USA).

The primers used in the experiment were designed by the study team using the FastPCR 6.0 [20] computer package program and the mRNA sequences of Homo sapiens-specific β-actin (F-AGCAAGAGAGGCATCCTCACC, R-ACAGGGATAGCACAGCCTGGA; NM_001101.5), *Bax* (F-GACATTGGACTTCCTCCGGGA, R-ACAAAGATGGTCACGGTCTGC; NM_001291428.2) *Bcl-2* (F-TGGACAACATCGCCCTGTGGA, R-TCACTTGTGGCCCAGATAGGCA; NM_000633.3), *caspase-3* (F-GCTCCTAGCGGATGGGTGCTA, R-GATTTCAAGGCGACGCCAACC; NM_004346.4), and *p53* (F-AAACCTACCAGGGCAGCTACG, R-CTCACAACCTCCGTCATGTGC; AB082923.1) genes were obtained from the NCBI website.

Differences between the expression levels of selected genes were examined using Bio-RAD CFX Manager 3.1 software on the Bio RAD real-time PCR device. The relative changes in the mRNA expression levels of target genes were calculated by the $2^{-\Delta\Delta}$ ct method based on the cycle threshold (Ct) values of the amplification curves obtained after the amplification process consisting of three steps: denaturation, primer adhesion and chain extension [21]. The calculated value was substituted into the $2^{-\Delta\Delta}$ ct formula for each gene and the mRNA expression level was determined as a fold decrease or increase. The β-actin gene was used as an endogenous control and the expression levels of other genes were corrected (normalization) according to the β-actin gene level of each sample.

Statistical Analyses

The obtained data were analyzed in GraphPad 8 program. The Kruskal-Wallis test was used for differences between groups in MTT and Comet analysis results, and Dunn's Multiple Comparison Test was used for multiple comparisons. In mRNA analyses, the difference between group means was evaluated with the one-way ANOVA test, and multiple comparisons were evaluated with Tukey's test. Results were given as mean ± standard deviation and $p<0.05$ was considered significant in all comparisons.

RESULTS

TbA and 5-FU Applications Reduced Cell Viability

Applied doses of 50 and 100 µM of TbA significantly reduced cell viability compared to the control group (Figure 1) ($p<0.05$). This reduction was determined to be 25% and 35% at 50 µM and 100 µM doses, respectively. Moreover, 100 µM TbA application showed a significant cytotoxic effect compared to the DMSO group ($p<0.05$). To determine the cytotoxic activity of TbA in A2780 cells, the activity of the standard chemotherapy drug 5-FU in cell cytotoxicity was also determined (Figure 2). Applied doses of 1-25 µM of 5-FU did not significantly affect viability in A2780 cells. On the other hand, 50 and 100 µM doses of 5-FU significantly decreased cell viability ($p<0.05$).

TbA Application Induced DNA Damage In Cancer Cells

The level of DNA damage in A2780 cells after TbA and 5-FU applications was shown in Figure 3. After 100 µM 5-FU application, the tail DNA % parameter did not alter significantly compared to the control. On the other hand, 100 µM TbA caused

a significant enhancement in the DNA damage level, and this change was significant compared to the control group (Figure 3; $p<0.05$). These results show that TbA exerts a genotoxic effect on human ovarian cancer cell lines by inducing DNA damage and that this effect may mediate cell death.

TbA Increased mRNA Expression Levels of Pro-apoptotic Genes

5-FU (100 µM) and TbA (50 and 100 µM doses) treatments up-regulated *Bax* (Figure 4A), *p53* (Figure 4B), and *caspase-3* (Figure 4C) mRNA expressions compared to controls, whereas down-regulated *Bcl-2* mRNA expression (Figure 4D) ($p<0.05$). This showed that TbA exerts a cytotoxic effect on human ovarian cancer cells by inducing apoptosis, especially at high doses.

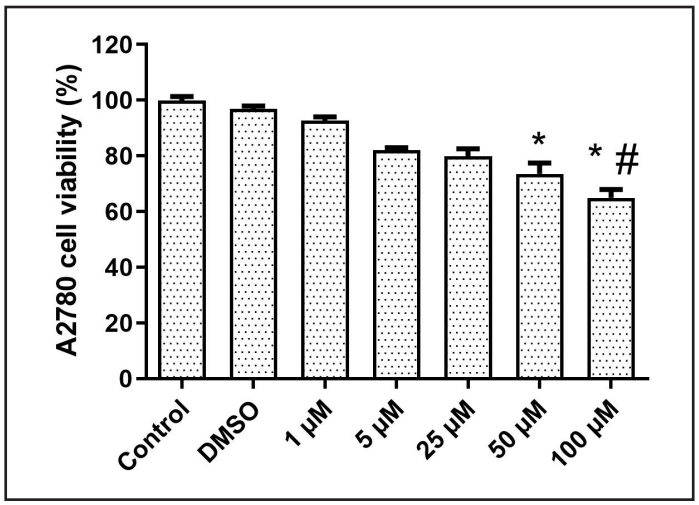

Figure 1. A2780 cell viability level after TbA application. Data are given as mean ± SD. *$p<0.05$ compared to control group, #$p<0.05$ compared to DMSO group

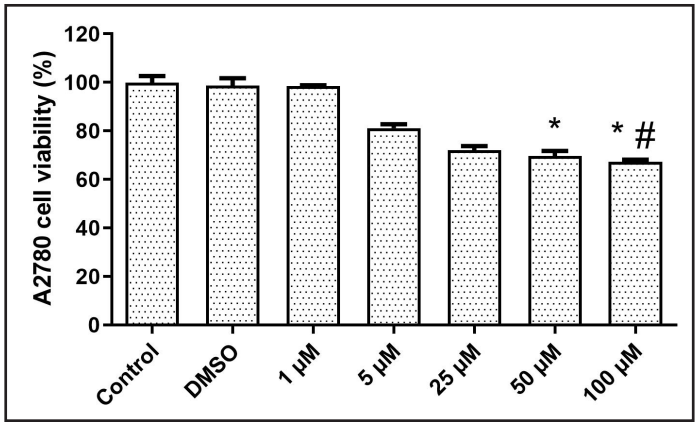

Figure 2. A2780 cell viability level after 5-FU application. Data are given as mean ± SD. *$p<0.05$ compared to control group, #$p<0.05$ compared to DMSO group

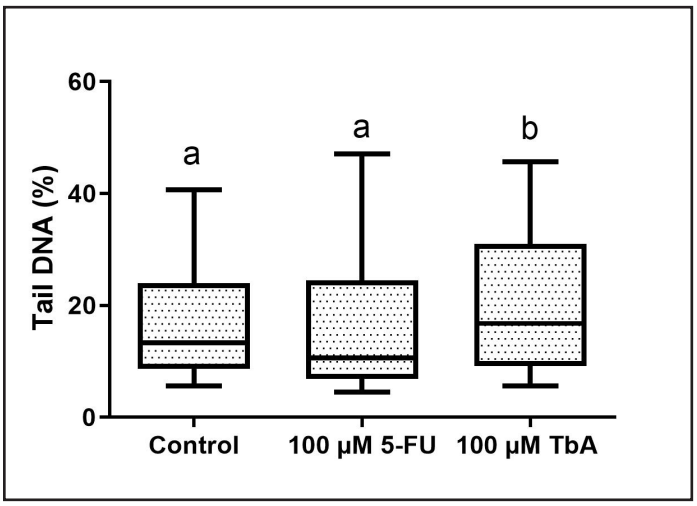

Figure 3. DNA damage level in A2780 cells after TbA (100 µM) and 5-FU (100 µM) application. Data are given as mean ± SD. Different letters (a,b) are statistically significant ($p<0.05$).

DISCUSSION

The main active components of *C. tubulosa* are phenylethanoid glycosides [22]. In recent years, the results of various studies have emphasized that TbA shows various pharmacological and biological activities [12,23]. However, the molecular and/or cellular mechanisms underlying these effects are not fully solved. This study demonstrated for the first time that TbA has genotoxic and cytotoxic effects on a human ovarian cancer cell line by inducing DNA damage and apoptosis.

In an in vitro study investigating the protective effect of TbB on SH-SY5Y neuronal cells, it was reported that approximately 45.6% of the cells underwent apoptosis death after incubation with TNF-α, whereas TbB (1, 10 and 100 mg/L) treatment decreased the cell death rate in a dose-dependent manner (30%, 19.5% and 6.2%, respectively) [15]. Similarly, it was reported that different concentrations of TbB (5, 10, 50 and 100 µM) increased the cell survival rate decreased by 1-methyl-4-phenylpyridinium ion in PC12 neuronal cells [24]. In addition, it has been reported that acteoside (26.7µM), a phenyletenoid glycoside, can induce DNA degradation in promyelocytic leukemia HL-60 cell lines [25]. Another phenyletenoid compound, verbascoside, has been shown to inhibit the growth of human colorectal cancer cell lines (HT-29, HCT-116, LoVo, and SW62) in a time- and dose-dependent manner at a concentration of 29-67 µM after 24, 48 and 72 hours of incubation at 25-100 µM [26]. In this study, like other phenyletenoid compounds, 24 h TbA treatment inhibited cell growth and showed antiproliferative effect in A2780 ovarian cancer cells at 50 and 100 µM amounts.

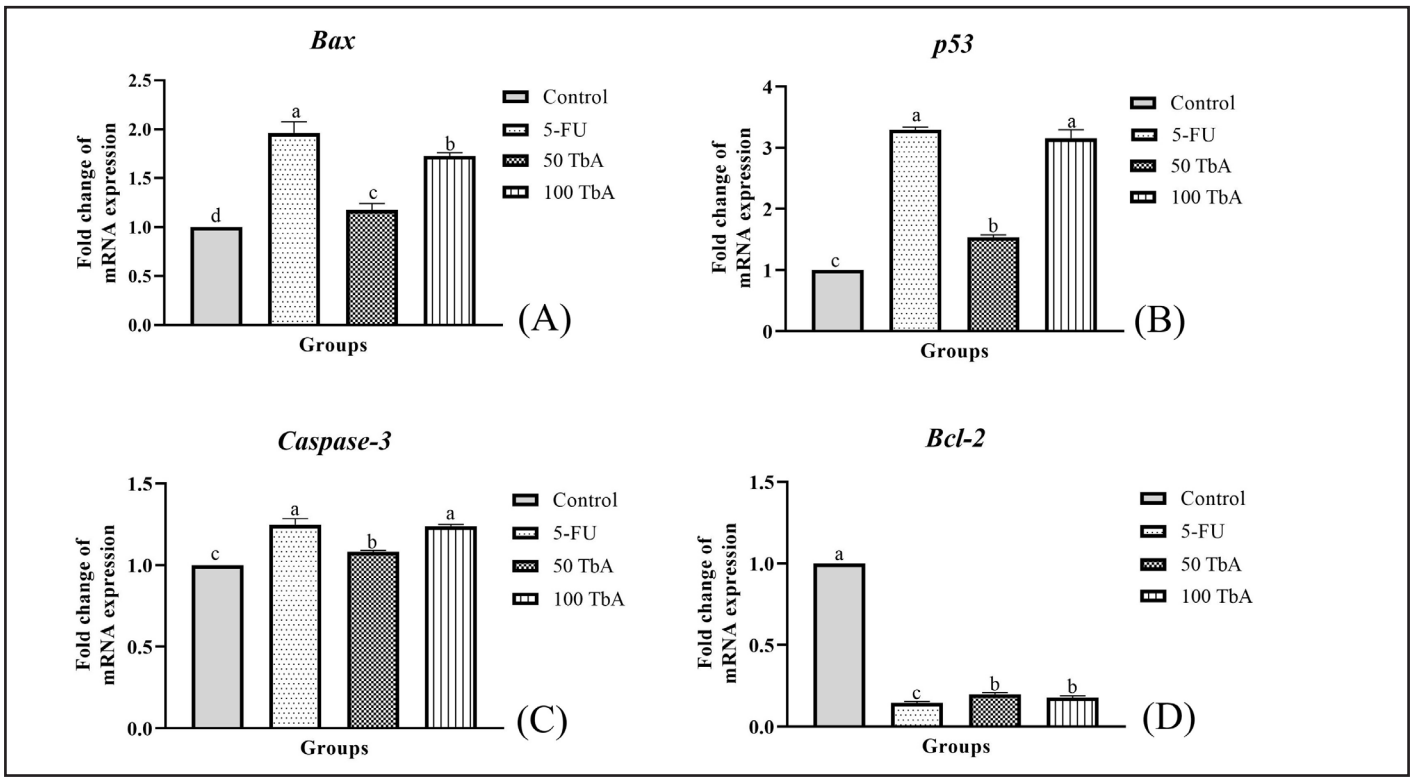

Figure 4. Effect of TbA (50 and 100 µM) and 5-FU (100 µM) treatment on Bax (A), p53 (B), caspase-3 (C) and Bcl-2 (D) expression levels in A2780 cells. Data are given as mean ± SD. Different letters (a,b,c) are statistically significant ($p<0.05$).

Whether Echinacoside from Cistanche and Echinacea plants causes DNA damage was investigated in cancer cell lines (SW480, MCF-7, SK-HEP-1, and 48 MG-63) and non-cancer cell lines (human normal liver-LO2, human embryonic kidney-HEK 293 and mouse fibroblast-NIH/3T3). These cell lines were exposed to Echinacoside 0, 15, 30, 60, and 80 µM for 5, 12 or 24 hours and examined by fluorescent immunostaining, it was reported that doses above 60 µM increased DNA damage in cancer cell lines and did not cause any change in non-cancer cell lines [27]. Furthermore, Dong et al [28] revealed that Echinacoside occurred a significant increase in 8-oxoG, intracellular oxidized guanine, and a dramatic increase in double-stranded DNA break binding protein (53BP1) in SW480 cancer cells. Similarly, as a result of Comet analysis in this study, it was seen that TbA had a genotoxic effect by causing DNA damage in A2780 ovarian cancer cell lines.

It has been shown in some studies that *C. tubulosa* phenylethanoid glycosides exhibit antitumor effects on various tumor cells [29,30]. Yuan et al [31] reported that phenylethanoid glycosides significantly inhibited the growth of HepG2 and BEL-7404 from cancer cells through mitogen-activated protein kinase and apoptosis. Also, in the study investigating the antitumor effect of *C. tubulosa* phenylethanoid glycosides on esophageal cancer (Eca-109), it was reported that the apoptotic process was associated with *Bcl-2* and showed effect by increasing *caspase-3, -7* and *-9* levels [32]. Some studies have shown that *Bcl-2*, *Bax*, and *caspase-3* play a main role in apoptosis in A2780 cells [5,33]. In this study, it was shown that TbA could induce *Bax* expression and up-regulate *caspase-3* while inhibiting *Bcl-2* expression in A2780 cells. Besides, the majority of tumors are associated with mutation of *p53* gene, and this may affect apoptosis by changing the regulation of *Bcl-2* [5]. In this study, it was found that TbA increased *p53* expression in A2780 cells. These results suggest that TbA may induce apoptosis through *caspase-3*-dependent apoptotic signaling as well as increasing *p53* expression in ovarian cancer.

CONCLUSIONS

In conclusion, it has been shown that TbA reduces cell viability in ovarian cancer and promotes apoptosis by activating *p53* and *caspase-3* pathways. This indicates that TbA may be a promising new choice for the treatment of ovarian cancer. Additionally, the underlying mechanism needs to be investigated in more detail in future studies.

Conflict of interest: The authors declare that they have no conflicts of interest.

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Ethical Approval: The authors state that in vitro studies with human cell cultures do not require ethical approval.

Author Contributions: Conception: AT; SI - Design: FZN, SG, YE - Supervision: AT, SI - Materials: FZN, YE- Data Collection and/or Processing: AT, SI, FZN, YE- Analysis and/or Interpretation: FZN, SG- Literature: AT, SI - Review: AT, SI - Writing: AT, SI - Critical Review: AT, SI

AT and SI conceptualized the present study, conducted the investigation, and were involved in data curation. FZN, SG, and YE analyzed and interpreted data. SI wrote the original draft. AT and SI wrote, reviewed and edited the manuscript. AT, FZN, YE and SI confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

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Original Research

Morphological and Topographical Features of the Radial Recurrent Artery and Its Possible Clinical Significance

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ABSTRACT

Objective: The anatomy of the radial recurrent artery (RRA) is very important for interventional procedures. The aim of this study was to investigate the morphological and topographic anatomy of the RRA.

Methods: The study was conducted on 20 human cadavers (14 males and 6 females, 40 upper limbs). The RRA was classified into 4 groups as follows according to the observed origin: RRA originated from the radial artery (RA) (Type A), the root of the RA (Type B), the brachial artery (BA) (Type C), and the ulnar artery (UA) (Type D). The relative positioning of the RRA in relation to the tendon of the biceps brachii muscle (TBB), in terms of the antero-posterior direction, was determined. The vertical distance of the origin point of the RRA to the intercondylar line and the diameters of this artery were determined. Morphometric evaluation was performed with a digital caliper. The obtained data were analyzed using SPSS version 21.00 software.

Results: The artery most commonly originated from the RA (Type A 47.5%, 19 extremities). This was followed by RA root (Type B 32.5%, 13 extremities), BA (Type C 17.5%, 7 extremities), and UA (Type D 2.5%, 1 extremity). The RRAs coursed anteriorly to the TBB in 38 extremities (95%) and passed behind the tendon in 2 extremities (5%). The vertical distance of the origin point of the RRA to the intercondylar line was meanly 32.20 ± 6.86 mm. The diameter of the artery at its origin point was meanly 2.57 ± 0.58 mm and just after its first branch was meanly 2.05 ± 0.48 mm. Our study documents a rare morphological variation of the RRA originating from the UA (Type D).

Conclusion: While many of our findings align with previous studies, this research presents novel anatomical findings and elucidates the superficial course and topographical positioning of the RRA to estimate its origin point.

Keywords: Radial recurrent artery, morphology, morphometry, anatomy, clinical significance

INTRODUCTION

The anatomy of the RRA is important in clinical applications such as plastic and reconstructive surgery, interventional radiology, and microsurgery used in the elbow area and forearm [1, 2]. It was reported that the RRA can be used as a fasciocutaneous flap in soft tissue defects occurring around the elbow joint, traumas, and burns, as well as a free fasciocutaneous or fascial flap in surgeries of the head and neck area [1, 3-5].

It was also reported that the RA is used as a graft in coronary artery bypass surgery and that the proximal border of this graft is determined by the origin of the RRA [6, 7]. It was shown previously that RRA can develop after RA graft removal and can feed the areas where the RA is distributed in the forearm [7].

Abnormal new vessels might develop from the RRA in some patients with chronic lateral epicondylitis. It is considered that these abnormal vessels might be the source of chronic pain. In such instances, the RRA can be considered as the target artery for transcatheter arterial embolization in therapeutic procedures [8, 9].

Another clinical aspect of the RRA pertains to its proximity to the tendon of the biceps brachii muscle (TBB). Based on this neighborhood, it was emphasized that the RRA should be considered in open surgeries involving the TBB [10]. Additionally, the RRA plays a vital role in terms of the anastomosis with the radial collateral branch of the deep brachial artery. This anastomosis can serve as an alternative blood supply to the forearm in case of BA occlusion [11].

Although the RRA is frequently utilized in clinical practice, the frequency of its variations [1] can significantly impact the safety and success of clinical applications associated with this artery [12-14]. Therefore, it can be reasonably hypothesized that the safety and success of any interventional procedure involving the RRA are contingent on a comprehensive understanding of the variations in this artery. In this context, the purpose of this cadaveric study is to discuss the morphological features of RRA and explore its topographic and superficial anatomy to elucidate the possible relationships with relevant clinical practice.

Main Points;

- The anatomy of the radial recurrent artery is very important for interventional procedures.
- With this study, we aimed to investigate the morphological and topographic anatomy of the radial recurrent artery.
- The artery most commonly originated from the radial artery (Type A 47.5%, 19 extremities).
- The vast majority of the radial recurrent arteries (38 extremities) coursed anteriorly to the tendon of biceps brachii muscle.
- The vertical distance of the origin point of the radial recurrent artery to the intercondylar line was meanly 32.20 ± 6.86 mm.
- A rare morphological variation of the radial recurrent artery originating from the ulnar artery (Type D) was observed in the current study

MATERIALS AND METHODS

Examinations of the RRAs were conducted on 40 samples (20 right and 20 left) of the upper extremities of the 20 human cadavers, which had been preserved using a mixture of formaldehyde, phenol, ethyl alcohol, glycerin and water. The cadavers were sourced from the Department of Anatomy at Istanbul University's Istanbul Faculty of Medicine. These cadavers had been previously utilized for dissection training for medical students between 2006 and 2017.

Six of these cadavers were female, 14 were male, and the ages ranged between 49 and 88 years. Two experienced anatomists (L.S. and O.G.) dissected the cadavers and carried out the evaluation of anatomical parameters. There was no gross pathology and/or deformity in any of the cadavers that might affect the measurements. Ethical approval for the study was granted by the Clinical Research Ethical Committee of Istanbul Faculty of Medicine of Istanbul University (IRB (Institutional Review Board) number: 2017/1121).

Dissection Procedure

The extremities were fixed with the elbow in extension and the forearm in the supine position as much as possible. Two skin incisions (each approximately 15 cm in length) were made from medial to lateral (approximately 5 cm proximal and distal to the elbow joint) and transverse to the extremity. The lateral ends of these incisions were combined with a third incision parallel to the long axis of the extremity and the skin in this region was carefully elevated medially. The superficial veins and superficial nerves were dissected in the subcutaneous tissue. The deep fascia and bicipital aponeurosis were dissected, and the BA, brachial veins, and the median nerve were uncovered just below. The division of the BA into its two terminal branches was determined by tracing its course. The deep veins that accompanied the brachial, ulnar, and radial arteries were carefully separated from the arteries and removed. Using classical anatomical landmarks, the RRA that proceeded between the superficial and deep branches of the radial nerve and ascended between the brachioradialis and brachialis

muscles superficially to the supinator muscle was identified. The morphology of the RRA was uncovered by following the trace of the RRA upward to the lateral epicondyle.

Morphological Evaluation

Each RRA was evaluated morphologically, and the subsequent classification was established based on the observed variations [5].

- Type A: RRA originates from the RA (see **Figure 1**)
- Type B: RRA originates from the root of the RA (see **Figure 2**)
- Type C: RRA originates from the BA (see **Figure 3**)
- Type D: RRA originates from the ulnar artery (UA) (see **Figure 4**)

Also, the relative positioning of the RRA in relation to the biceps brachii muscle tendon, in terms of the antero-posterior direction, was determined.

Morphometric Evaluation

- Vertical distance of the origin points of the RRA to the intercondylar line (DICL) (see **Figure 5**)
- Forearm length (FL): The perpendicular distance between the lateral epicondyle and the styloid process of the radius was measured (see **Figure 5**) and the ratio of DICL to FL was calculated.
- Perpendicular distance of the origin of the RRA to the point of bifurcation of the BA (DBA=Distance to BA) (see **Figure 5**). Please note that this distance was not measured when the RRA originated from the root of the RA.
- Diameter of the RRA at its origin (DRRA1=Diameter of RRA's origin) and the diameter immediately after it gave its first branch (DRRA2= Diameter of RRA after second branch) (see **Figure 5**).

Morphometric assessments were performed by using a digital caliper (Mitutoyo Company, Kawasaki-shi, Kanagawa, Japan). The widest transverse distance was employed as the reference when measuring the arteries diameters.

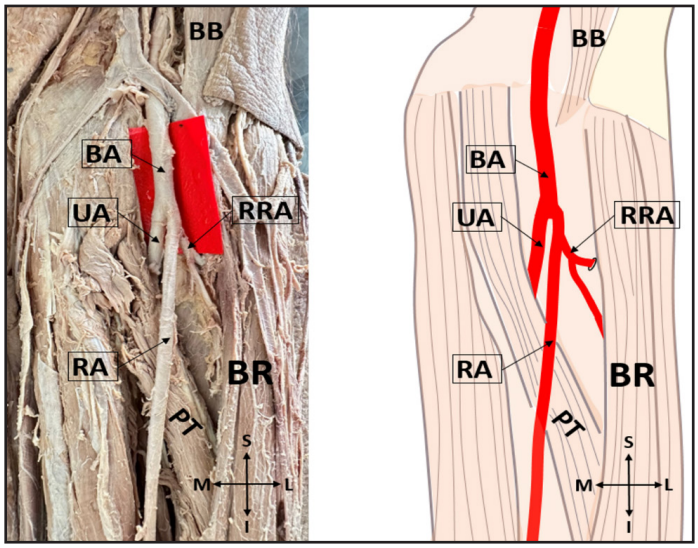

Figure 1. Radial recurrent artery originating from the radial artery (Type A) in the cadaver on the left side and its schematic illustration on the right (anterior view, left). Brachial artery *(BA)*, radial artery (*RA*), ulnar artery (*UA*), radial recurrent artery (*RRA*), biceps brachii muscle (*BB*), brachioradialis muscle (*BR*), pronator teres muscle (*PT*), and directional references (*M* for medial, *L* for lateral, *S* for superior, *I* for inferior).

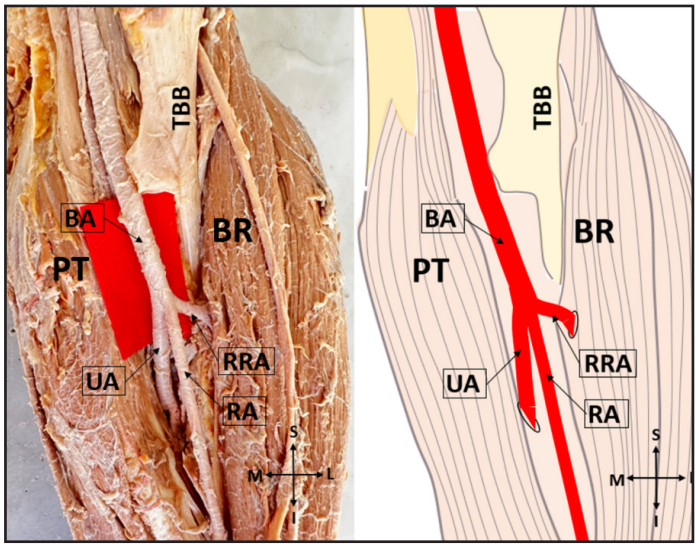

Figure 2. Radial recurrent artery originating from the root of the radial artery (Type B) in the cadaver on the left side, and its schematic illustration on the right (anterior view, left). Brachial artery (*BA*), radial artery (*RA*), ulnar artery (*UA*), radial recurrent artery (*RRA*), tendon of biceps brachii muscle (*TBB*), brachioradialis muscle (*BR*), pronator teres muscle (*PT*), and directional references (*M* for medial, *L* for lateral, *S* for superior, *I* for inferior).

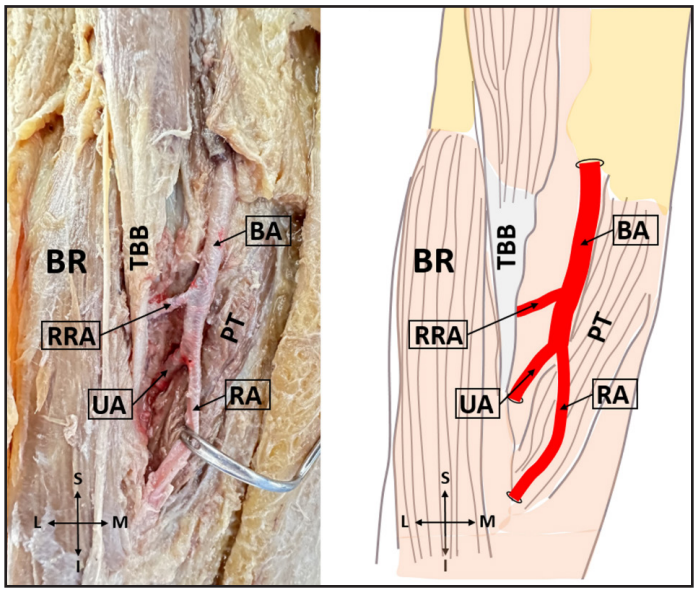

Figure 3. Radial recurrent artery originating from the brachial artery (Type C) in the cadaver on the left side, and its schematic illustration on the right (anterior view, right). Brachial artery (*BA*), radial artery (*RA*), ulnar artery (*UA*), radial recurrent artery (*RRA*), tendon of biceps brachii muscle (*TBB*), brachioradialis muscle (*BR*), pronator teres muscle (*PT*), and directional references (*M* for medial, *L* for lateral, *S* for superior, *I* for inferior).

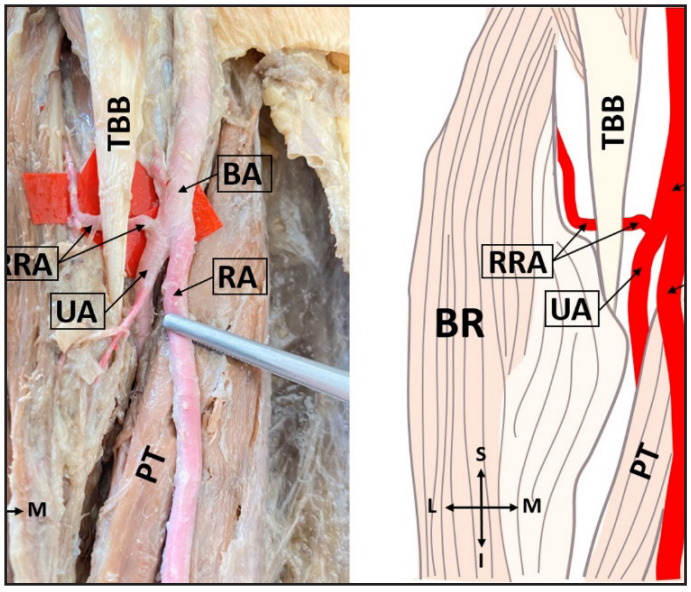

Figure 4. Radial recurrent artery originating from the ulnar artery (Type D) in the cadaver on the left side, and its schematic illustration on the right (anterior view, right). Brachial artery (*BA*), radial artery (*RA*), ulnar artery (*UA*), radial recurrent artery (*RRA*), tendon of biceps brachii muscle (*TBB*), brachioradialis muscle (*BR*), pronator teres muscle (*PT*), and directional references (*M* for medial, *L* for lateral, *S* for superior, *I* for inferior). Please note that the radial artery was replaced medially from its normal anatomical position for the photography.

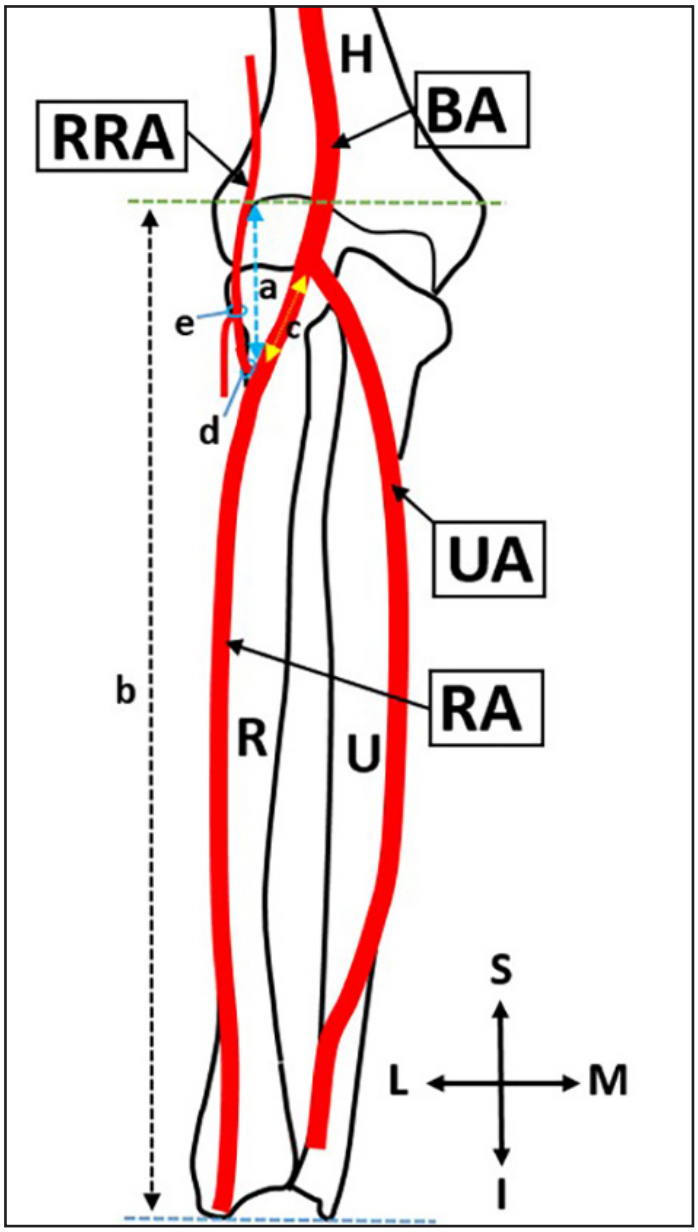

Figure 5. A schematic illustration of morphometric measurements related to the radial recurrent artery (anterior view, right). Brachial artery (*BA*), radial artery (*RA*), ulnar artery (*UA*), radial recurrent artery (*RRA*), labeled measurements including the vertical distance of the origin point of RRA to the intercondylar line (*a, DICL*), the perpendicular distance between the lateral epicondyle and the styloid process of the radius (*b, FL*), the perpendicular distance of the origin of the RRA to the point of bifurcation of the brachial artery (*c, DBA*), the diameter of the RRA at its origin (*d, DRRA1*), the diameter of the RRA just after its first branch (*e, DRRA2*), humerus (*H*), radius (*R*), ulna (*U*), and directional references (*M* for medial, *L* for lateral, *S* for superior, *I* for inferior), the dotted green line represents the intercondylar line and the dotted blue line indicates the line passing transversely from the styloid process of the radius.

Statistical Evaluation

The SPSS version 21.00 (IBM Corp.) software was used in the statistical analysis of the categorical and measurement values. Frequency (n) and percentage (%) values were used for categorical variables. Descriptive statistics such as mean and standard deviation were applied for the variables in the measurements. The Shapiro-Wilk Test was used to test whether the measured values were normally distributed. Following this test, the student t-test was used to compare the normally distributed data concerning side and gender groups with the relevant group.

The Mann-Whitney U-Test was used to compare non-normally distributed data among side and gender groups with the relevant groups. The Kruskal-Wallis Analysis of Variance was used to evaluate the DRRA1 value according to Types (A, B, C, D). Note that the Type D variable was excluded from the analysis due to the presence of only one side in the Type D.

RESULTS

Morphological Features

No aneurysms or structural abnormalities were detected in the BA, RA, UA, or RRA. The distribution of RRAs by type was as follows. Type A, 47.5% (19 extremities); Type B, 32.5% (13 extremities); Type C, 17.5% (7 extremities); and Type D, 2.5% (1 limb). The distribution of numbers and frequency of types of RRA in terms of gender and side is shown in Table 1. RRAs coursed anteriorly to the TBB in 38 extremities (95%) and passed behind the tendon in 2 extremities (5%).

Table 1. Distribution of numbers and frequency of types of the radial recurrent artery (RRA) in terms of gender and side

	n (side)	Type A	Type B	Type C	Type D
Male	28	14 (50%)	11 (39.3%)	2 (7.%)	1 (3.6%)
Female	12	5 (41.7%)	2 (16.6%)	5 (41.7%)	-
Right	20	11 (55%)	6 (30%)	3 (15%)	-
Left	20	8 (40%)	7 (35%)	4 (20%)	1 (5%)
Total	40	19 (47.5%)	13 (32.5%)	7 (17.5%)	1 (2.5%)

Type A: RRA originates from the radial artery, **Type B**: RRA originates from the root of the radial artery, **Type C**: RRA originates from the brachial artery, **Type D**: RRA originates from the ulnar artery

Table 2. The values obtained according to the sides

	Right Side (n=20) (mm)		Left Side (n=20) (mm)		
Parameters	**Mean**	**± SD**	**Mean**	**± SD**	**P value**
DICL	32.82	8.33	31.59	5.15	0.57
FL	254.63	18.15	251.51	16.62	0.57
DRRA2	2.11	0.55	2.00	0.40	0.48
	Median	**Min-Max**	**Median**	**Min-Max**	**P value**
DBA[a]	3.35	0.00-11.89	3.55	0.00-28.00	0.74
DRRA1[a]	2.55	2.03-3.78	2.54	1.59-3.44	0.30

[a]Median and minimum (min) / maximum (max) values are given for values that did not have a normal distribution for the sides.

mm: millimeter; **DICL**: The vertical distance of the origin point of the radial recurrent artery (RRA) to the intercondylar line; **FL (Forearm length):** The perpendicular distance between the lateral epicondyle and the styloid process of the radiu; **DRRA2**: The diameter of the RRA immediately after it gives its first branch; **DBA**: The perpendicular distance of the origin of the RRA to the point of bifurcation of the brachial artery; **DRRA1**: The diameter of the RRA at its origin; **SD**: Standard deviation

Morphometric Features

The mean DICL, FL, and DBA were 32.20 ± 6.86 mm, 253.07 ± 17.25 mm, 4.06 ± 4.01 mm, respectively. Similarly, the mean DRRA1 and DRRA2 were 2.57 ± 0.58 mm and 2.05 ± 0.48 mm, respectively. Comparisons based on sides are presented in Table 2, and comparisons based on gender are shown in Table 3. The calculated ratio of DICL value to FL was calculated as 1:7.85. Since p=0.88 was detected in the evaluation of DRRA1 value according to Types (A, B, C, D), no significant differences were observed in this regard (p> 0.05). Furthermore, the number of samples was small in our study, and no statistically significant differences were detected in the comparison of the sides and genders in almost all the morphometric data.

DISCUSSION

Morphological Features

Types of RRA

When the studies in the literature conducted on RRA were reviewed, it was found that there was no standard among researchers (Table 4). Type A, Type B, Type C, and Type D morphological properties were detected at the highest rates, respectively, by Gupta et al. [15], Zeltser and Strauch [10], Nasr [16], and Zeltser and Strauch [10]. In the present study, RRA in Type A, Type B, Type C, and Type D morphology was found to be 47.5% (19 out of 40 extremities), 32.5% (13 out of 40 extremities), and 17.5% (7 out of 40 extremities), respectively and 2.5% (1 in 40 extremities).

Although it was found that our RRA value in Type A morphology was close to the results of the study conducted by Zeltser and Strauch [10], our results regarding Type B RRA origin were similar and compatible with those reported by Hamahata et al. [5]. However, the rate of Type C RRA in our study represents the highest value in the literature and differs from previous findings. For instance, Adachi [17] reported that the RRA was separated from the BA or superficial brachial artery in 25 of 311 upper extremities (8.03%), and the RRA was separated from the distal border of the BA or superficial brachial artery in 15 upper extremities (4.82%). We did not encounter the superficial brachial artery mentioned by Adachi [17] in our study.

The rate of Type D RRA morphology in our study did not align with the results reported by Hamahata et al. [5], or Zeltser and Strauch [10]. The reason why our RRA results in Type C and Type D morphological features were incompatible with previous studies might be that these studies were conducted on samples from different races and/or the sample populations. Some interpretation differences in morphological classifications might also affect the results of the studies. For example, Adachi [17] introduced three subgroups for the classification of Type B RRA origin while Vazquez et al. [1] did not observe this variation in their study. The variation in findings by Vazquez et al. [1] may be attributed to the grouping of Type B and Type A origins together. In their study conducted on 120 upper limbs, Haładaj et al. [18] observed that RRA originated from the radial artery in 81.6%, the posterior radioulnar division (called "trifurcation of the brachial artery") in 9.2%, the brachioradial artery (radial artery of high origin) in 5%, and the cubital crossover (anastomosis between the brachioradial and "normal" brachial artery) in 4.2%. We did not observe the posterior radioulnar division, brachioradial artery, or cubital crossover in our study. We think that the possible reason is due to the small sample size in the study.

Hamahata et al. [5] reported that they used the free RA flap, modified with RRA, in their facial salvage operations. According to their findings, Type A group of RRAs was preferred for this flap type due to its ease of dissection. The Type B was the second most preferred, as it required careful surgical incision and sutures to protect the separation site. They also reported that RRAs with Type C and Type D morphology were unsuitable for surgical operations. In our study, the RRA rate in Type C and Type D morphological features was higher than those reported by Hamahata et al. [5], and therefore, we believe that RRAs with these morphologies are less likely to be used in facial surgical operations of this kind.

The RA can be used as a graft in coronary artery bypass surgery [6, 7]. The origin point of RRA is the proximal border of the RA graft [6, 7]. Given our observation of RRAs with Type C and Type D morphological characteristics that do not originate from the RA, it is crucial for surgeons to be aware of these potential variations when utilizing the RA as a graft in coronary artery bypass surgery. Failure to recognize such variations could result in inadvertent dissection of the BA, as the proximal border might be challenging to ascertain in these cases. Awareness of these anatomical nuances is essential to ensure the success and safety of surgical procedures involving the RA graft.

Relationship of RRA with the TBB

It is important that there are very few studies that evaluated the relationship between the RRA and the TBB, and that their results are not consistently aligned. Adachi [17]'s study on 311 upper

Table 3. The values obtained according to the gender

	Male (n=28) (mm)		Female (n=12) (mm)		P value
Parameters	**Mean**	**± SD**	**Mean**	**± SD**	
FL	258.50	15.15	240.40	15.55	0.001*
DRRA1	2.66	0.60	2.34	0.49	0.11
DRRA2	2.15	0.51	1.83	0.31	0.05
	Median	**Min-Max**	**Median**	**Min-Max**	**P value**
DICL[a]	31.75	10.49-41.54	32.10	18.70-47.92	0.59
DBA[a]	2.84	0.00-28.00	4.37	0.00-12.38	0.99

[a]Median and minimum (min) / maximum (max) values are given for values that did not have a normal distribution.

*A statistically significant difference was detected when comparing FL values according to gender (**p<0.001**).

mm: millimeter; **SD:** Standard deviation; **FL (Forearm length)**: The perpendicular distance between the lateral epicondyle and the styloid process of the radius; **DRRA1**: The diameter of the RRA at its origin; **DRRA2**: The diameter of the RRA immediately after it gives its first branch; **DICL**: The vertical distance of the origin point of the radial recurrent artery (RRA) to the intercondylar line; **DBA**: The perpendicular distance of the origin of the RRA to the point of bifurcation of the brachial artery.

Table 4. Studies conducted on radial recurrent artery in the present and previous reports

Studies	Sampling Count (Sides)	Place of the Study	Type A		Type B		Type C		Type D	
			Side	Percentage (%)	Side	Percentage (%)	Side	Percentage (%)	Side	Percentage (%)
[a]**Adachi [17]**	311	Japan	231	74.27%	32	10.28%	40	12.86%	0	0%
[b]**Gupta et al. [15]**	75	North India	65	87%	0	0%	9	12%	0	0%
[c]**Nasr [16]**	100	N/A	83	83%	0	0%	15	15%	0	0%
Hamahata et al. [5]	18	Japan	11	61%	6	33.3%	0	0%	1	5.6%
[d]**Vasquez et al. [1]**	332	N/A	215	64.8%	0	0%	24	7.2%	0	0%
Zeltser and Strauch [10]	17	N/A	7	44%	9	55%	0	0%	1	5.8%
[e]**Haładaj et al. [18]**	120	N/A	98	81.6%	-	-	-	-	-	-
Our Study	40	Turkey	19	47.5%	13	32.5%	7	17.5%	1	2.5%

Type A: RRA originates from the radial artery; **Type B:** RRA originates from the root of the radial artery; **Type C:** RRA originates from the brachial artery; **Type D:** RRA originates from the ulnar artery.

[a]Adachi (1928) divided the 32 limbs into 3 subgroups those separated from the proximal part of the root of the radial artery (16 limbs), those separated from the level of the radial artery (13 limbs), and those separated from the distal part of the root of the radial artery (3 limbs).

[b]Gupta et al. (2012) reported no RRAs on one side (1.3%).

[c]Nasr (2012) reported no RRAs on 2 sides (2%).

[d]Vasquez et al. (2013) reported that there were RRAs that did not fit the types reported above.

[e]Haładaj et al. (2018) reported that the RRA originated from the posterior radioulnar division (called "trifurcation of the brachial artery") in 9.2% (11/120), directly from the brachioradial artery in 5% (6/120), and the cubital crossover in 4.2% (5/120).

extremities, for instance, found that the RRA passed in front of the TBB in 80.06% of cases and behind the TBB in 17.36% of cases. He also emphasized that the RRA, which separates from the RA or the level of origin of the RA, always passes in front of the TBB. He pointed out that in other cases that originate from the root (separated from the proximal root of the RA or the distal part of the root of the RA), the RRA always passes behind the TBB.

In a study involving 332 upper extremities, Vazquez et al. [1] reported that the RRA was localized in front of the TBB in 91.26% of cases with the remaining 8.73% showing the RRA behind the TBB. Another study conducted by Zeltser and Strauch [10] showed very well the relationship of the BA, the RA and its recurrent branches, and venous branches with the TBB. Zeltser and Strauch [10] reported in their study conducted on 17 cadaveric upper extremities that they detected main RRA and additional RRA. They noted that in cases with a single RRA, all of them crossed the TBB anteriorly, whereas in samples with additional RRAs, 47% (8 of 17 sides) crossed the TBB posteriorly.

In the present study, we observed that the RRA passed in front of the TBB in 95% of the examined extremities, while in 5% of cases, it passed and behind the tendon. The presence of additional RRA was not evaluated in our study.

The values obtained in the present study are not compatible with the values reported in the literature. The reason for this discrepancy might be that previous studies were conducted in different races and/or differences in sample populations. Bone protrusions, muscles, tendons, and even muscle fibers can be used as guides in invasive procedures [19, 20]. In this context, the TBB may serve as a good landmark when harvesting RRA flaps. According to the results of the present study, although it was found that the majority of the RRA passes in front of the TBB, a significant part of it passes behind the tendon (5%). In this respect, we think that knowing this when harvesting the RRA flap might facilitate invasive procedures and help prevent potential complications in the area, such as unanticipated bleeding.

Morphometric Features

In their case report, Wysiadecki et al. [2] and Patnaik et al. [21] reported DICL values of 39 mm and 30 mm, respectively. No other data on DICL values were found in the literature. In the present study, the average DICL was determined to be 32.20 ± 6.86 mm, a result largely consistent with previous studies. According to our findings, the origin of the RRA is situated approximately 3 cm distal to the intercondylar line. This suggests that our average DICL value may serve as a valuable reference for clinicians in locating the origin of the artery during interventional procedures involving the RRA.

The average FL value in our study was measured at 253.07 ± 17.25 mm. A statistically significant difference was observed when comparing FL values by gender (p=0.0003). The ratio of DICL value to FL was calculated as 1:7.85. This ratio indicated that, the origin of the RRA corresponds to roughly 1/8 of the proximal FL. Such a ratio was not found in the existing literature. We believe that this ratio holds practical value for estimating the RRA's origin quickly and superficially.

In the literature, only one study by Zeltser and Strauch [10] was identified regarding the DBA value. Their study, conducted on 17 upper extremities, reported an average DBA value of 2 mm in 2 extremities, 5 mm in 4 extremities, and 7.6 mm in 2 extremities. In the present study, the researchers found the average DBA value to be 4.06 ± 4.01 mm. We think that knowing the average DBA value might help surgeons in terms of preserving the RRA because the RRA origin point determines the proximal border when harvesting the radial artery graft for coronary artery bypass surgery [6, 7]. We also believe that this value might be important in terms of practically detecting the origin point of the RRA. In other words, we believe that the RRA is easily accessible at a distance of approximately 0.5 cm distal to the RA after the BA and RA have been identified during surgery.

In the present study, we measured the diameter of the RRA from two different points, DRRA1 and DRRA2, resulting in measurements of 2.57 ± 0.58 mm and 2.05 ± 0.48 mm, respectively. In their study on 18 upper extremities, Hamahata et al. [5] calculated the average diameter of the RRA at a distance of 20 mm from the bifurcation of the RA as 1.84 ± 0.59 mm and emphasized that the most suitable point for dissection of the artery was 20 mm from the bifurcation of the RA, and the artery gave off some branches after 20 mm. There is a discrepancy between the results of the present study and the study of Hamahata et al. [5]. The reason for this might be that the diameter measurements of the RRA were made more proximally and/or the samples studied belonged to different races in the present study.

Luther et al. [22] identified 32 transradial neurointerventions (TRA) in which patients had radial artery loops. They found that

patients with smaller caliber RRAs (RRA diameters ≤2 mm) were more prone to TRA failure in the presence of a radial artery loop. In this context, considering that our average RRA1 and RRA2 values were greater than 2 mm, it can be said that TRA can be successful in the presence of a radial artery loop. We would not have knowledge of whether our cadavers had radial artery loops or not. So, we could not make any comment. Consequently, the mean RRA1 and RRA2 values may be important in predicting TRA failure in the presence of a radial artery loop.

No statistically significant differences were detected in the comparison of the sides and genders in almost all the morphometric data (the number of samples was small). We think that the lack of such a difference might mean that invasive procedures for the RRA can be performed irrespective of side and gender.

Limitations

The most important limitation of this study was that it had a small size sample (20 cadavers). Therefore, we think that we could not observe the variations reported in the literature (posterior radioulnar division, brachioradial artery, etc.). If we had studied a large sample size, we could have observed many variations of RRA. Additionally, statistical analysis could have been more meaningful.

The fact that the study was conducted on embalmed cadavers previously used in dissection training of medical students can be considered as one of the limitations. Although this does not mean that the results of the study are not valid, fresh frozen cadavers or fresh unembalmed cadavers may provide more objective data. The ages of the specimens included in the study can also be considered among the limitations. Our findings were obtained from cadavers ages ranged between 49 and 88 years. Therefore, we do not know whether the findings we observed are age-related. Our results could have been more meaningful if the study had been conducted in a balanced age group (for example, ages ranged between 30 and 40 years). Moreover, we did not have a clinical presentation of the specimens. If we had, it would be valuable to reveal their relationship with the clinical presentation.

CONCLUSION

The present study investigated the morphological characteristics, topographic anatomy and superficial projection of the RRA. Unlike previous studies, the present study documented the existence of a rare morphological variation of Type D, originating from the UA. The prevalence of RRAs passing in front of the TBB is higher than in previous literature. Also, the study expanded upon the available morphometric data for RRAs, offering a more comprehensive understanding of this artery's anatomical features. By analyzing the collected data according to sides and genders, the study provides a nuanced perspective on the variations and relationships between different morphological and morphometric characteristics of the RRA.

Finally, the study described the topographic location of the superficial projection of the RRA in relation to the intercondylar line and forearm length, facilitating a practical estimation of its origin. These findings contribute to the broader understanding of the RRA's anatomical features and may aid clinicians and surgeons in various medical interventions involving the RRA. Overall, this study sheds light on previously unexplored aspects of the RRA, enhancing our knowledge of its anatomical variations and clinical relevance.

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Review

Hyperinsulinemic Hypoglycemia in Childhood

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ABSTRACT

Hyperinsulinemic Hypoglycemia (HH) is the most common cause of permanent hypoglycemia, especially in the neonatal period. Childhood HH is mostly related to genes encoding proteins in the insulin secretion pathways, and may also be seen in syndromes such as Beckwidth Wiedemann, Kabuki, and Turner. The majority of congenital HH cases are the result of KATP channel gene defect. Most of these cases are unresponsive to diazoxide treatment. In this review, recent genetic studies and recent updates in treatment options in childhood HH are reviewed.

Keywords: Hyperinsulinemic Hypoglycemia, Childhood, KATP gene, Diazoxide, Octreotide

INRODUCTION

Hyperinsulinemic Hypoglycemia (HH) is the most common cause of severe and persistent hypoglycemia resulting from impaired suppression of insulin secretion from pancreatic beta cells. The presence of measurable insulin in the blood sample taken when blood glucose is <50 mg/dl, along with low free fatty acid level and low blood ketone value, make the diagnosis of HH [1,2]. Insulin has a plasma life of 6 minutes and is secreted pulsatilely. For this reason, insulin may not be measured high in HH cases sometimes. The serum C-peptide level is more stable and has a 24-hour lifespan. It is a more reliable parameter for the diagnosis of HH than insulin [2,3]. In addition, the need for >6-8 mg/kg/min dextrose infusion to ensure normoglycemia and a positive response to glucagon injection support the diagnosis of HH. The development of hypoglycemia after protein intake or after exercise in older children should also bring HH to mind.
Depending on the age group, hypoglycemia may cause adrenergic symptoms such as feeding problem, hypotonia, feeling of hunger, weakness, sweating, palpitation, and nervousness. As the severity of hypoglycemia increases, neuroglycopenic findings, convulsions, loss of consciousness and permanent neurological sequelae, and even death may occur due to the inability to meet the glucose requirement of the brain. Babies with HH are usually macrosomic, and some of them may have cardiomyopathy and hepatomegaly due to glycogen storage [1-3].

Insulin is the main regulator of blood sugar. It increases the use of blood sugar by providing the passage of cells. At the same time, it increases the storage of glycogen in the liver and muscles, while suppressing endogenous glucose production pathways such as gluconeogenesis and glycogenolysis. Insulin also inhibits lipolysis in adipose tissue and suppresses ketone formation.

Glucose, which increases in the blood after meals, enters the pancreatic beta cell via GLUT-2. It is converted to glucose-6-phosphate (G6P) by the enzyme glucokinase (GCK). As glucose 6 phosphate is metabolized by glycolysis, energy (ATP) is released. When the ATP/ADP ratio increases, ATP-sensitive potassium channels (KATP) are closed. These channels consist

of two proteins called sulfonylurea receptor 1 (SUR1) and Kir6.2 (Inward rectifier potassium channel) encoded by ABCC8 and KCNJ11 genes. It is normally responsible for potassium flow out of the cell. With the closure of the KATP channel, a membrane potential (depolarization) occurs in the beta cell. Voltage sensitive calcium channels open due to the membrane potential formed between the inside and outside of the cell. Calcium flows into the cell. Increased intracellular calcium causes cytosolic contractions in insulin-stored granules and insulin release by exocytosis (Figure 1).

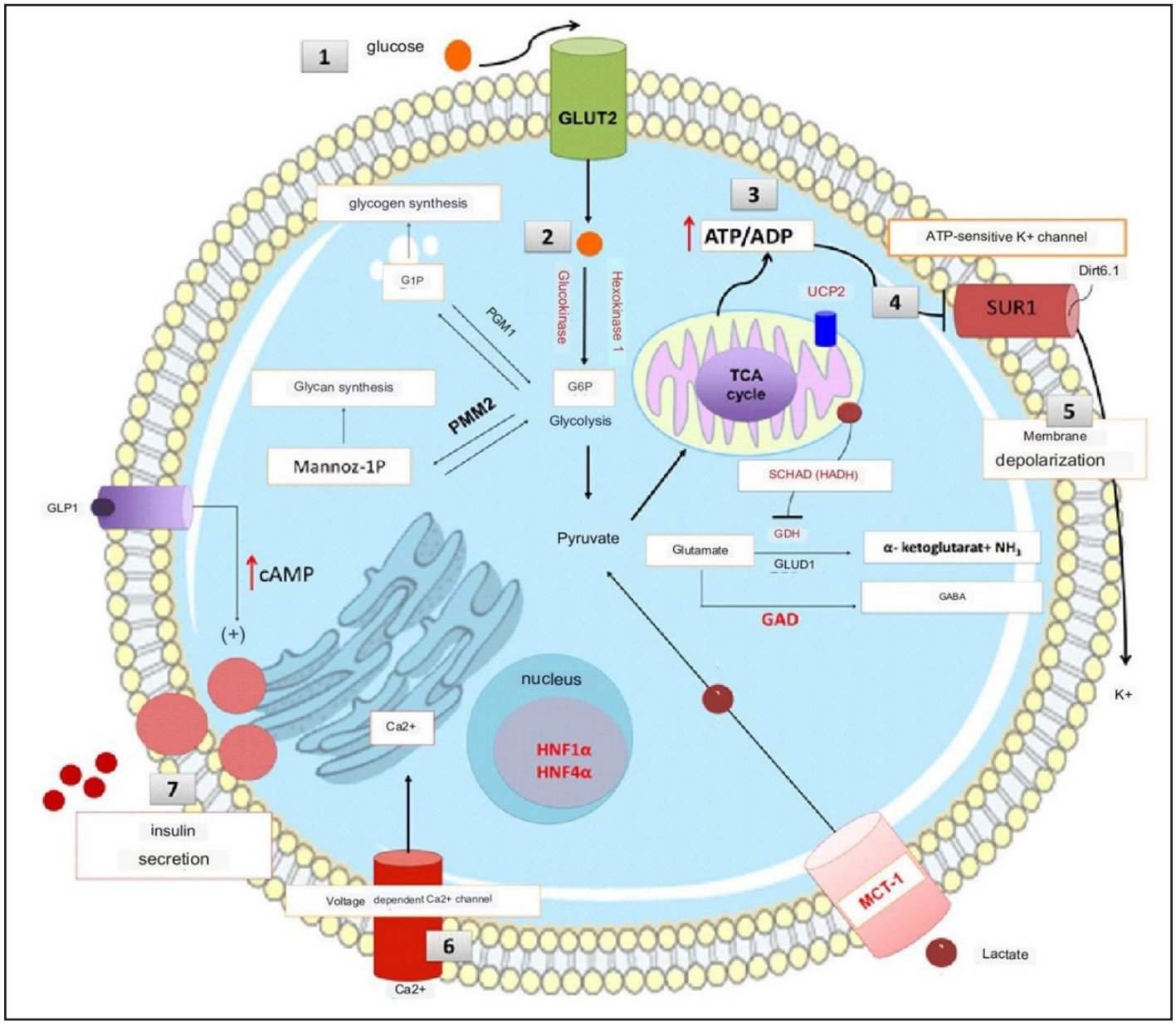

Figure 1. Regulation of insulin release from pancreatic β-cell and sites of gene mutations involved in the genetics etiology of hyperinsulinaemic hypoglycaemia

SUR1: sulphonlyurea receptor 1, **Kir6.2:** inwardly rectifying potassium channel 6.2, **K:** potassium, **MCT1:** monocarboxylate transporter 1, **Glu:** glucose, **P:** phosphorus; **PGM1:** phosphoglucomutase 1, **PMM2:** phosphomannose-mutase 2, **UCP2:** mitochondrial uncoupling protein 2, **NH3:** ammonia, **GDH:** glutamate dehydrogenase, **GLUD1:** glutamate dehydrogenase 1 gene, **SCHAD:** short-chain L-3-hydroxyacyl-CoA dehydrogenase, **HADH:** hydroxy-acyl-CoA dehydrogenase, **HNF1A and 4A:** hepatocyte nuclear factor 1A and 4A, **Ca+2:** calcium; **GAD:** glutamate decarboxylase enzyme, **GABA:** γ-aminobutyric acid, **GLP1:** glucagon like peptide 1, **cAMP:** cyclic adenosine monophosphate (amplifier for the exocytosis of insulin secreting granule. [1].

Insulin has a blood sugar lowering effect. In normal situations, when blood sugar begins to fall below the threshold value (85-90 mg/dl in plasma), insulin secretion begins to decrease, and when it falls below 50 mg/dl, insulin secretion must decrease to undetectable levels. All kinds of events that affect the functioning of this cycle, which plays a role in the release of insulin from β-cells, cause severe hypoglycemia, as a result of which insulin secretion cannot be suppressed. For these reasons, if normoglycemia (> 60 mg/dl) cannot be achieved in 6-8 hours of fasting, especially in newborns after 48-72 hours, permanent hypoglycemia should be mentioned and its cause should be investigated [4,5].

Hyperinsulinemic Hypoglycemia

In particular, HH of genetic origin is usually seen in newborns, but it can also be seen in infancy or older ages. Patients who are protein sensitive and develop hypoglycemia after exercise show clinical signs in later months. Hyperinsulinemic Hypoglycemia due to insulinoma can present clinically at any age, including adult age.

Hyperinsulinemic Hypoglycemia can be temporary or permanent, sometimes accompanied by syndromes. Temporary HH can be seen in the baby of diabetic mother, Rh incompatibility, erythroblastosis fetalis, intrauterine growth retardation, SGA, drugs such as propronalol, sulfonylurea taken by the mother, or hyperglycemia due to stress in the mother. The main mechanism in these cases is transient hyperinsulinism. They usually resolve spontaneously within the first 1-2 months, rarely, hyperinsulinemia may persist for more than 6 months [6]. Hyperinsulinemic Hypoglycemia can be seen together with various syndromes. Hyperinsulinemic Hypoglycemia has been reported in syndromic channelopathies such as overgrowth syndromes (such as Beckwith-Wiedemann syndrome (BWS) and Sotos syndrome), monogenic or chromosomal developmental syndromes with postnatal growth retardation (Turner syndrome, Kabuki syndrome, etc.), congenital glycosylation syndromes and Timoty syndrome [7].

Beckwith-Wiedemann syndrome is the most common cause of HH. BWS is a type of overgrowth syndrome that includes macrosomia, macroglossia, neonatal hypoglycemia, hemi-hypertrophy, and omphalocele. Patients with BWS are predisposed to develop embryonal malignancies such as Wilms' tumor, hepatoblastoma, neuroblastoma, and rhabdomyosarcoma. Hyperinsulinemic Hypoglycemia develops in around 50% of BWS patients and is usually transitory. However, in some circumstances, it may produce HH that is resistant to diazoxide and, in rare cases, may necessitate pancreatectomy [8]. In certain BWS patients, HH may be the primary clinical finding, with no other symptoms of the disease [9]. Beckwith-Wiedemann syndrome is caused by genetic and/or epigenetic defects that alter the expression of imprinting genes on chromosome 11's short arm (11p15.5) [10]. The presence of the KATP gene on the short arm of the 11th chromosome appears to facilitate the onset of hyperinsulinism [7].

Kabuki Make Up syndrome has been reported as the second most common syndrome causing HH in some series. Kabuki syndrome is a rare hereditary multisystem disorder marked by developmental delays, large palpebral fissures, lateral epicanthus, permanent fingertip pads and dermatoglyphic abnormalities, as well as several congenital skeletal and visceral malformations. Kabuki syndrome has been linked to mutations on the X chromosome in the KMT2D (75%) and KDM6A (3-5%) genes. Both genes encode proteins that regulate histones, so they are in the group of chromatin regulation disorders [7]. Especially in Kabuki syndrome due to KMD6A gene mutation, 56% of neonatal hypoglycemia and 28% of HH can be found to affect the β-cell function of this gene, but the exact mechanism is not clear yet [11]. The HH seen in Kabuki syndrome responds to diazoxide and usually resolves within the first 2 years. However, cases in which pancreatectomy had to be performed have also been reported [12].

Turner syndrome is a relatively common (1/2500 female birth) syndrome, characterized by short stature and ovarian insufficiency. The association of cases with Turner syndrome and HH has been reported, and when the cases in some series are evaluated, it has been predicted that HH is seen 50 times more often than the normal population [7]. Moreover, in a study in which HH was found in 9 out of 69 patients with Turner syndrome, they stated Turner syndrome as the third most common syndrome in which HH was detected [12]. In cases of Turner syndrome, KDM6A "haploinsufficiency" induced by mosaic X chromosome monosomy was assumed to be the etiology of HH. Most of the cases are responsive to diazoxide, and the need for medication usually disappears within 1 year, but cases that went to pancreatectomy have also been reported [7].

Tablo 1. Etiology of Hyperinsulinemic Hypoglisemia in Childhood [2,3]

Transient hyperinsulinaemic hypoglycaemia
• Mother with diabetes (before and during gestation) • Maternal use of sulfonylureas or intrapartum intravenous glucose infusion • Intrauterine growth restriction • Perinatal asphyxia • Rhesus haemolytic disease • Erythroblastosis fetalis • HNF4A, HNF1A mutation
Persistent hyperinsulinaemic hypoglycaemia
• Congenital hyperinsulinism (genetic hyperinsulinaemic hypoglycaemia ABCC8, KCNJ11, KCNQ1, CACNA1D, SLC16A1, GLUD1, GCK, HADH, HNF4A, HNF1A FOXA2 EIF2S3 UCP2 HK1 • Insulinoma
Syndromic or metabolic causes of hyperinsulinism
Prenatal and postnatal overgrowth syndromes: • Beckwith-Wiedemann syndrome • Soto's syndrome • Simpson-Golabi-Behmel syndrome • Perlman syndrome Chromosomal abnormality syndromes: • Trisomy 13 (Patau syndrome) • Mosaic Turner syndrome
Contiguous gene deletion aff ecting the ABCC8 gene: • Usher syndrome Syndromes leading to abnormalities in calcium homoeostasis: • Timothy syndrome
Insulin receptor mutation:
• Insulin resistance syndrome (leprechaunism)
Congenital disorders of glycosylation
• Types 1a, 1b, and 1d (PMM2, PGM1)

HNF1A: Hepatocyte nuclear factor 1A; **HNF4A:** Hepatocyte nuclear factor 4A; **ABCC8:** ATP Binding Cassette Subfamily C Member 8; **KCNJ11:** Potassium Voltage-Gated Channel Subfamily J Member 1; **KCNQ1:** Potassium Voltage-Gated Channel Subfamily Q Member 1; **CACNA1D:** Calcium Voltage-Gated Channel Subunit Alpha1 D; **SLC16A1:** solute carrier family 16 member; **GLUD1:** glutamate dehydrogenase 1; **HADH:** hydroxyacyl-CoA dehydrogenase; **GCK:** glucokinase; **UCP2:** uncoupling protein 2; **HK1:** hexokinase 1; **PGM1:** phosphoglucomutase 1; **PMM2:** phosphomannomutase 2, **FOXA2:** forkhead box protein A2; **EIF2S3:** Eukaryotic translation initiation factor 2 subunit 3.

Genetic Basis of Persistent Hyperinsulinemic Hypoglycemia

Persistent HH is mostly due to genes encoding pancreatic β-cell membrane ion channels and carrier proteins (ABCC8, KCNJ11, KCNQ1, SLC16A1, CACNA1D), enzymes in glucose metabolic pathways (GLUD1, GCK, HADH, UCD2, HK1, PMM2, PGM1) and transcription factors in insulin production (HNF4A, HNF1A, FOXA2). To date, mutations in nearly 15 genes, some of which have been presented as a single case, have been shown to cause HH [1-3]. In Table 1, the genes detected in HH cases are given as a table [3]. However, in approximately half of the cases, a genetic cause cannot be demonstrated [1].

Recessive loss-of-function mutations in KCNJ11 and ABCC8 genes, which encode two major proteins of the KATP channel, Kir6.2 and SUR1, are the most common cause of CHI (Congenital hyperinsulinism). Mutations in ABCC8 and KCNJ11 genes are responsible for approximately half of CHI cases [2]. ABCC8 gene mutations encoding the SUR1 protein constitute the majority of these in our country [13]. ABCC8/KCNJ11 recessive inactivating mutations are mutations that completely disrupt the synthesis of KATP channel proteins, thus channel formation and activity (class I) or reduce channel activity (class II), so cause uncontrolled insulin release despite severe hypoglycemia [14]. Mutations in these genes are also the most common cause of diffuse β-cell hyperplasia unresponsive to medical therapy [15]. Again, paternally inherited recessive mutations of KATP channel genes cause HH due to focal β-cell hyperplasia as a result of somatic mutation in pancreatic islet cells and loss of maternal allele (Paternal uniparental disomy, UPD). Dominant inactivating ABCC8 and KCNJ11 mutations usually cause milder phenotype HH, although cases unresponsive to medical treatment have also been reported [16].

KCNQ1 gene mutations cause cardiac arrhythmia (hereditary long QT syndrome), deafness, and gastrointestinal system disorders. Recently, HH has been reported in individuals with hereditary long QT syndrome caused by KCNQ1 mutations [17]. In these patients, uncontrolled insulin release and hypoglycemic episodes are seen later in the test during the long oral glucose

tolerance test. Although the role of Kv7.1 channels encoded by the KCNQ1 gene in glucose metabolism has not been fully explained, it has been suggested that this channel may regulate insulin secretion by playing a role in the repolarization of the plasma membrane.

The "Calcium Voltage-Gated Channel Subunit Alpha1 D (CACNA1D)" gene encodes L-type voltage-sensitive calcium channels, and mutations in this gene affect insulin secretion in pancreatic β-cells. Recently, the CACNA1D mutation was demonstrated in a patient with HH, cardiac disorders and severe hypotonia. This mutation activates L-type voltage-dependent Ca2+ channels, causing the channel to remain open even at lower membrane potential, causing uncontrolled calcium entry into the cell and insulin secretion [18].

Monocarboxylate carrier protein 1 (MCT1) are carrier proteins that allow monocarboxylate molecules such as lactate and pyruvate to be taken into the cell, and from there, pyruvate to be transported into the mitochondria and enter the "Kreb's cycle". The MCT1 protein is encoded by the SLC16A1 gene. Under normal conditions, the expression of the MCT1 protein in the β-cell is low. This results in low lactate-pyruvate levels in the β-cell and minimal effect on insulin secretion. Activating dominant mutations in the promoter region of the SLC16A1 gene cause MCT1 expression in the β-cell, lactate and pyruvate uptake into the cell, and pyruvate entering the Krebs' cycle, resulting in increased ATP synthesis and thus uncontrolled insulin secretion [19]. This form of HH, defined as exercise-induced hyperinsulinism, is characterized by autosomal dominant inheritance, inappropriate insulin secretion after anaerobic exercise and pyruvate loading. In these cases, which usually respond to diazoxide treatment, it is usually sufficient to avoid heavy exercises and there is no need for a continuous drug treatment. However, in some cases, diazoxide alone cannot prevent hypoglycemia, and it may be necessary to avoid heavy anaerobic exercise and to take carbohydrate-containing foods before, during and/or after exercise [20].

The Glutamate Dehydrogenase 1 (GLUD1) gene catalyzes the synthesis of α-ketoglutarate, a substrate for the Krebs cycle, in pancreatic β-cells. Thus, it causes an increase in the ratio of ATP: ADP, which activates the KATP channel in the cell, depolarization of the cell membrane and increase in exocytosis insulin secretion. GDH also catalyzes the deamination of L-glutamate and its conversion to α-ketoglutarate and ammonia in the liver and kidney. Activating GLUD1 mutations have been reported as the second most common cause of HH [1]. GLUD1 mutations cause protein/leucine-induced hyperinsulinism/hyperamonemia syndrome (HI/HA) [21]. In these patients, 3-5 times higher ammonia level is observed due to increased ammonia production in the kidneys. It does not cause ammonia toxicity symptoms such as hyperammonemia, drowsiness, headache, vomiting, coma in these patients. However, epileptic seizures may be seen in some patients with mutations, especially in the 6th and 7th exons. Since these seizures can occur without hypoglycemia, it is thought that the mutation may have a direct effect on the brain [22]. Since HI/HA syndrome generally has a milder clinical course compared to KATP gene mutations, the diagnosis can be made after the neonatal period. In normal birth weight infants, persistent but asymptomatic hyperammonemia is characterized by fasting or postprandial protein/leucine-induced hypoglycemia. Neurological findings such as epilepsy and learning difficulties are more common in patients with HI/HA syndrome than other causes of hyperinsulinism.

Recessive inactivating mutations of the HADH gene cause a decrease in mitochondrial L-3-hydroxyacyl-coenzyme A dehydrogenase (HADH) enzyme levels, and the inhibitory effect on GDH is lost, thus causing HH. In HADH deficiency, HADH gene mutations make protein sensitive HH. However, since the increase in GDH activity is limited to pancreatic islets, the increase in ammonia level seen in cases with HI/HA syndrome is not seen here. Patients may present with a heterogeneous clinical picture ranging from mild or late-onset, fasting or protein/leucine-sensitive hypoglycemia to severe hypoglycemia immediately after birth [23,24]. Congenital hyperinsulinism due to HADH gene mutations almost always responds to diazoxide treatment. However, in order to prevent protein-induced HH in these patients, patients should also be advised to avoid meals containing pure protein.

The glucokinase enzyme encoded by the GCK gene is a hexokinase (Hexokinase IV) that phosphorylates the glucose entering the beta cell to form G6P. This enzymatic reaction constitutes the rate limiting step for glycolysis. Because of this feature, it plays a glucosensor role for glucose-stimulated insulin secretion from the β-cell. Heterozygous (dominant) activating mutations in the GCK gene increase the enzyme's affinity for glucose. Enzyme activity continues even at low glucose levels, thereby increasing ATP production. The increased ATP/ADP ratio causes the KATP channels in the pancreatic β-cell

membrane to remain closed for a long time and uncontrolled insulin release. Activating heterozygous mutations in the GCK gene cause HH with autosomal dominant inheritance [25]. The onset of the disease may occur in a wide age range from the neonatal period to the adulthood [26]. The severity of clinical findings varies among affected individuals. There is usually a family history of hypoglycemia. However, since the severity of the disease may be different in affected individuals, the picture may be quieter in previous individuals. Affected individuals may even become ill without being aware of it [27]. Although most GCK mutations lead to HH that responds to diazoxide therapy, some patients may need octreotide administration or pancreatectomy [26].

In addition to GDH1, HADH and GCK, which are found in the metabolic pathways of glucose, HH is also seen due to uncoupling 2, (UCP2), Hexokinase 1 (HK1), and PGM1 and PMM2 gene mutations that cause congenital glycation disorders. Theoretically, this group of patients is expected to be responsive to diazoxide, since the KATP channel proteins are intact.

Hepatocyte nuclear factor 1α and 4α (HNF1α and HNF4α), which act as transcription factors for nuclear hormone receptors encoded by the HNF1A and HNF4A genes, are also expressed in β-cells that regulate insulin secretion. Heterozygous inactivating mutations of these genes cause two contrasting clinical pictures; While they cause HH in newborns and infants, they cause MODY type diabetes (MODY type 1 and 3), which is called early-onset monogenic diabetes in the later stages of life. Hyperinsulinemic Hypoglycemia patients due to HNF1A/HNF4A mutations are typically macrosomic and may cause disease in a clinical spectrum ranging from mild transient hypoglycemia to severe HH responsive to diazoxide [28,29]. Although HH due to HNF1A and HNF4A mutations is rare, it has been reported as one of the most common causes of diazoxide-sensitive HH in some series [29].

Cases of HH due to Forkhead box A2 transcription factor (FOXA2), also known as hepatocyte nuclear factor 3β (HNF3β), have been reported. The FOXA2 gene is one of the positive regulators of the pdx1 gene, which has a role in pancreatic development [30]. The FOXA2 gene is also involved in the expression of KCNJ11 and ABCC8. On the other hand, it has been reported that the FOXA2 gene is a binding point in the intronic region of the HADH gene and plays a role in the activation of the HADH gene. The first FOXA2 mutation, which causes pituitary dysfunction and clinical findings accompanied by HH, was shown in a patient with congenital hypopituitarism, HH, and organ anomalies differentiated from the endoderm [31].

Histopathology in Hyperinsulinemic Hypoglycemia

Congenital HH is histologically divided into two subgroups as diffuse and focal disease. The diffuse form is typically characterized by enlargement and hyperplasia of β-cells. In the focal form, there is nodular hyperplasia consisting of ductal and acinar complexes surrounded by normal pancreatic tissue. The diffuse form cases, which constitute 40-50% of the cases, are usually seen due to recessive or dominant inherited ABCC8, KCNJ11, GLUD1, GCK, HADH, SLC16A1, HNF4A, HNF1A and UCP2 gene mutations. Focal forms generally develop as a result of paternal unidisomy, loss of maternal 11p15 somatic allele and ABCC8/KCNJ11 gene mutation [2].

It is possible to distinguish between diffuse and focal forms with ^{18}F-DOPA-PET/CT imaging. This provides us a chance of surgical cure, especially in focal forms [32].

Treatment and Management in Hyperinsulinemic Hypoglycemia

The primary goal in the treatment of HH is to bring blood sugar to the normoglycemic level as soon as possible, and to allow sufficient ketone production if possible [1,2]. Because glucose and ketone bodies are the main and alternative energy sources of the brain. Since lipolysis is suppressed at high insulin levels, ketone production is also suppressed, thus increasing the risk of brain damage and sequelae [33,34]. For this reason, it should be aimed to increase blood sugar to at least 60-65 mg/dl and above, which is the safe range, and to control inappropriate insulin secretion.

Emergency Treatment of Hyperinsulinemic Hypoglycemia

When hypoglycemia is detected, after critical blood samples are taken, solutions containing 0.2 mg/kg glucose (2 cc/kg 10% dextrose) are injected intravenously to bring blood sugar back to normal as soon as possible. Blood glucose is measured within 5-10 minutes after the first bolus, and a bolus is given again if necessary. If it still does not improve, higher doses and repeated pushes are applied. On the other hand, even if normoglycemia is achieved, fluid containing dextrose is started with a glucose infusion rate of 6-8 mg/kg/min and above. The amount of dextrose in the fluid and the glucose infusion rate are titrated according to the blood glucose level obtained in

repeated measurements. An important point to note here is that bolus dextrose push-ups stimulate insulin release and there is a risk of rebound hypoglycemia. For this reason, it is appropriate to avoid boluses whenever possible and to start dextrose fluid infusion immediately after the first push if intravenous dextrose is needed.

Intramuscular glucagon can be life-saving in cases such as symptomatic hypoglycemia, hypoglycemic seizures, or patients with vascular access issues by boosting blood sugar within minutes. Each injection should have a dose of 0.5-1 mg [1,2]. While glucagon primarily raises blood sugar through glycogenolysis in the liver, it also creates an immediate energy source for brain tissue by stimulating gluconeogenesis, ketogenesis, and lipolysis [35].

Frequent feeding of the patient according to the fasting tolerance period may also help for normoglycemia. However, severe anorexia usually occurs in HH patients, especially with the effect of diazoxide treatment. Another important problem is vomiting and gastroesophageal reflux, which are frequently seen in these patients. Antireflux treatment for gastroesophageal reflux, percutaneous gastrostomy and, in some cases, antireflux surgery may be required [36]. On the other hand, while switching to oral nutrition, insulin secretagogues secreted from the gastrointestinal tract may increase hypoglycemia and intravenous glucose requirement in these patients.

Long-term Treatment

Medical treatment

The mechanism of action and possible side effects of drugs used in the treatment of HH are summarized in the table 2. Diazoxide is a potent inhibitor of insulin secretion and is the first-line drug of medical treatment in HH [1-3]. Diazoxide binds to the KATP channel SUR1 subunit, thereby inhibiting insulin secretion by channel's opening and showing its activity. Most of the cases of diffuse HH caused by homozygous or compound heterozygous mutations in ABCC8 and KCNJ11 genes in which the KATP channel structure and/or function is impaired, and focal HH caused by paternally inherited heterozygous mutations of the same genes are unresponsive to diazoxide. If normoglycemia is still not achieved at a dose of 15 mg/kg/day for 5 days, it is considered unresponsive to diazoxide. The most common acute side effect is fluid and salt retention. Water retention is more common in newborns and infants and may cause congestive heart failure [1,37]. It is frequently used in combination with chlorothiazide (7-10 mg/kg/day and in 2 divided doses), a thiazide diuretic, to protect against fluid retention and have a synergistic effect against hyperinsulinism. However, there is no need to routinely start chlorothiazide in patients who do not have clinical signs of fluid retention, except in the newborn and infancy period.

Although nifedipine, a calcium channel blocker, is a promising molecule due to the role of voltage-dependent calcium channels in the insulin release mechanism from beta cells, its success in CHI treatment in clinical practice has been limited [38,39].

Octreotide is accepted as the second alternative drug after diazoxide in HH. By binding to somatostatin receptors 2 and 5 (SSTR2 and SSTR5), it provides a potent inhibitory effect on insulin release from pancreatic beta cells. The recommended starting dose for octreotide is 5 μg/kg/day administered subcutaneously, given in 4-6 divided doses. The maximum treatment dose is accepted as 35-40 μg/kg/day [1-3].

In patients who cannot achieve normoglycemia with conventional treatments, surgical pancreatectomy is required. In some cases, normoglycemia was achieved with Sirolimus, an mTOR inhibitor, before surgery and the need for pancreatectomy was eliminated. The treatment dose is started in two equally divided doses at a dose of 0.5-1 mg/m2/day and titrated with the weekly sirolimus level. Sirolimus blood level is targeted as 5-15 ng/mL [40]. Due to its serious side effects, sirolimus is still recommended to be used as the last option of medical therapy in the treatment of HH. In addition, it was reported that the chance of success with sirolimus was not as expected in HH series that included a small number of diazoxide-resistant patients [41,42]. Long-acting somatostatin analogs (Octreotide/Lanreotide LAR) are a new treatment option administered deep intramuscularly every 28 days. Especially in chronic treatment, it is a candidate to be the first choice for patients who have compliance problems in daily use of diazoxide and octreotide. It has similar side effects to short-acting octreotide. It appears to be more effective than conventional treatments [2].

Surgical Approach

If normoglycemia cannot be achieved with current treatments in HH, surgical pancreatectomy should be planned. In cases of diffuse HH unresponsive to treatments, >95% of the pancreas is removed. If a focal lesion is detected, cure is achieved by removing only the focal lesion [1,2]. In a study including 105

cases with 58 diffuse and 47 focal lesions who underwent surgery; diffuse cases underwent near-total pancreatectomy, hyperglycemia was observed in 53% of cases, and 10 required a second operation, and the need for postoperative medical treatment continued in 59%, but this need was reported to have passed within 5 years. No post-operative hyperglycemia developed in focal patients and all were cured. In the same study, 91% of diffuse HH patients who underwent near-total pancreatectomy developed diabetes requiring insulin in their 14th year [32].

Tablo 2. Medications for the treatment of hyperinsulinaemic hypoglycemia [1]

	Route	Dose	Mode of action	Side effects
Conventional Medicines				
Diazoxide	Oral	5-20 mg/kg/day, divided into three doses	Binds to the SUR1 subunit of KATP channels, opening them and inhibiting insulin secretion. To function effectively, the KATP channel must be intact.	Water and salt retention, hypertrichosis, and loss of appetite are all common symptoms. Cardiac failure, pulmonary hypertension, hyperuricaemia, blood dyscrasias (bone marrow suppression, anaemia, eosinophilia, etc.), and paradoxical hypoglycemia are uncommon.
Chlorothiazide	Oral	7-10 mg/kg/day, divided into two doses	Prevents fluid retention and has a synergistic impact on KATP channels with diazoxide to reduce insulin secretion.	Hyponatraemia, hypokalaemia
Nifedipine	Oral	0.25-2.5 mg/kg/day in 2-3 split doses	Inhibits Ca-channels of the β-cell membrane Ca-channels in the -cell membrane are inhibited.	Hypotension
Octreotide	s.c	5-35 g/kg/day, split into 3-4 doses or administered as a continuous subcutaneous infusion	SSTR2 and SSTR5 activation limits calcium mobilization and cholinergic activity, decreases insulin gene promoter activity, and decreases insulin production and release.	Acute symptoms include anorexia, nausea, abdominal pain, diarrhoea, drug-induced hepatitis, increased liver enzymes, long QT syndrome, tachyphylaxis, and necrotizing enterocolitis. Long-term effects include decreased intestinal motility, bile sludge, and gallstone formation, as well as suppression of pituitary hormones (growth hormone, TSH).
Glucagon	bolus s.c/i.m or infusion s.c/i.v	0.02 mg/kg dosage or infusion rate of 5-10 g/kg/hour	G-protein-coupled adenylate cyclase activity raises cAMP. Glycogenolysis and gluconeogenesis are stimulated.	High doses (>20 g/kg/hour) cause nausea, vomiting, skin rash, and rebound hypoglycemia due to paradoxical stimulation of insulin secretion.
New medicines				
Rapamycin (everolimus, sirolimus)	Oral	An initial dose of 1 mg/m2 per day may require dose adjustment based on blood sirolimus concentration, with the goal of keeping it between 5-15 ng/mL.	Inhibitor of mTOR. Inhibit insulin release and -cell growth via many methods that are still unknown.	Immunological suppression, mucositis, hyperlipidemia, increase of liver enzymes, thrombocytosis, and decreased immunological response to BCG vaccine were all seen.

Octreotide LAR/ Lanreotide	deep s.c	The dose is estimated using the cumulative current multi-injection dose of octreotide and administered as a single dose every four weeks, for a total dose of 15-60 mg/four weeks.	These long-acting somatostatin analogues work in the same way as daily multidose octreotide.	Similar to octreotide injections given on a daily basis. However, long-term outcomes are unknown.
Abbreviations: SUR1 is for sulphonlyurea receptor 1, KATP stands for adenosine triphosphate-sensitive potassium channels, s.c. stands for subcutaneous, i.m. stands for intramuscular, and i.v. stands for intravenous. SSTR2 stands for somatostatin receptors 2, and SSTR5 stands for somatostatin receptors 5. TSH stands for thyroid-stimulating hormone. BCG stands for Bacillus Calmette-Guérin, mTOR stands for mammalian target of rapamycin, and LAR stands for long-acting release				

New Approaches in Hyperinsulinemic Hypoglycemia

Glucagon-like peptide 1 (GLP-1) is an incretin hormone that stimulates insulin secretion during satiety. In studies with Exendin (9-39), which is a GLP-1 receptor antagonist, significant increases in fasting glucose levels were obtained [43].

Today, various drug studies are still ongoing for HH. The results of studies such as monoclonal insulin receptor antibody (RZ358), SST5 receptor agonist (CRN0477), GLP1 receptor antagonist (exendin 9-39), Glucagon analogue (dasiglucagon), Long-acting glucagon analogue (HM15136) seem to open up horizons in the management of HH.

In conclusion, HH is the most common cause of permanent hypoglycemia in newborns. Rapid correction of hypoglycemia and maintenance of normoglycemia are vital because of potential brain damage and the risk of neuromotor developmental delay and epilepsy in the long term. It is vital to follow the developments in HH, which is an important entity in pediatric practice, and to take appropriate initiatives without wasting time.

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Review

Current Bio-based Cements and Radioactive Opacifiers in Endodontic Approaches: A Review of the Materials Used in Clinical Practice

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ABSTRACT

Objective: This study aims to evaluate the importance of endodontic root canal sealers in filling cavities and irregularities in root canals with the primary goal of minimizing or eliminating bacterial residues. Despite this crucial objective, it's noteworthy that several conventional sealers have been linked to adverse effects, such as impaired wound healing, inflammation, and bone resorption. Therefore, there is a constant search for an optimal sealer that can effectively mimic the properties of lost tissue while maintaining an acceptable level of biological, physicochemical and biocompatible properties. The present study analyzes bioceramic cement's properties in endodontics through a comprehensive review of the available literature. Also, to evaluate the beneficial properties and characteristics of the biomaterials highlighted in this work.

Methods: The present study used a systematic review approach to conduct a comprehensive literature search to find relevant publications on bioceramic cement properties in the endodontics field. Articles were retrieved using MeSH keywords and digital searches of journal websites. The selected studies were examined to extract data on sealability, bioactivity, pH, cytotoxicity, color change, radiopacity, edge adaptation, adhesive strength, antibacterial properties and biocompatibility.

Results: The results of the reviewed research show that bioceramic endodontic cement has favorable properties for the therapeutic treatment of root canals. The literature highlights the material's biocompatibility, low cytotoxicity, bioactivity, radiopacity, appropriate pH value, favorable edge adaptation, high adhesive strength, practical sealability, antibacterial properties and minimal color change.

Conclusion: Research results to date indicate that biomaterials used in endodontics have beneficial properties for root canal therapy and mimicking natural tissue regeneration. The beneficial properties of these materials, such as their biocompatibility, bioactivity, radiopacity, pH stability, edge conformability, adhesion strength, sealability and antibacterial properties, make it a promising replacement for traditional sealers. Further studies are needed to investigate the extended clinical effectiveness of the above intervention and to refine its composition to improve the outcomes associated with endodontic therapies.

Keywords: Bioceramics, Biomimetic materials, Endodontics, Radiopacifiers.

INTRODUCTION

Endodontic treatment aims to prevent and cure apical periodontitis by destroying the microbial ecosystem through chemical-surgical or chemical-mechanical preparations. After cleaning and shaping, the filling must seal the root canal system as tightly as possible. Although zinc oxide eugenol (ZOE) based, calcium hydroxide based, glass ionomer-based, resin-based, and bioceramic sealers have been used to fill root canals, problems with biocompatibility, suboptimal sealability and long-term stability have been reported [1]. Therefore, to better serve patients undergoing root canal treatment with better outcomes and better adhesion between obturation materials and the canal walls, there is an urgent need for an improved endodontic cement that effectively addresses these challenges [2]. Physical properties such as setting time, radiopacity and solubility, and chemical properties such as pH and biocompatibility are critical to selecting the filling material. Bioceramic material is often used as endodontic cement in dental restorative procedures due to its remarkable properties and compatibility with dental tissues. Bioceramic-based cements are promising because of their excellent biocompatibility, practical sealing ability that prevents microbial activity, and ability to promote tissue regeneration in dental restorative procedures. Increase the success rate by improving the sealing ability or blocking any reinfection passage, thus ensuring the longevity of the treated teeth. Bioceramic materials are mainly composed of calcium silicates and have been proven to have excellent biocompatibility with the surrounding tissue. This leads to the formation of an appetite layer during the setting process, which influences the bond between dentin and the filling material [3].

Main Points;

- Several new endodontic biomaterials have emerged in recent research studies.
- A comprehensive review of the literature has been conducted to categorize these biomaterials based on their composition and intended applications.
- The assessment aims to identify their biological and physicochemical properties, biocompatibility, cytotoxicity, bioactivity, antimicrobial effects, and ability to penetrate dentinal tubules, all of which are associated with these novel biomaterials.
- Additionally, the research includes the determination of their radioopacity, radiodensity characteristics, and the presence of radio-opacifier additives.
- It is evident that further in vitro and in vivo investigations, along with well-designed long-term clinical studies, are required to elucidate the mechanisms and validate the sustainability of these novel endodontic biomaterials in practical dental applications.

Root fillings are known to emerge coronally and come into contact with the oral flora, as numerous studies have shown. In in vitro and in vivo studies, dyes and bacteria were found to penetrate filled canals within three months and bacterial endotoxin within 21 days. Conventional root canal sealers possess inherent drawbacks, such as undergoing volumetric shrinkage upon curing and dissolution when exposed to tissue fluids. These issues can lead to the formation of voids within the sealing material, potentially facilitating the escape of microorganisms. In the context of endodontic therapy, a fundamental tenet is the complete three-dimensional obturation of the endodontic spaces, ensuring their permanent isolation from the root canal contents, thereby mitigating irritation of periapical tissues and cross-infection reactions. However, despite advancements in technology, the efficacy of bioceramic root canal sealers remains uncertain owing to limited scientific understanding and research in this area [4]. Endodontic cement often contains radiopaque means for radiographic visibility. Recent research has shown that endodontic cement radiopacifiers improve clinical outcomes and diagnostics [5, 6]. Bismuth oxide, zirconium oxide and barium sulfate increase the radiopacity of endodontic cement [6, 7]. This helps radiographs show cement placement, assessment of root canal filling, and possible complications. Endodontic cement's radiopacity helps monitor healing, assess treatment success and identify problems at follow-up visits. Clear visualization of the cementum allows assessment of its integration with surrounding structures, identification of voids or leaks, and detection of complications such as periapical pathology or root fractures. Recent literature emphasizes the importance of selecting radiopaque additives that provide sufficient radiopacity without compromising material properties or biocompatibility [8]. To optimize performance and patient safety, radiopacifier studies examine cement properties such as setting time, pH, solubility, and antimicrobial properties [9]. Recent studies have examined how radiopacifiers affect the biocompatibility of endodontic cement and tissue response; the use of bismuth oxide as a radiopacifier in endodontic materials has been widely used. Gandolfi et al. showed in vitro that the cement's biocompatibility may also be reduced or impaired. [10, 11]. Radiopacifiers that maintain biocompatibility and minimize effects on periapical tissue are under investigation[8] .

To achieve these objectives, one must consider the filling technique and the material, always considering its biological, physical, and chemical properties. The physical properties stand out: setting time, radiopacity, film thickness, solubility, flow, and dimensional stability were performed according to the American National Standard Institute/American Dental Association (ANSI/ADA) specification no. 57/2000 and ISO 6876/2001 [12].

Although these materials have good biological properties, there is a limited amount of work evaluating the physicochemical properties of bioceramic endodontic cement. Thus, it is opportune to carry out the present research to make possible and safe the use of these materials in clinical practice. The current work will discuss information related to the bioceramic cement used for endodontic cement.

Aim and Motivations of the Present Contribution

Bioceramic contains endodontic cement, which yielded many improved performances and has gained importance lately in endodontic practice due to its unique properties, as pointed out. Bioceramics'' impact on treating various pulpal or periradicular infections shows fast improvement. Researchers have emphasized that these biocompatible materials could significantly serve as valuable elements for several functions for different endodontic treatments, obtaining beneficial results and showing great promise in the treatment prognosis [13].

Hence, there is a need to address further the detailed characteristics of endodontic bioceramic sealers or cement and their radiodense contents. When the current literature is evaluated, there is a lack of a comprehensive review article about bioceramics used in endodontic practice, which comprehensively evaluates the materials in all aspects and their radio-opacifier additives. This review aimed to evaluate chemical, biological, physical, and mechanical progress, recent research outputs, and prospective endodontics applications.

MATERIALS AND METHODS

Eligibility Criteria

After discussing the research question, the aims of the study, and potential methodological limitations, the reviewers agreed on a set of inclusion and exclusion criteria. Studies analyzing the effects of different radiopaque additives on the physical properties and radiopacity of bioceramic-based materials used in endodontics were considered appropriate. The bioactivity potential of these root canal sealers was also evaluated, along with in vitro studies investigating the potential cytotoxic or inflammatory effects of radioactive additives in bioceramic-based materials and how these can be minimized. In addition, in vivo biocompatibility tests were performed. Bioceramic cement was tested for its effectiveness in endodontics. The benefits of bioceramic cement were evaluated based on a review of the existing literature on the subject. These benefits were analyzed regarding biocompatibility, cytotoxicity, bioactivity, radiopacity, pH, marginal conformability, bond strength, sealability, antibacterial properties and possible color change. This research aimed to investigate the use of bioceramic cement for root canal therapy and determine its effectiveness and potential for advancing endodontic practices.

Search Strategy

This study thoroughly examines bioceramics' role in endodontics by conducting a comprehensive literature search. To gather relevant articles, an electronic survey was conducted using databases like PubMed, Scopus, Web of Science, and Google Scholar, and MeSH keywords like" dental materials+endodontics"," bioceramic+root canal sealer"," bioceramics+endodontic"," cements+endodontic"," hydraulic cements+endodontic", and"radiopacity" or"radioopacifier". Only peer-reviewed articles written in English were included, and a preliminary screening was conducted to ensure that met the inclusion criteria. After checking all studies for relevance, a total of 75 articles were selected for this study. Based on the PRISMA guidelines, Figure 1 shows the selection criteria used for our study. As part of our analysis, we assessed 51 articles for their research quality. Of the selected articles, 88% were original research papers. As of May 23, 2023, the study's findings revealed that a significant amount of research was conducted on bioceramic root canal sealers, as indicated in Figure 2 (a-c).

RESULT AND DISCUSSION

Bioceramic Materials Used in Endodontics

Bioceramics exhibit excellent bioactivity and biocompatibility, inducing a regenerative response in the human body and promoting hydroxyapatite formation. It can be osteoinductive and absorb osteoinductive substances, making it ideal for root cement in endodontics [14]. The material includes dicalcium and tricalcium silicates, calcium phosphates, zirconium oxide, and calcium hydroxide [15, 16]. Bioceramic cement is popular due to its high pH, biocompatibility, non-resorption, low cytotoxicity, increased root resistance, and stability. First-

generation bioceramics, such as alumina and zirconia, had excellent mechanical properties. The second generation, such as calcium phosphate-based hydroxyapatite and bioactive glass (BG) cement, adhere to the living bone without causing inflammation or toxicity [17]. BG cement has healthcare and modern biomaterial-driven medicine. In endodontic practice, biomaterials are used for various treatment procedures, such as pulp capping for vital pulp treatment, root canal filling as an intracanal sealer, and extraarticular repair material [18], Figure 3 shows The use of biomaterials in endodontic practice. The common types of bioceramic materials used in endodontics include:

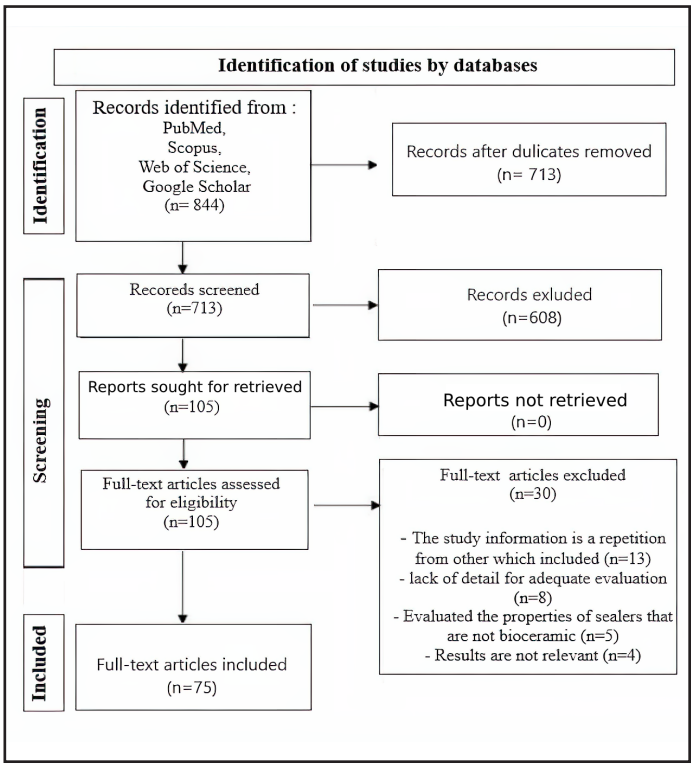

Figure 1. A PRISMA flowchart illustrating the collection and curation of data from the web.

Calcium Silicate Containing Cements

Mineral trioxide aggregate (MTA) cement is a bioactive cement introduced in the 1990s as a root-end filling material for endodontic treatment. Since then, its use has expanded to various clinical applications such as pulp capping, apexification, perforation, and furcation repair. MTA is composed of finely ground Portland cement, bismuth oxide, and other minerals and has a unique chemical composition that provides excellent sealing ability, biocompatibility, and the ability to induce complex tissue formation [19].

MTA comprises tricalcium aluminate, dicalcium silicate, calcium oxide, silicon dioxide, tricalcium silicate (66.1%), aluminium oxide, and Bi2O3 as a radiopacifier [20]. MTA has some of the ideal properties of repair material. However, Portland cement and MTA may contain heavy metals in their composition, with arsenic levels higher than the safe limit specified by ISO 9917-1 of 2007. In addition, there is evidence that Bi2O3 interferes with the hydration mechanism[21]. They were promoting failures in the microstructure of Portland cement. Consequently, there is an increase in porosity, resulting in a decrease in the strength of the material [22].

The replacement of MTA by tricalcium silicate has been evaluated, resulting in materials with promising physicochemical properties [20]. Replacing Portland cement with tricalcium silicate allows better control over impurities and heavy metal inclusions found in Portland cement.

Modifications in MTA composition gave rise to MTA Plus (MTAP) (Avalon Biomed Inc., Bradenton, FL, USA), a commercially available material based on tricalcium silicate in powder-liquid or gel form. According to the manufacturer, it is indicated for vital pulp therapies (pulp capping and pulpotomy) and endodontic procedures (repair of perforations and resorptions, apexification, apexogenesis, root canal, and retro filling). Its composition comprises tricalcium silicate, dicalcium silicate, and Bi2O3 [23]. As a material used in pulpotomy teeth with incomplete root formation, MTAP induces the release of calcium hydroxide as a by-product and the formation of calcium phosphate when in contact with tissue fluid; however, it causes staining in contact with sodium hypochlorite due to Bi2O3. Biodentine cement is a calcium silicate-based material that has gained attention in dentistry due to its beneficial properties and clinical applications. It is biocompatible, meaning it is well-tolerated by the surrounding tissues. It also has bioactive properties, promoting the formation of hydroxyapatite and facilitating the remineralization of dentin [24]. Biodentine cement offers several advantages compared to other materials. It has similar mechanical properties to natural dentin, making it a suitable substitute[24]. It also has a simplified handling process, with a powder and liquid component that can be easily mixed. Biodentine can be used in various clinical scenarios, including direct and indirect pulp capping, apexification, and dentin substitutes for restorations [25]. Table 1 shows the common calcium silicate-containing cement.

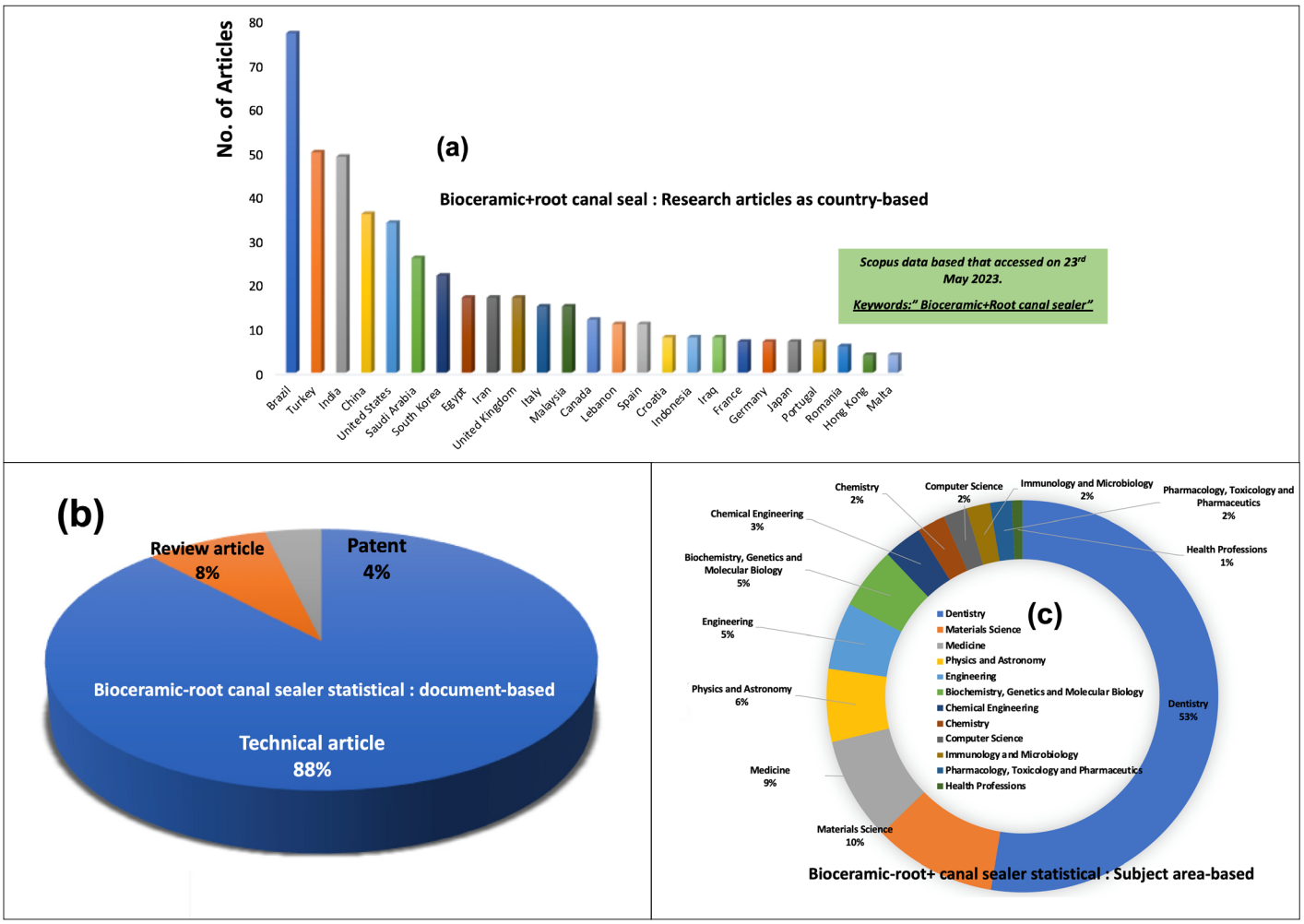

Figure 2. Bioceramic, root canal sealer statistical analysis on (**a**) research article: country-based, (**b**) document –based and (**c**) subject area-based. After screening all studies for relevance, a total of 75 articles were selected for this study. Of the selected articles, 88% were original research papers. As of May 23, 2023, the findings of the study revealed that there had been a significant amount of research conducted on bioceramic root canal sealer, as indicated in Figure 2 (**a-c**).

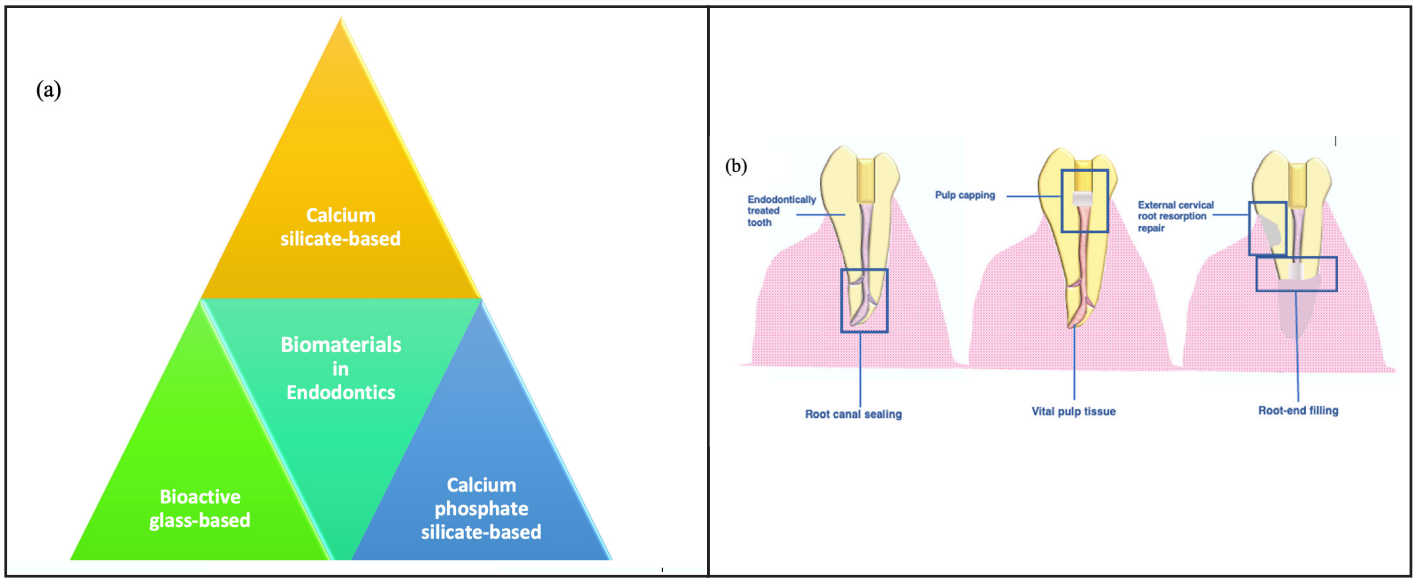

Figure 3. The use of biomaterials in endodontic practice

Table 1. Common types calcium silicate containing used in endodontics.

Product	Company	Composition	Radioopacifier additive	Product format	Form	Conditions of use
ProRoot MTA	DENTSPLY Tulsa Dental Specialties	Tricalcium silicate , dicalcium silicate , calcium dialuminate , and calcium sulfate dehydrated .	Bismuth oxide	Powder + liquid (water)	Cement	Pulp capping, pulpotomy, apexification, perforation repair, root-end filling
MTA Angelus	Angelus Dental Solutions	Tricalcium silicate, dicalcium silicate, tricalcium aluminate, tetracalcium aluminoferrite, calcium sulfate and bismuth oxide	Bismuth oxide	Powder + liquid (water)	Cement	Pulp capping, pulpotomy, apexification, perforation repair, root-end filling
MTA Repair HP	Angelus Dental Solutions	MTA Repair HH.P.is based on the formulation of conventional MTA but contains calcium tungstate as radiopacifier and a mixing liquid with a plasticizer agent	Calcium tungstate	Powder + liquid (water with polymer plasticizer)	Cement	Pulp capping, pulpotomy, apexification, perforation repair, root-end filling
MTA Plus	Prevest DenPro Limited	Similar in composition to ProRoot MTA (Dentsply) but is ground finer.	Bismuth oxide	Powder + liquid (water with proprietary polymer)	Root canal sealer	Pulp capping. Cavity lining. Pulpotomies.
MTA Flow	Ultradent Products, Inc.	Extremely fine, inorganic powder of tricalcium and dicalcium silicate	Bismuth oxide	Powder + liquid (water-based gel)	Cement	Pulpotomies, pulp capping, rootend filling, apexification, perforation repair, and root resorption
MTA Vitalcem	Brasseler USA	Has a composition similar to that of conventional MTA	Zirconium dioxide	Powder + liquid (calcium chloride and water)	Cement	Vital pulp therapy
Biodentine	Septodont	Tricalcium silicate, dicalcium silicate, calcium carbonate and oxide filler, iron oxide shade, and water	Zirconium oxide.	Powder + liquid (calcium chloride , hydro-soluble polymer)	Cement	Pulp capping. Indirect pulp capping. Revitalization. Root canal obturation. Root canal retreatment. Simple tooth extraction. Vital pulp therapy.
EndoSequence Root Repair Material	Brasseler USA	Dicalcium silicate , tricalcium silicate , calcium hydroxide , fillers	Zirconium oxide.	Single componet(paste or putty)	Cement	Repair of root canals and root perforations
TotalFill BC Sealer	FKG Dentaire	Dicalcium silicate , tricalcium silicate , calcium hydroxide , fillers	Zirconium oxide.	Single componet(ready to use)	Root canal sealer	Permanent obturation of the root canal
Ceraseal	Meta Biomed	tricalcium silicates, dicalcium silicates, calcium aluminates,	Zirconium oxide.	Powder + liquid (water)	Root canal sealer	permanent root canal sealing and for the permanent root canal filling
Dia root bio sealer	DIADENT GROUP INTERNATIONAL	Calcium Silicates, calcium aluminates, Calcium Oxide, and iron oxide	Zirconium Oxide	ready to use	Root canal sealer	Permanent root canal obturation

Calcium Phosphate-Silicate Containing Cements

Developing new biomaterials for orthopedics and dentistry based on calcium phosphate cement is relevant since they have a chemical composition similar to the mineral phase of bones and teeth and a series of advantages derived from their process. Hydraulic cement can be defined as mixtures of inorganic materials that set and develop mechanical strength by chemically reacting with water and forming hydrates [26].

The cement's consolidation occurs in two stages: setting and hardening. When mixed with water, a plastic paste is formed that loses elasticity with time and increases its mechanical strength so that if molded or remixed with water, the plasticity is restored or re-established. In the second stage, consolidation occurs, usually accompanied by the loss of water permeability, where the maximum resistance value is reached after several hours, days, or months [27].

The setting and hardening of cement involve a chemical reaction (dissolution, precipitation, or hydrolysis). The setting is a weak colloidal stage in crystal lattice development, while hardening leads to an irreversible crystal structure. This theory is based on LeChatelier's principle, which explains hardening through crystallization. Introducing the concept of an early colloidal stage explains cement measurement and setting phenomena [28].

The time during which the cement paste behaves eminently in a thixotropic form, called setting time, is strictly related to the working time, to the time available to prepare and place the cement definitively. The method used to measure this setting time, considering the time from which the needle used does not completely penetrate the cement mass, is performed by a device called Vicat [29]. Among the range of advanced endodontic sealers available, BioAggregate, iRoot SP, and Cerafill RCS stand out with distinctive qualities. BioAggregate impresses with its rapid setting time and potent antimicrobial effects, making it ideal for vital pulp therapy[30]. iRoot SP, on the other hand, focuses on biomineralization and sealing ability, along with efficient flow in intricate canals, and BioRoot RCS emphasizes silicone-based stability, dentin adhesion, and dimensional reliability [31]. The selection among these sealers hinges on the specific clinical context, necessitating carefully considering each product's unique strengths and suitability for the intended endodontic procedure.

There are many different types of Calcium Phosphate commercially available, including Cem-Ostetic (Calcium phosphate powder with Sterile water), MBCP® putty (β-TCP with Hydrogel), α-BSM (amorphous calcium phosphate, dicalcium phosphate dehydrate with Unbuffered aqueous saline solution), KyphOs™ FS (TCP, Mg3 (PO4)2, MgHPO4, SrCO3 with H2O, (NH4)2HPO4)), JectOS® (TCP and dicalcium phosphate dehydrate) and Quick Set Mimix,(Calcium phosphate powders, Na3C6H5O7•2H2O with Citric acid aqueous solution, etc. [32]. Due to their superior performance to brushite CPCs, it can be seen that the majority are apatite CPCs. Apatite CPCs are relatively non-degradable, which has limited their ability to be used more frequently in clinical settings. Therefore, to increase their clinical use, it is crucial to improve apatite CPC degradation. The common type of calcium phosphate-silicate-containing cement is detailed in Table 2.

Table 2. Common types of calcium phosphate silicate-containing cement used in endodontics.

Product	Company	Composition	Radioopacifier additive	Product format	Form	Conditions of use
BioAggregate	Innovative BioCeramix	Tricalcium silicate, tantalum oxide, calcium phosphate, silicon dioxide	Tantalum oxide	Powder + liquid (water)	Cement	Root repair, pulp capping, or pulpotomy procedures.
iRoot S.P.	Innovative BioCeramix	Tricalcium silicate, zirconium oxide, dicalcium silicate, calcium sulfate, calcium phosphate monobasic, and filler agent	Zirconium oxide.	Single componet(paste or putty)	Root canal sealer	Obturation of the root canal system
Cerafill rcs	PREVEST DENPRO LIMITED	Calcium Silicates, Calcium Phosphates, Bioactive Glasses, Calcium Sulphate, Calcium Oxide	Zirconium Oxide	ready to use	Root canal sealer	Permanent root canal obturation

The Bioactive Glass containing cement

The BG was developed in the 1960s, and the glass compositions of $Na_2O–CaO–SiO_2$ were explored from 1969 to 1971 to find a material implanted in the human body without forming scar tissue around the device. The 45S5 formulation, 45% SiO_2, 24.5% Na_2O, 24.5% CaO, and 6% P_2O_5 (wt %), which has a high Na_2O and CaO content and relatively high CaO/P2O5 ratio that makes the surface of the material highly reactive in a physiological environment. Due to its proximity to the ternary eutectic, this composition was chosen because of its high concentration of calcium oxide[33]. BGs are not allowed to be referred to by the trademarked term Bioglass. Biological Glass® is distinguished by silica (SiO2), a network-forming oxide in the form of the SiO4 tetrahedron, as the fundamental unit of the glass network. An oxygen link may be bridged between teams by –Si–O–Si– bridging oxygen bonds, allowing for the construction of a 3D network. As network moderators, sodium and calcium break up the network and form non-bridging oxygen bonds [34].

Glass type Na2O-CaO-SiO2-P2O5 in specific proportions [35], with silica (SiO2) component 50% mol%. BG has been used in the field of orthopedics for decades. After implantation of BG in a defect near the bone, several reactions on the material surface release critical concentrations of soluble P, Ca, Si, and Na ions, which induce favourable extracellular and intracellular responses, leading to the rapid formation of bone [35].

Commercially available root canal sealers that contain BG are available on the market. Some bioseal materials include GuttaFlow Bioseal (GFB), which consists of gutta-percha and polydimethylsiloxane, platinum catalyzer, zirconium dioxide, and BG. Despite its low porosity, solubility, alkalization capacity, dentin penetration, and cytocompatibility, GFB is cytocompatible [36]. The limited published evidence is currently available on the mechanism of the mentioned sealer hardening or its ability to seal and be removed for re-entry [17]. Nishika Canal Sealer BG (CS-BG) is the second product; there is compelling evidence about its removability, biocompatibility, physicochemical properties, and sealing ability. Bone and dentin-pulp complex regeneration were the original goals of the created CS-BG biomaterials .CS-BG's Paste A includes fatty acids, silica dioxide, and bismuth subcarbonate, while Paste B has magnesium oxide, dioxide, and calcium silicate glass (a type of BG). These paste are dispensed in a 1:1 ratio through a double syringe and can be easily mixed. A plastic spatula is recommended instead of a stainless-steel one due to the paste's corrosive nature. To prevent the hardening of CS-BG paste due to exposure to heat or moisture, store the syringes in a resealable aluminium foil bag and then keep the bag in a cool, non-freezing storage location, with temperatures ranging between 1-10°C [17]. Table 3 lists common types BG BG-containing cement used in endodontics

Table 3. Lists common bioactive glass containing cement used in endodontics.

Product	Company	Composition	Radioopacifier additive	Product format	Form	Conditions of use
GuttaFlow Bioseal (GFB)	Coltène/Whaledent AG, Altstätten, Switzerland	Gutta-percha, polydimethylsiloxane, platinum catalyzer and BB.G.	Zirconium dioxide	Ready to use	Root canal sealer	Filling material, It acts as a sealer between the core material and root canal walls.
Nishika Canal Sealer BB.G.(CS-BG)	Nishika Canal Sealer BB.G.(CS-BG)	Two-phased paste; Paste A consists of fatty acids, , and silica dioxide, whereas Paste B consists of magnesium oxide, calcium silicate glass (a type of BB.G., and silica dioxide	Bismuth subcarbonate	Ready to use	Root canal sealer	Root canal sealer during endodontic treatment, not as a general filling material.

Hybrid Cement Containing Bioceramic Material

Hybrid endodontic cement incorporating bioceramic materials has gained significant attention recently due to its enhanced properties and clinical benefits. These cements combine traditional endodontic cements' advantages with bioceramics' unique characteristics, resulting in improved sealing ability, biocompatibility, and tissue regeneration potential [37].

One such example is the combination of bioceramic particles with resin-based materials. This hybrid approach combines the adhesive properties of resins with the bioactivity and antimicrobial properties of bioceramics. The resulting cement exhibits strong dentin bonding, reduced microleakage, and the ability to stimulate mineral deposition for tissue healing[38, 39].

Incorporating bioceramics into hybrid cement contributes to their radiopacity, allowing for better post-operative assessment and follow-up. Furthermore, the alkaline pH of bioceramics in this cement can help neutralize acidity within the root canal system and promote an environment unfavourable for bacterial growth [40].

Each sealer offers unique benefits and characteristics, catering to diverse clinical scenarios and contributing to the ever-evolving landscape of endodontic treatment.

Characterization of Sealers and Cement Used for Endodontic

Pulp capping

The material used to cover the pulp significantly impacts the success of vital pulp therapies. A pulp capping material must be both biocompatible and antibacterial to be effective. This remedy is designed to help differentiate oral pulp cells and repair dentin [41]. Pro-Root MTA (Dentsply-Sirona, New York, USA) was the first hydraulic calcium silicate cement, followed by several others [42]. The development in the marketed materials is due to the biocompatibility and long-term survival of MTA and the demand for materials with better handling, less risk of discolouration, better sealing and lower prices [43]. Resin-based calcium silicate cements are among the latest developments for pulp capping. These materials can reduce staining and adhere to the tooth structure, improving sealers performance. Adding resins can achieve better binding to resin composites and resin-modified glass ionomers on top, minimizing treatment time and reducing leakage and early loss filling. The main disadvantage of this cement is the lack of biocompatibility of the monomers with essential pulp tissue, which can prevent the formation of a complete rigid tissue barrier in the exposed area [41].

Intracanal

After biomechanical preparation and disinfection, gutta-percha intracanal sealers are used for permanent root canal filling. Epoxy resin-based sealers are the"gold standard" due to their excellent sealing, low solubility, short setting time, and cost [44]. Bioceramics' bioactive properties can template a tissue-material bond, unlike epoxy resin-based sealers. Compared to other endodontic materials, they are more biocompatible and less cytotoxic [45].

This material forms dentin bridges, is biocompatible, has an alkaline pH, and does not promote inflammation, making it promising for root perforations, retrograde fillings, and pulpal exposure treatment. In 1998, the FDA approved the MTA. Since 1993, MTA has been used in surgical and non-surgical dental applications. MTA Fillapex seals root canals with salicylate resin and MTA. Its thin film and high flow rate reach the lateral and accessory canals. Antibacterial and biocompatible MTA and salicylate resin comprise 13% of the product. After 2 hours of setup, the working time is 23 minutes.[46]

DiaRoot BioAggregate is a root canal repair material composed of ceramic nano-particles. DiaRoot Bioaggregate is non-toxic, tooth-coloured, easy to apply, expands by 20% during curing, is highly hydrophilic, and chemically bonds to dentin. It is a biocompatible pure white powder mixed with BioAggregate to form a paste. DiaRoot Bio Sealer is a pre-mixed bioceramic calcium silicate-based MTA sealer used with DiaRoot BioAggregate. It was used for Root perforations, direct capping, apexification, internal root resorptions, and retrograde root canal filling .[47]

AH-Plus Bioceramic sealer reacts with collagen's exposed amino groups to form covalent bonds and can replace dentine surface treatment or dentine glue. Dual-cure resin-based sealers bind dentine better than AH-Plus. Later, hydrophilic methacrylate resin sealers could moisten canal walls and enter dentinal tubules. AH-Plus was the least soluble, radiopacity, and setting time sealer. AH-Plus BC contains zirconium oxide, iron oxide, and calcium tungstate. Zirconium and iron oxide in AH-Plus BC made it more radiopaque than barium sulfate sealers. Since epoxy amines polymerize slowly, AH-Plus takes time to set [48].

Extraradicular

Dentinal resorption, according to Costa et al.[49], is the loss of hard dental tissue (cementum or dentin) due to odontoclast action, whether physiological (first tooth exfoliation) or pathological. The resorption of alveolar bone involves clastic cells. This can be caused by physiological, pathological, or other factors. Non-mineralized tissues like pre-dentin, the odontoblastic layer and pre-cementum on both sides of the tooth root protect the tooth root.

Dentin apical inflammation and oscillating forces were essential factors in the pathogenesis of dental caries, according to Lee et al. [50]. The most common reason was tooth movement caused by orthodontic treatment. Surface resorption is a sign of periodontal ligament or root surface damage being repaired by healthy tissue nearby. It is self-limiting because the ligament can regenerate new fibers [51]. The main cause is decayed and infected dental pulp. Bacteria may produce acids and proteases that destroy the bone matrix components, or they may stimulate the production of osteolytic factor, which promotes osteoclastic activity, as two potential mechanisms for bacterial-induced resorption. Endotoxin (lipopolysaccharides) has been linked to tooth resorption, with osteolytic factor induction serving as the prevailing mechanism. These substances represent the gram-negative bacteria's outer surface [52].

Internal resorption is treated with calcium hydroxide paste injected into the canal and resorption lacuna. To remove necrotic tissue from the lacuna, calcium hydroxide and sodium hypochlorite are used sequentially to induce necrotization. The preferred treatments for lateral resorption are pulp removal, root canal debridement, and calcium hydroxide application. After medication, warm gutta-percha can be used to compact the defect [53, 54].

MTA Repair HP (Angelus Industrial de Produtos Odotontológicos S/A, Londrna, PR, Brazil) has developed tricalcium silicate-based products. MTA Repair HP incorporates calcium tungstate to replace the bismuth oxide radiopacifier. Furthermore, MTA Repair HP has more flexibility than its predecessor, white MTA-Angelus, which improves handling and insertion into the tooth. These materials are recommended for treating dental pulps (pulp capping, cavity lining, and pulpotomies) and root canals (perforation repair, root resorption, and apexification). However, it should be noted that the cytotoxicity of the material employed during acute pulp treatment, perforation repair, and retrograde filling may impact the survival of dental or periradicular cells, resulting in cell death through apoptosis or necrosis. As a result, it is critical to avoid using toxic dental materials on pulpal and periodontal cells [55].

External cervical resorption (ECR), the loss of dental hard tissue due to odontoclast activity, includes a dynamic process that affects dental, periodontal, and pulpal tissues in the following stages. ECR has recently received significant attention due to enhanced micro-CT, histological, and radiographic CBCT detection tools. However, it is noted that further research is required to determine the causes and consequences of several potential contributing elements. The most impacted teeth are the maxillary central incisor, maxillary canine, maxillary lateral incisor, mandibular first molar, and maxillary first molar. The analogous processes in the ECR process are commencement, progression, and resorption, followed by reparative phases. Resorption, healing, or remodeling may occur simultaneously in different regions of the diseased tooth. Improved CBCT analysis accuracy leads to more accurate detection and assessment of ECR and the decision of the optimum treatment method [14].

The rationale for non-surgical therapy of perforation is to avoid periradicular irritation. This may be performed by immediately sealing the perforation with a non-irritating material that will provide a sufficient seal to prevent microbial penetration. Even if a non-toxic and biocompatible material is utilized to repair a furcal perforation, the significant lesion may cause lasting damage to the periodontal attachment mechanism at the furcation site [56]. The prognosis is dismal in the event of a late and faulty repair. To keep such teeth, adequate and early treatment of the concerned teeth is required. In large perforations, the total closure of the hole with a sealing substance is problematic because it constantly permits irritants to access the furcation region—perforations at the gingival sulcus cause chronic inflammation and sulcular epithelial down growth into the defect. Coronally situated perforations, particularly furcal perforations, are more severe than those in the middle and apical thirds of a canal [56]. Bioceramics, such as mineral trioxide aggregate (MTA) and Biodentine, have been used to treat ECR. Nonsurgical repair using bioceramic putty is an effective treatment option for ECR. MTA has been used as a filling material in ECR cases, and its use successfully manages ECR with a stable outcome. The use of MTA in ECR cases involves filling the resorptive defect with MTA. In one case report, fibre post placement using flowable composite resin and MTA was used to fill the resorptive

defect. The use of MTA provides better conditions to access the resorption process. Effective management and appropriate treatment can only be carried out if the true nature and exact location of the ECR lesion are known. MTA has been widely used in pulp capping, apexogenesis, pulpotomy, and perforation repairs. MTA is the best material for repairing gaps. Strip, lateral, and furcation holes have all been effectively repaired by MTA, as shown by many trials with long-term follow-up. MTA's various benefits include its excellent sealing feature, biocompatibility, bacteriostatic or bactericidal qualities, radiopacity, and the ability to set in the presence of blood or moisture. Cementum formation is facilitated by cementoblasts, which MTA initiates. MTA's disadvantages include a longer setting time, challenging handling, and the possibility of discolouration[56]. Table 1 shows the characterization of different types of bioceramic sealer and cement used in endodontic practical.

Biological and Physicochemical Properties of Sealers/ Cement

Root canal filling is the endodontic treatment aiming to fill the newly decontaminated root canal system to prevent bacterial microleakage from the oral environment and apical and periapical tissues. This filling is considered one of the keys to the success of endodontic therapy.

One of the purposes of obturation is to prevent microorganisms from proliferating within the root canal system, making them impermeable and preventing the passage of microorganisms from the oral cavity or apical tissues to the canals. In addition, due to its flow, the cement reaches regions of the isthmus, secondary channels, accessories, and variable extensions in the dentinal tubules, reducing marginal microleakage and repairing periradicular tissues and conditions for the maintenance of periapical health [57].

An ideal cement's physical, chemical, and biological properties are good sealing, biocompatibility, antimicrobial activity, dimensional stability, insoluble in the oral environment and tissue fluids, adequate flow and low viscosity, and filling irregularities and spaces between the cones. Bonding dentinal walls and cement, ease of manipulation and insertion in the canal, radiopacity, not changing the color of the dental crown, adequate setting time, adaptation and adhesion to the root canal walls, being reabsorbed in the periapex when extravasated, stimulating or allow deposition of repair tissue and ease of removal when necessary[57].

Dentinal Tubule Penetration

The long-term success prognosis of the root canal treatment includes some essential steps, such as dimensional obturation of the shaped and disinfected root canal system; the sealers should adhere to the material and dentinal walls to avoid voids at the dentine-sealer interface [58].

The penetrability of sealers used in endodontic practice into dentinal tubules and anatomically complex areas directly relates to the flow property. The voids and leakage after root canal treatment may disturb the healing process. A moderate flow is desirable to access areas that need to be filled and not leak into the periapical region [59]. Regarding the sealer penetration, outcomes are inconsistent with the pressure on the obturation material during application to thrust the sealer into the tubules; however, several studies could not find a relationship between the type of obturation and penetration depth [60, 61].

There is no reliable information that sealers were labelled with any other fluorescent dye, except for the studies mixed with rhodamine B. one study utilized calcium as a marker [62], which was lower than those expected from previous studies. However, this technique is also not yet validated, and no other method is available except for confocal scanning[62]. Due to its resorption resistance and dimensional stability, AH Plus (DENTSPLY DeTrey, Konstanz, Germany) has been the gold standard material for hydrophobic epoxy resin-based sealers. However, it has drawbacks, including the possibility of mutagenicity, cytotoxicity, and an inflammatory reaction[63].

Furthermore, its hydrophobicity prevents the hydrophilic channel from being filled. Retained dental moisture, in particular, may cause errors in AH-Plus adherence to the canal walls; besides, a renewed sealer-based calcium silicate, a recently introduced sealer, Total Fill BC Sealer HiFlow (TFHF) (FKG Dentaire, St. Maur de Fossés, Switzerland) on the market that recommended usage for warm obturation techniques [64, 65].

Evaluating the filling quality with tubule penetration of bioceramic endodontic sealers is limited and leads to inconsistent results. Akçay et al.[66] compared the dentinal tubule penetration of various root canal sealers after the application of different final irrigation techniques, namely, conventional needle irrigation (CI), Er:YAG laser with photon-induced-photoacoustic streaming activation (PIPS), and passive ultrasonic activation (PUI). The

sealer types investigated were AH Plus, iRoot SP, MTA Fillapex, and GuttaFlow Bioseal. A total of 156 human mandibular premolars were examined, and the samples were sectioned at 2, 5, and 8 mm from the apex to assess the dentinal tubule penetration using a laser scanning confocal microscope. The results showed that iRoot SP exhibited a significantly higher penetration area than the other groups, whereas there were no significant differences between AH Plus, MTA Fillapex, and GF Bioseal. PIPS and PUI had significantly higher penetration than CI. Statistically significant differences were also determined at each root canal third, with the coronal third showing the highest penetration and the apical third the lowest. The study concluded that the selection of root canal sealer, final irrigation procedure, and root canal third significantly affected the dentinal tubule penetration area, and the use of iRoot with PIPS tip or PUI seems advantageous in dentinal tubule penetration. Fernández et al. [67] evaluated the ability of a calcium silicate-based sealer (iRoot SP) and an epoxy resin-based sealer (Topseal) using two gutta-percha filling techniques to fill artificial lateral canals (ALCs) in extracted human teeth and penetration of sealer and/or gutta-percha into the ALCs. The results indicated that the calcium silicate-based sealer with continuous wave of condensation was more effective in artificially filling lateral canals than the single-point technique. The epoxy resin-based sealer with both filling techniques was effective in artificially filling lateral canals. The apical third was associated with the lowest acceptable filling, followed by the middle and coronal thirds.

Başoğlu et al. [68] compared the penetration characteristics of two commonly used root canal sealers, Ah Plus and MTA Fillapex, following irrigation activation with different techniques, namely sonic, passive ultrasonic, SWEEPS, and XP-Endo Finisher, using confocal microscopy.160 mandibular premolar teeth were randomly allocated to four groups and eight subgroups. Confocal microscopy was used to examine three sections at different levels, and statistical analysis found significant differences in material, device, and region. The results suggested that SWEEPS as an irrigation activation technique holds promise in enhancing dentin tubule penetration by root canal sealer.

Antimicrobial Properties

Antimicrobial activity can increase the success rate of treatments in the endodontic practice, as they eliminate residual infections, whether bacteria arising from the treatment of the dental element or infiltrated later. However, evidence was insufficient on bioceramic-based sealers' long-term sealability or prognosis [69]; if a sealer used in a root canal system has antimicrobial activity, it can reduce a load of residual microorganisms [70] and may provide support in preventing secondary infections [71] .

It was reported that the freshly mixed root canal sealers are effective against some microorganisms; thus, the effectiveness between 2 and 7 days later was not reported; within this research, a significant number have focused on comparing the various materials in vitro[72]. The literature demonstrated that Enterococcus faecalis (*E. faecalis*) is one of the microorganisms in necrotic pulp, especially in teeth with secondary root canal infection [73]. Other microorganisms, including *M. luteus, E. coli, S. aureus, P. aeruginosa, C. albicans*, and *S. mutans*, have also been used to evaluate the antibacterial effects of endodontic sealers [3].

Analyzing the antibacterial impact is relevant to clinical practice. Commonly used models to evaluate antibacterial activity are the direct contact test (DCT), agar diffusion test (ADT), and modified direct contact test (MDCT)[74]. Hasna et al. [75] evaluated the antibiofilm action, biocompatibility, morphological structure, chemical composition, and radiopacity of five mineral oxides (5MO), mineral trioxide aggregate repair high plasticity (MTA Repair HP), and mineral trioxide aggregate (MTA) cements. The findings indicate that 5MO, MTA Repair HP, and MTA were effective against five anaerobic microorganisms and demonstrated biocompatibility with mouse macrophage and osteoblast cultures. It also possessed adequate radiopacity for clinical usage. Jerez-Olate et al. [76] evaluated the antibacterial efficiency of calcium silicate repair cement and sealers against a dual-species planktonic aerobic model with varying ageings and the capacity to suppress the establishment of a 21-day-old multispecies anaerobic biofilm. The bactericidal effectiveness of MTA Angelus, Pro-Root MTA, Biodentine, TotalFill BC, and BioRoot RCS against a dual-species aerobic planktonic model was investigated using the MDCT. SEM and CLSM were used to investigate the capacity to suppress biofilm development. Biodentine and BioRoot RCS exhibited a stronger antibacterial effect, and Biodentine maintained its antibacterial action in vitro. Antibiofilm action was more significant in MTA Pro-Root and Biodentine.

Setting Times and Behavior in the Biological Environment

Setting times of endodontic cement" refers to the time it takes

for the cement to harden and become stable. The setting times of endodontic cement vary depending on the type of cement and the clinical application. Generally, the optimal start time is between 4-8 minutes, and the final time is 10-15 minutes. Quick hardening is convenient, but the surgeon needs enough time to mold and implant it in the surgical site [77]. The particle size in the initial powder is also essential for the cement's setting and final properties. Smaller particles lead to faster dissolution and more excellent hardening rates due to the precipitation of a new phase through a precipitation dissolution mechanism [78].

Queiroz et al.[79] evaluated tricalcium silicate-based experimental materials, associated with different radiopacifiers such as zirconium oxide (ZrO2), calcium tungstate (CaWO4), or niobium oxide (Nb2O5), in comparison with MTA Repair HP (Angelus). The results showed that all the materials presented alkaline pH, antibacterial activity, low solubility, and no cytotoxic effects. The highest alkaline phosphatase activity occurred in 14 days, especially to TCS, TCS + ZrO2, and TCS + CaWO4. TCS + ZrO2, TCS + Nb2O5, and MTAHP had higher mineralized nodule formation than those of the negative control. After 7 days, there was no difference in mRNA expression for ALP, when compared to NC. However, after 14 days, there was no overexpressed ALP mRNA, especially TCS + Nb2O5, in relation to the CN. All the materials presented antimicrobial action. Lucas et al. [80], evaluated the physicochemical properties and dentin bond strength of the tricalcium silicate-based Biodentine in comparison to white MTA and zinc oxide eugenol-based cement (ZOE). The materials assessed included White MTA, ZOE cement, and Biodentine. The data were analyzed using ANOVA and Tukey-Krammer post-hoc test. Biodentine presented the shortest initial and final setting time, radiopacity that does not agree with ISO 6876:2012 specifications, higher compressive strength after 21 days, and higher dentin bond strength in comparison to white MTA and ZOE. Both MTA and Biodentine produced an alkaline environment compared to ZOE. It can be concluded that Biodentine exhibited faster setting, higher long-term compressive strength and bond strength to the apical dentin than MTA and ZOE.

Adding MTA to water and propylene glycol at various concentrations produced a smooth mixture, as reported by Natu et al. [81]. However, adding reduces the water available for the hydration reaction, resulting in extended first-setting periods. This can lead to a longer wait time before restoring the tooth and increased solubility, impairing the material's sealing ability. On the other hand, PP.G. improves flowability, enhancing the root canal system's ability to adapt to abnormalities and increasing the material's capacity to infiltrate perforations. However, handling and injecting the mixture into the root canal may present new challenges. Bramante et al. [82], MTA and clinker with 5% calcium sulfate had the slowest initial and final setting times for Portland cement, respectively. MTA's initial setting time was significantly longer than other materials, which is concerning. The only factor that extended the setting time compared to pure clinker was the inclusion of 5% calcium sulfate. The 5% calcium sulfate clinker had a much longer final setting time. A cement designed for biomedical applications must be set and hardened under physiological conditions with high humidity. Experimental techniques have allowed the design of stable cement formulations that can be submerged in a liquid phase immediately after mixing, solving some applicability issues [83].

Biocompatibility

Dental materials must be biocompatible to avoid toxicity to living tissues, particularly when in contact with bone cells in the periapical region. Various filling cement with unique compositions are available, and biocompatibility studies have been conducted in vitro and in vivo. A new generation of in vitro study methods and filling cement has recently emerged[84]. The cement's biocompatibility is crucial for resolving pre-existing bone lesions or preventing inflammatory reactions in healthy bone tissues in the periapical region [39].

Bramante et al. [82] examined the inflammatory reaction, presence of foreign body giant cells, and tissue regeneration around the implanted materials (Portland cement clinker with or without 2% or 5% calcium sulfate, and MTA-CPM) after 15, 30, and 60 days. The inflammatory reaction was graded on a scale from 0 to 3, while the presence of foreign body giant cells and tissue regeneration were assessed qualitatively. The results were then compared and analyzed using statistical tests, such as ANOVA and Tukey's test, to determine the significance of the findings. The materials that showed less inflammatory reaction, fewer foreign body giant cells, and better tissue regeneration were considered more biocompatible. Inflammatory cells and blood vessels were few. Biodentine has better cytocompatibility with primary human osteoblasts than MTA, as seen by increased cell survival, adhesion, and proliferation. Human osteoblast-like cell line MG63 has shown similar biocompatibility to MTA and Biodentine; both promote survivability, bonding, and

proliferation of MG63 cells. This may be because MTA and Biodentine have comparable heterogeneous morphology, surface roughness, and particle size [85]. MTA encourages Saos-2 cells to adhere, disseminate, proliferate, and secrete collagen.

According to Tanomaru-Filho [86], MTA increases ALP activity, calcified nodule development, osteogenic differentiation, and Saos-2 cell line differentiation. Widbiller et al. [87] evaluated the suitability of a new tricalcium silicate cement, Biodentine™, for use in dentistry by comparing its cytocompatibility and ability to induce differentiation and mineralization in three-dimensional cultures of dental pulp stem cells with mineral trioxide aggregate (MTA). The result showed that the cell viability was highest on the tricalcium silicate cement, followed by MTA, while viability on glass ionomer cement and dentin disks was significantly lower. Alkaline phosphatase activity was lower in cells on new tricalcium silicate cement compared to MTA, but the expression patterns of marker genes associated with mineralization were alike between the two materials. The results indicated that the new tricalcium silicate cement is cytocompatible and bioactive, confirming its suitability as an alternative to MTA in vital pulp therapy.

Regarding cytotoxicity, EndoSequence Root Repair Material and MTA show similarly low levels of cytotoxicity and cytokine expressions (IL-1b, IL-6, and IL-8). It was investigated by Fayyad [83] that two bioceramic-based materials, BioAggregate and iRoot (Innovative Bioceramix (IBC) Vancouver, Canada), showed acceptable biocompatibility and cytotoxic effects on human fibroblast MRC-5 cells, which was concentration-dependent). For the in vitro biocompatibility of White ProRoot MTA and iRoot, iRoot was found to be biocompatible and did not cause any significant cytotoxic effects, even though it promoted significantly lower viability than MTA after 48 hours of exposure; iRoot did not cause any significant cytotoxic effects because cell viability was greater than 70% of the control group in most tests[88].

Bioactivity

Bioactive materials induce a desired host tissue response using biomimetic approaches; in tissue engineering, the term refers to the biomaterial's ability to induce cellular effects via active ions biologically and substances released from its surface. In contrast, in biomaterial science, the term describes the biomaterial's ability to form the mineral hydroxyl apatite on its surface in vivo and in vitro[89].

The elongated shape of human dental pulp stem cells (DPSCs) induces calcified deposition in the presence of MTA in a simulated pulp capping model, confirms MTA's excellent bioactivity, and justifies its use in pulp capping.

In a comparative study conducted by Luo et al., the bioactivity of Biodentine and iRoot FS was assessed concerning their interactions with human periodontal ligament cells (PDLCs). The investigation revealed that both Biodentine and iRoot FS elicited an enhancement in the adhesion of human PDLCs. Notably, iRoot FS exhibited a superior capacity in comparison to Biodentine for facilitating the viability, proliferation, and osteoblastic differentiation of human PDLCs [90].

Chang et al.[91] studied the bioactivity and biocompatibility of four root canal sealers (Sealapex (Kerr Corporation), MTA Fillapex, iRoot SP ARS (Dentsply-Sankin KK) better to understand iRootSP'ss bioactivity to human PDLCs. All the tested sealers proved safe for human PDLCs while boosting Alp activity and causing mineralization nodules to form. Dubey et al. [92]proposed using graphene nanosheets to enhance dental cement physicomechanical properties and bioactivity. Biodentine and Endocem Zr were tested with the addition of Gp-NSs and found to have increased hardness and decreased setting times without sacrificing any of their fundamental properties.

Radiopacity

Radiopacity is a fundamental property because, radiographically, it will allow the professional to verify the correct root canal filling by the filling materials showing the correct apical limit of obturation and future controls to ascertain the success of endodontic therapy [93].

This property became standardized for dental restorative materials with the ISO 6876–2012 standard, which established that these materials must have a radiopacity equal to or greater than the radiographic density of dentin, equivalent to 3 mm of aluminium. When the radiopacity of the restorative material is lower than that of dentin, the differential diagnosis through imaging is compromised [94].

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is lower than that of dentin, the differential diagnosis through imaging is compromised [8]. More recent studies on the radiopacity of cement have used digital radiography or digitized images [95-97] , as this digital radiography requires less exposure time and eliminates the stage of chemical processing, responsible for variations in image quality, in addition to allowing better observation of density and radiographic contrast. The substances in their composition define the radiopacifying characteristics [8]. Many factors, such as material thickness, X-ray beam angle, type of radiographic film or a digital system used, and changes in the powder-to-liquid ratio during material handling, can affect this response of luting agents, but their composition seems to be the most essential. Among the oxides most used for this purpose, bismuth oxide is present in the composition of MTA Angelus and Pro-Root MTA. However, several studies have shown that this radio pacifier can increase the porosity of MTA, consequently decreasing the compressive strength and altering the cement hydration process[98]. This oxide has also been pointed out as probable for the dental darkening verified for MTA in contact with dental structures [8].

Radiopacifier Additives in the Endodontic Bioceramics

The radiopacity of endodontic materials is a critical physical feature. Endodontic cement must have a radiopacity larger than 3.0 mm about the aluminium scale, as specified by the ISO 6876 standard[99]. According to ANSI/ADA standard no.57, all endodontic cement must be 2.0 mm more radiopaque than dentin or bone [100]. Biomaterials with low radiopacity may result in incorrect analysis.

Additionally, radiopacity is essential for some spinal compression fracture therapies involving cement injections. In this case, it is critical to correctly identify the biomaterial after application to avoid material leakage into the spine or veins [101]. Radiopacity is an essential property to determine the materials used in the root canal system, such as those used to treat vital organs and distinguish them from natural tissues[29]. Thus, a radiopacity agent must be added to EndoBinder (Binderware, Sao Carlos, SP, Brazil) to enable radiographic visualization of the cement and the quality of the root canal filling and differentiate the cement from neighbouring anatomic structures. The ideal radiopacity agent should be inert, contaminant-free, and nontoxic, and it should be supplied in the smallest amount feasible, without forgetting that this smallest amount must be constituted of elements with large atomic numbers. By adding a proper amount of particles containing heavy metals such as bismuth (Z=83), silver (Z=47), and zinc (Z=30), the radiopacity of the material may be altered [7, 102, 103]. Zinc oxide is a non-toxic component of dental materials used in prosthetic and implant dentistry. Specific formulations include zinc oxide impregnated with silver to boost its radiopacity. The disadvantage is that silver is prone to discolor dentine. This is a particular issue for the coronal access cavity and impairs the look of the tooth. Bismuth compounds have substituted silver in sealer formulations, a well-known radiopacifying agent[104]. MTA has a large amount of bismuth oxide and exhibits a high radiopacity due to this compound's X-ray absorption. Nonetheless, specific studies have shown that this addition might increase porosity and decrease the mechanical strength of samples made of cement[98]. Thus, creating a new cement component based on rare earth elements with a high radiopacity that aids in the hydration and setting of the cement might improve its use.

CONCLUSION

According to current scientific information, bioceramics are essential to endodontics advancements. This conclusion is based on the existing body of knowledge. Bioceramics often need to be improved to benefit from their unique properties. Antimicrobial and radio-opacifier agents, such as silver compounds and many trace elements, have been integrated into bioceramics, as described above. Scientists assess dentists'' treatment progress using a biocompatible combination in various treatment procedures. The effectiveness of the method was the primary focus of the investigations. Because of this, scientists were eager to investigate commercial bioceramic products in endodontics.

On the other hand, research has shown that changing market trends and the need for better patient outcomes are essential determinants of technological change. The advantages of the bioceramic cement based on the literature in this review started with similarity to biological tissue, improved biocompatibility, intrinsic osteoinductive capacity because they can absorb osteoinductive substances during bone healing, resorbable lattices provide a regenerative scaffold that dissolves as the body rebuilds tissue excellent hermetic seal, chemical bond, and radiopacity, and these properties are critical to biomimicry of lost tissue, for the term biomimetic endodontics. In contrast, precipitation in situ after setting resulted in bacterial sequestration, which was the reason for the limitation. The nanocrystals inhibit bacterial adherence in bioceramic powders with a 1-3 nm diameter. Fluoride ions may be found in apatite crystals, resulting in a nanomaterial with antibacterial

characteristics. Solubilization of the repair may be compromised if the lead content or solubility is increased.

Tooth cracking may occur as a result of excessive bioceramic cement setting expansion. Biomineralization by the use of bioceramic cement is less successful. Developing new bioceramic formulations moves at a glacial pace, and more clinical research is urgently needed to speed things up. Healthy teeth are essential to overall health because teeth are the primary component of the oral cavity. Besides endodontics, bioceramics have also been used in surgical and prosthodontic procedures; its properties during endodontic formation can preserve a more tooth structure.

The main limitation of the current study is that most of the included studies are technical and laboratory preclinical studies. Laboratory studies present the preliminary results to improve clinical conditions. However, to guide the interpretation of the benefits offered by bioceramics, it should be noted that each test performed on these materials has limitations. Although the current studies are promising for these materials, whose clinical use has recently become widespread worldwide, long-term clinical results are lacking in the literature. Given all its benefits, they appear to have a bright future in dentistry. These materials have the potential to revolutionize endodontics with further research. There is a lack of data in this field to support the above-described and identified future research avenues for bioceramics in endodontics. However, conduct extensive clinical research on these scientific aspects in upcoming work.

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Case Report

Preoperative Assessment for Coronary Artery Bypass Graft: Going Back to the Basics

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ABSTRACT

As doctors increasingly rely on technological advancements, ever-improving laboratory techniques, and imaging modalities, the importance of a thorough physical evaluation and bedside manner has been noticeably diminished. When it comes to patients scheduled for coronary artery bypass graft (CABG), the preoperative bilateral arm blood pressure (BP) measurement is often omitted; thus, sinister signs of atherosclerotic disease like the brachial gradient are neglected and the surgical plan is jeopardized. We present the case of a 72-year-old male listed for CABG, stressing the necessity of meticulous clinical examination and the routine use of color Doppler ultrasonography (CDUS), prior to surgery. This case aims to highlight the major role of thorough preoperative clinical assessment. The bilateral BP measurement and CDUS are two reliable, expeditious, noninvasive preoperative screening methods, which can detect patients with generalized atherosclerosis, altering, if necessary, the surgical plan, and aiming for the best possible outcome, without complications.

Keywords: clinical examination, arm pressure gradient, cardiac surgery, subclavian artery stenosis, color doppler ultrasonography

INTRODUCTION

The role of comprehensive physical examination and bedside skills has been remarkably lessened nowadays, as physicians tend to depend more on technological advancements, and the ever-improving laboratory and imaging modalities [1]. It seems that technology has evolved from its initial complementing role, into a popular and dominant diagnostic tool, prevailing over clinical skills [1]. In addition, the global Covid-19 pandemic, which severely disrupted medical education, delivered another blow to the already fading from the mid-20th century clinical examination [2]. This is particularly evident in high volume clinical centers, where patients scheduled for coronary artery bypass graft (CABG) are not routinely submitted to preoperative bilateral arm blood pressure (BP) measurement. Important and potentially tailoring-treatment information is therefore neglected [3].

A typical example is the brachial gradient, a sinister sign of advanced atherosclerotic disease, which may alert the physician for the need to revise his surgical plan. Differential arm pressure will dictate further preoperative evaluation, which can be carried out either with color Doppler ultrasonography (CDUS), or CT angiography [4]. The examination of the subclavian artery (SCA), the left internal thoracic artery (LITA) and the postoperative evaluation of graft patency can be performed using the well-established CDUS technique [4].

We report the case of an elderly patient, with the aim to emphasize on the necessity of the meticulous clinical examination and the routine use of CDUS, prior to CABG surgery.

CASE PRESENTATION

A 72-year-old male, a heavy smoker with no prior medical history, was listed for CABG surgery at our cardiac surgery clinic. During preoperative clinical examination, we found a significant difference in systolic BP between the left (80mmHg) and right (130mm Hg) arm. Following the clinic's protocol, the patient underwent a CDUS for the initial assessment of probable subclavian and/or internal thoracic arteries disease.

Both internal mammary arteries were patent. Notably, there was a systolic flow reversal in the LITA with low/modest peak systolic velocities (~20 cm/s). This was particularly evident in the vessel's proximal end, adjacent to the SCA, along with collateral flow through the left intercostal arteries (Figure 1). Regarding the left SCA, the CDUS exhibited low upstroke waveforms (tardus parvus with peak systolic velocities around 40 cm/s) post the occlusion, which corroborate with significant stenosis/occlusion of the proximal left SCA. In addition, there was reversal flow in the left vertebral artery, accompanied by significant peak systolic velocities in the right vertebral artery (120 cm/s); those findings are consistent with subclavian steal syndrome. Subsequently, a preoperative CT carotid angiography confirmed the proximal left SCA occlusion (Figure 2). The patient, who displayed no evidence of arm claudication, underwent a triple CABG procedure with the right ITA deployed to the left anterior descending artery and the two saphenous vein grafts to an obtuse marginal branch and the right coronary artery, respectively. His postoperative course was uneventful, and the patient was discharged on the 6th postoperative day.

DISCUSSION

CDUS is an easily applicable, noninvasive functional technique for preoperative screening. Up to 7% of the CABG patients have severe SCA disease, according to medical literature [5]. Patel and colleagues in an attempt to decipher its prevalence, showed that the occurrence of significant SCA disease, proximal to the origin of the LITA (stenosis > 50% of luminal diameter), accounted for 5% of the cases [6]. In the same paper, the authors highlight the infrequency of patients' routine evaluation during coronary angiography, prior to CABG, which leads to failure to preoperatively document cases of LITA disease [6].

LITA is the most preferred conduit for coronary revascularization, as it has exhibited superior long-term patency [7]. However, SCA stenosis or occlusion proximal to the origin of LITA may severely compromise the graft's flow, even if the internal thoracic artery (ITA) per se, is an otherwise disease-free graft. Therefore, this can potentially result in retrograde flow or steal phenomenon from the coronary circulation, with resultant myocardial ischemia [8].

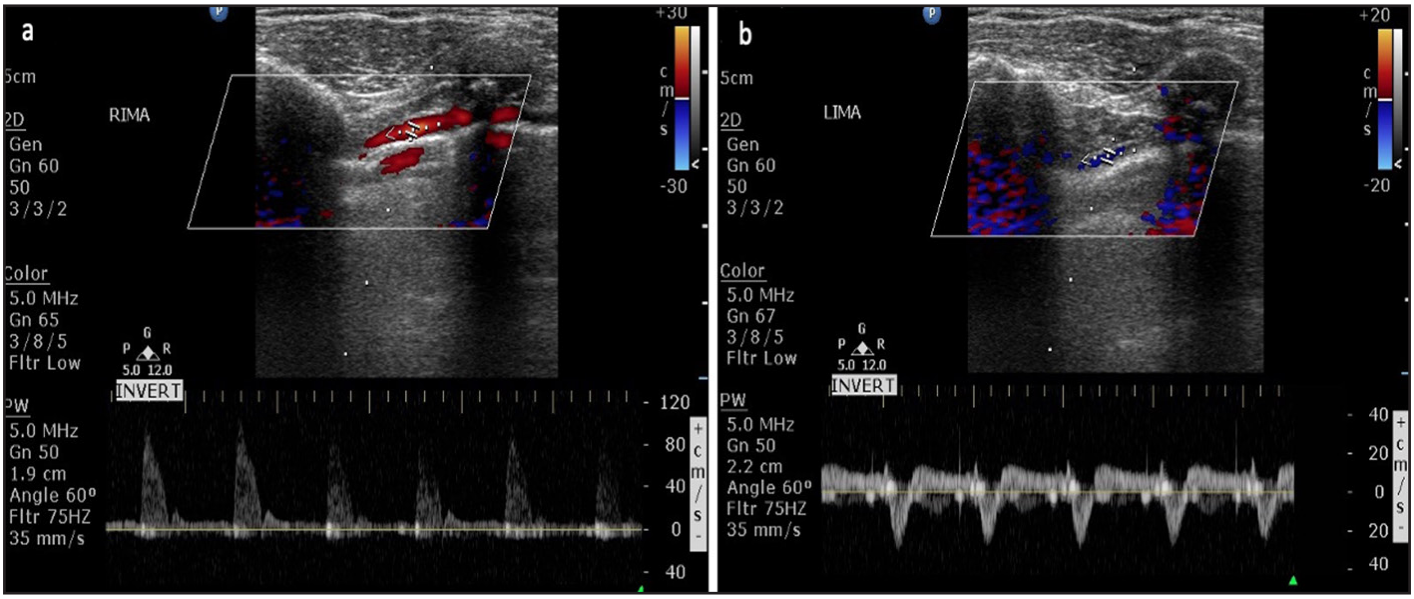

Figure 1a. CDUS of the right ITA; the artery is patent with normal systolic velocities (~80-100 cm/sec). **b.** CDUS of the LITA depicts low maximum systolic velocities (~20 cm/s) and reversal of flow in the systolic phase. ITA: internal thoracic artery, LITA: left internal thoracic artery

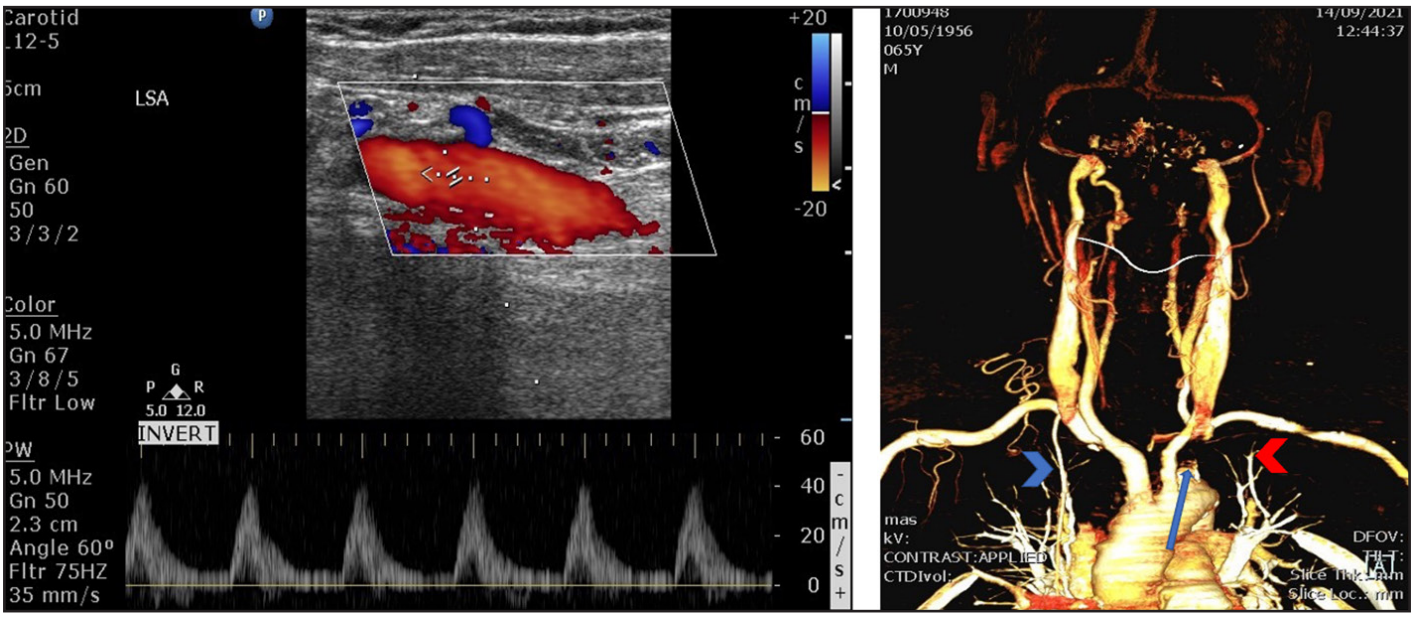

Figure 2. Left side: CDUS of the left SCA shows low upstroke waveforms (tardus parvus with peak systolic velocities of ~40 cm/s). **Right side:** CT angiogram of the carotids; proximal left SCA occlusion is demarcated by a blue arrow. Right ITA is visible (blue chevron) while the LITA is not depicted (red chevron). CDUS: color Doppler ultrasonography, SCA: subclavian artery, ITA: internal thoracic artery, LITA: left internal thoracic artery

In the preoperative setting of CABG, this can have major impact on surgical planning, especially on the grafts' arrangement. The need for surgeons' awareness cannot be overemphasized, since detection of optimal ITA flow prior to CABG can reduce complications caused by post-ITA graft ischemia [4, 6].

The preoperative routine of bilateral arm BP measurement is a simple method to enhance the safety of CABG [3]. This elementary, yet highly important examination can identify, in time, patients with SCA and LITA disease, shaping the surgical plan to achieve optimal revascularization and reduce the likelihood of unfavorable peri- and postoperative complications [9].

CONCLUSIONS

This case raises awareness of the crucial role of an in-depth preoperative clinical assessment, aiming especially to young doctors and nursing staff. In addition, it underscores the efficiency and convenience of CDUS, which represents a cost and time-effective, noninvasive modality for the evaluation of the subclavian and internal thoracic arteries flow and patency. The devoutly performed routine-medical-examination will not only provide a far more tailored treatment, but it will also diminish any chance of severe complications or even death, due to management and surgical mishaps.

Conflict of Interest: None.

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Consent: In order to publish our case with its accompanied images, we received a signed consent form from our patient.

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All authors had access to the data and a role in writing the manuscript.

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Case Report

Multiple Eruptive Dermatofibromas in a Patient with Primary Sjögren's Syndrome

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ABSTRACT

Multiple eruptive dermatofibromas (MEDF) are rare tumors and thought to be associated with the disturbances in the immune system. In our 40-year-old case, 5 nodules have developed in a 4-month period. The patient was diagnosed by a dermatologist to have MEDF, and referred to rheumatology outpatient clinic because of the symptoms such as dry mouth, and polyarthralgia. After clinical and laboratory evaluation, the diagnosis of primary Sjögren's syndrome (SS) was made. According to the best of our knowledge, this case is the first reported association between MEDF and primary SS. Therefore, when the diagnosis of MEDF is made, SS should also been included in the comprehensive evaluation of associated diseases.

Keywords: Sjögren's syndrome, dermatofibroma, dermal tumor, autoimmune diseases, fibrous histiocytoma

INTRODUCTION

Dermatofibroma (DF) is a common benign tumor among tumors of skin origin [1]. Although this tumor often occurs as a single lesion, more commonly on the upper extremities of young adults, the occurrence of multiple eruptive DF (MEDF) is rare. MEDFs have been associated with autoimmune diseases (AID), immunosuppressive therapy, HIV (human immunodeficiency virus) infection, hematological malignancies, pregnancy, and other conditions. Although the exact etiopathogenesis of MEDFs is not known, it is thought to be associated with the altered state of the immune system [2]. Sjögren's syndrome (SS) is a chronic inflammatory AID that often presents with dry eyes and mouth due to functional involvement of the exocrine glands [3]. SS consists of two forms: primary SS, characterized by sicca symptoms unrelated to AIDs; and secondary SS, which is characterized by symptoms associated with other AIDs, particularly systemic lupus erythematosus (SLE) [3]. We present a female patient who developed MEDF associated with primary SS.

CASE

A 40-year-old female patient applied to the dermatology clinic due to the presence of multiple asymptomatic papules and nodules developed in a four-month period. The patient has no known disease and no history of drug use. Clinical examination revealed five well-circumscribed, hyperpigmented, firm, brown and round nodules with a diameter of 3-10 mm in the left-right upper arm, right leg and abdomen (figure 1). In the skin biopsy performed, there are uniform spindle cells arranged in long fascicles parallel to the epidermis. Immunohistochemical examination showed the expression of factor XIIIa and vimentin. When evaluated together with the anamnesis, physical examination and skin biopsy it was found to be compatible with DF by the dermatologist. The patient diagnosed with DF was referred to the rheumatology clinic for the evaluation of AIDs. In the rheumatological evaluation, the patient had dry mouth, dry eyes and especially metacarpophalangeal and proximal interphalangeal joint pain of both hands for one year. No signs

of arthritis were found in the physical examination. In the Schirmer's test, the paper was wetted <5 mm in both eyes. In laboratory examination, antinuclear antibodies test was found to be positive (1:100 homogeneous). Extracted nuclear antigens were all negative. The patient was undergone a minor salivary gland biopsy which was reported as having focus score ≥1 and diagnosed as SS.

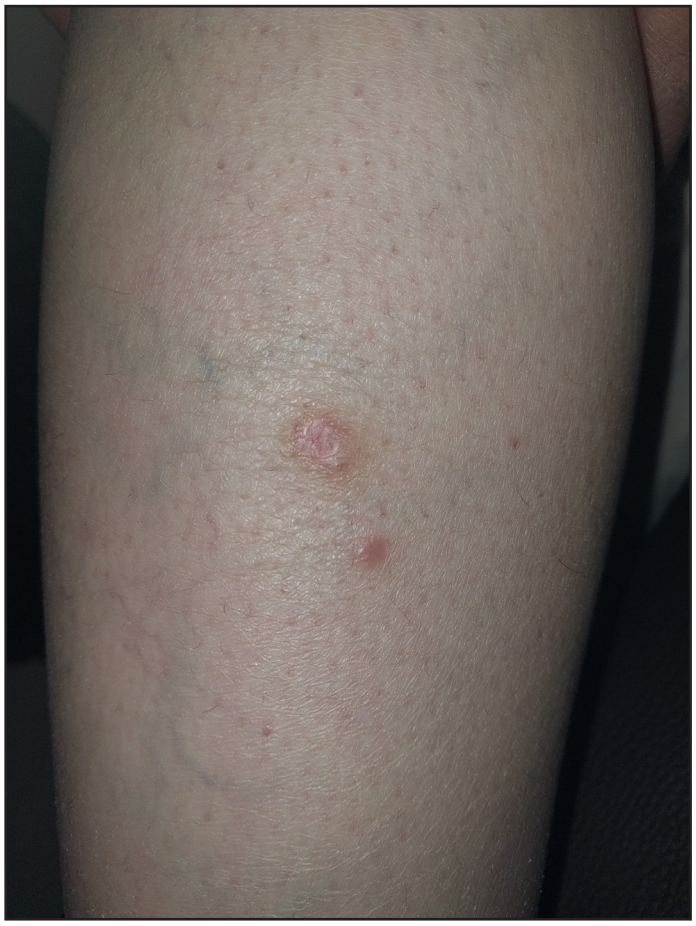

Figure 1. Hyperpigmented, brown and round dermal nodules are shown on the left-right upper arm, right leg and abdomen.

DISCUSSION

Dermatofibroma, also called benign fibrous histiocytoma, is one of the most common cutaneous soft-tissue lesions. It is known as a benign dermal proliferation of fibroblasts [4]. Although the pathogenesis is unknown, they can sometimes occur as a result of trauma or infection. DF occur most often in adults and are most commonly located on the lower extremities [4]. Diagnosis is usually based upon clinical appearance and history. If the lesion is longstanding, characteristically it should have no history of rapid change. Excision for histopathologic examination is indicated for any changing or bleeding lesion or when the lesion is suspicious for malignancy [5]. Usually, no treatment is required unless the lesion is symptomatic [6]. MEDF was first described in 1970 and is generally defined as the occurrence of at least 5 DFs within a four-month period. MEDF is significantly less common in patients with DF, and a significant proportion of MEDF is thought to be associated with a systemic condition [7]. The most frequently associated conditions associated with MEDF have been reported in the literature as AID (SLE, dermatomyositis), immunosuppressive drugs, HIV infection and hematological malignancies [8]. Of the MEDF considering its association with immune-mediated diseases or immune suppression states, it is thought to be strongly associated with immune system mechanisms [8]. Nestle et al. [9] states that DF arises as a result of an immune system in which dermal dendritic cells are prominent. According to this hypothesis, the development of MEDFs in immune-deficient conditions may be facilitated by inhibition of down-regulatory T cells; alternatively, MEDFs may develop as an exaggerated response to a putative pathogen that cannot be cleared by the suppressed immune system [9]. SLE is the most common autoimmune disorder associated with MEDFs. There are numerous cases of MEDF reported in patients with SLE or in patients with SLE and SS, but no cases of MEDF associated with primary SS have been reported [10]. Although we made the diagnosis of SS after the skin findings of DF in our case, the symptoms of SS had been begun months ago. Thus, MEDF might be considered as associated with SS. This case is one of the rare cases in the literature that emphasizes the potential relationship between primary SS and MEDF.

CONCLUSION

All these data show that the theoretical relationship between the incidence of MEDF and immunosuppression is not accidental. While any attempt so far to provide a solid interpretation has been purely speculative, dermatologists and rheumatologists should be aware that MEDF may be a sign of a disturbance in immune system, and all patients should evaluated for the presence of an associated systemic disease.

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DT. Data interpretation: SY. Drafting manuscript: MNK, SY. Revising manuscript: all authors.

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Letter to Editor

Artificial Intelligence in the Diagnosis of Maxillofacial Disorders

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Dear Editor,

Recently, studies and research have focused on the use of artificial intelligence (AI) in medical science [1,2]. It is probable that the healthcare industry, especially radiology, is a step or two ahead of the curve when using convolutional neural networks in clinical practice. The number of investigations into the use of radiography in daily life continues to grow, as does the number of accessible methods that have already impacted the issue of patient care, both of which are on the rise. In addition, there is a whole area devoted to Medical Imaging using AI. Additionally, a dedicated domain has emerged, focusing on the synergy between AI and Medical Imaging, particularly in the context of diagnosing Maxillofacial Disorders.

The diagnosis is made based on the patient's medical history, linked testing, and other susceptible variables, known to be risk factors for human memory retention. AI from human professionals performs much better than human specialists when using primary health data [3]. A study indicated that by using AI in conjunction with clinical diagnostics, the accuracy and efficiency of diagnosis might be significantly improved.

Recently, machine learning techniques have been used to diagnose several illnesses, including tumors, cancer, and metastases, among others. These algorithms demonstrated excellent reliability in distinguishing periapical cysts from keratocystic odontogenic tumors when manually created parameters [4] were used in their development. However when these approaches, such as convolutional neural network (CNN), were employed to examine the cytological pictures gathered, they revealed an inadequate performance error in identifying malignant lesions of the mouth. Although these results are hopeful, existing AI algorithms for diagnosing oral and maxillofacial lesions predominantly rely only on a single type of data, cytopathological reports. Using models that include the patient's medical history is critical for a more accurate analysis [5].

Deep learning (DL) and CNN have made significant contributions to AI in caries and endodontics because of their capacity to automate waste categorization and classification. To

classify radiographs or photographs, several criteria, including comparable qualities, are used to separate them into many discontinuous sections [6]. This process results in predictable data being generated from unpredictable data. Using understanding network (U-Net), the DL categorizes the cone beam computed tomography (CBCT) vertices into “lesions,” “tooth structures,” “bones,” “restorative materials,” and “backgrounds,” with the findings comparable to the diagnosis of total lesions. Apical is a company that supplies doctors [7]. Distal caries lesions may also be detected by DL using imaging data [8].

The clinical signs and symptoms that the patient exhibits are crucial in diagnosing temporomandibular disorders (TMD). It is a method for converting spoken language into an ordered computer language known as speech processing. It was found that constructing a software model based on the sorts of words used in the patient’s speech and the size of their mouth was more successful than using the actual mode [9]. A full degree of agreement between AI and the physician is shown in AI’s identification of condyle morphology.

Reviewing these articles was instructive since it provided us with an opportunity to observe the diverse range of approaches that have been created and assessed across a diverse range of images and experiences. However, it is important to note that no one has determined how these approaches will be integrated into a clinical workflow or, more importantly, whether and how they will impact radiologists’ diagnostic accuracy and efficiency, and consequently, patient outcomes. Therefore, it is difficult to predict which ones will be implemented in a clinical environment. As underscored by the study findings, continued research endeavors are imperative to harness the full potential of AI in transforming the landscape of diagnosing Maxillofacial Disorders.

Best regards,

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Letter to Editor

Dermoscopic Diagnosis of a Non-Pigmented Skin Tumor: Eccrine Poroma

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Dear Editor,

Eccrine poroma stands as a benign adnexal neoplasm that originates from the acrosyringium. It typically emerges as solitary, flesh-colored, or erythematous papules, plaques, or nodules, primarily appearing in areas with sweat glands. As well as pigmented lesions, dermoscopy has proven to enhance the clinical diagnosis of numerous non-pigmented skin tumors, including eccrine poroma. Herein we present a case of eccrine poroma located on the dorsal aspect of the left foot, with characteristic dermoscopic features. A 60-year-old woman was admitted with an asymptomatic lesion on the dorsum of her left foot, which had manifested approximately four years before. Clinically, the lesion presented as a well-circumscribed, violaceous, 0.9x0.9 cm papule (Figure 1a). Dermoscopic examination revealed flower-like and leaf-like vascular patterns, white interlacing areas, glomerular vessels, and milky red globules (Figures 1c-d). The lesion was excised and histopathologic findings were consistent with eccrine poroma (Figure 1b). Eccrine poroma (EP) is an adnexal tumor originating in the intraepidermal part of the eccrine sweat gland duct. Dermoscopy and histopathology help to differentiate EP from pyogenic granuloma, seborrheic keratosis, verruca vulgaris, basal cell carcinoma, squamous cell carcinoma, and amelanotic melanoma. Well-defined dermoscopic features in EP are: White interlacing areas around vessels, milky-red globules, flower-like and leaf-like vascular patterns, glomerular vessels, hairpin vessels, yellow structureless areas, poorly visualized vessels, and well-circumscribed globular or lacuna-like structures separated by white to pink mesh bands [1-3]. Histologically, EP manifests as a well-contained tumor constituted of proliferative cuboidal or poroid cells, often extending from the basal epidermis into the dermal layer. Shave, electrosurgical destruction or simple excision may be the treatment of lesions, depending on the depth of the lesion.

Kind Regards

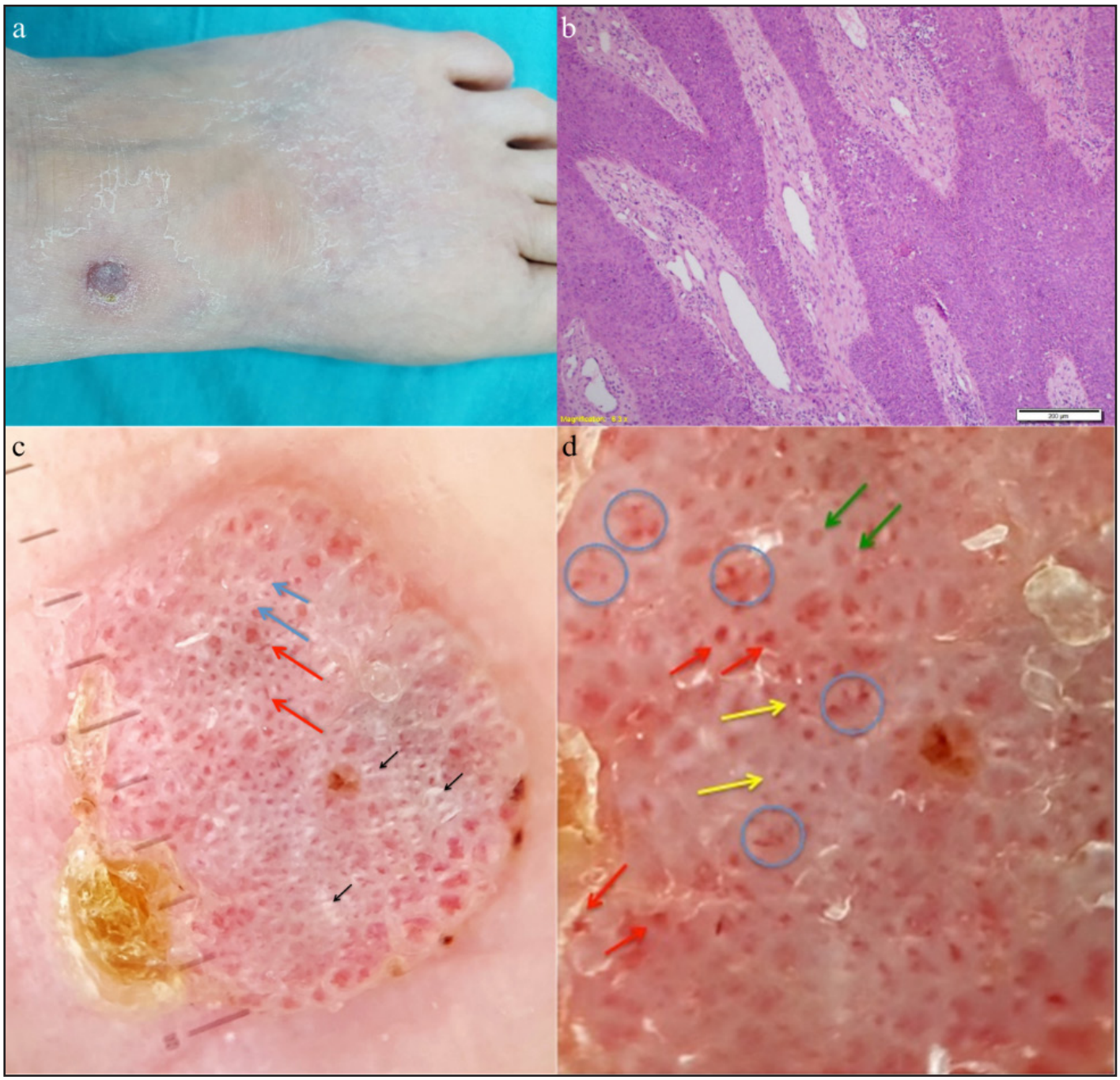

Figure 1. a) A 0.9x0.9 cm violaceous, well-circumscribed papule on the left foot dorsum. b) Histology: Anastomosing cords of cuboidal cells extended into the dermis. c) Fine scales all around the lesion and a small ex-ulcer on the periphery are seen. There are white-pink halos surrounding the vessels and white interlacing areas (black arrows), circular vessels (blue arrows) and thin linear vessels (red arrows). d) Dermoscopic image showing the flower-like and leaf-like vascular patterns (blue circles), glomeruloid vessels (red arrows), dotted vessels (yellow arrows) and milky red globules (green arrows).

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Letter to Editor

From Ancient Mythology to Modern Technology: The Historical Evolution of Artificial Intelligence

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Dear Editors,

Recently, a fascinating article detailing the evolution of Artificial Intelligence (AI) was published, as the article describes it with the bronze creature Talos from ancient Greek myths and the groundbreaking DaVinci Si and DaVinci Xi surgical systems of the modern era [1]. The interaction of ancient myths and medical technology shows how far we have come but how deeply we remain connected to our past.

As the article emphasizes, Talos, described in ancient Greek literature, may be the world's first recorded concept of an automaton with Artificial Intelligence capability. Talos, created by the god Hephaestus, used his bronze form and inner fire to patrol the coasts of Crete and deter invaders [2]. The story of a machine that moves independently, driven by an energy source and following specific instructions, is astoundingly pioneering [2]. It is interesting to think that robots, an idea we usually think of as a modern concept, were present in the imagination of ancient civilizations.

Leonardo da Vinci's robot knight is a moving tribute to the versatile genius of the Renaissance [3]. However, since its operation depends on external intelligence, it is not a direct precursor to the AI we know today. The article makes this distinction.

However, A claim that has been presented is open to discussion. Naming AI surgical tower da Vinci is misleading, article suggests. Although Leonardo's automaton was not autonomous, its extensive notebooks testify to a questioning and innovative mind that was always pushing boundaries. In this sense, it feels appropriate to mention the name of Da Vinci, a technological marvel.

The underlying sentiment is concurred with. Hephaestus' creation of Talos offers a more direct line to the concept of autonomous machines and perhaps it deserves a more prominent position in the story of the history of Artificial Intelligence.

Highlighting this overlooked intersection of mythology, history, and technology is commendable. Such discussions highlight the importance of understanding our past while understanding our rapidly evolving present and future.

Sincerely yours,

Keywords: Artificial intelligence, Ancient Mythology, Leonardo da Vinci's Automation, Medical Technology Integration.

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Letter to Editor

AI as a Co-Author? We Should Also Ask Philosophical (and Ethical) Questions

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Dear Editors,

With great interest and attention, I read authors' short but stimulating editorial articles, which also contain extremely valuable questions [1]. The questions posed by the authors and the emphasis on referred articles reflecting different perspectives seem to reflect content about the practical benefits, risks, and inevitability of the use of technology. In addition, it is seen that the valuable comments on the article focus on issues such as the risk of harm and publication ethics issues in academic use of AI [2,3].

Considering the discourse of the article's authors, which invites discussion, it is seen that questions that go beyond mere responsibility or practical benefits or risks should also be asked. In this context, I think that questions based on philosophical and ethical foundations should also be asked. For example, What is AI's ontological position as a writer is a very fundamental question. That is, does AI reflect a "particular" individual/entity as "a writer", or does it reflect cognitive domination that has the power to access and process the knowledge of entire humanity easily, or does AI reflect a collective mind or "universal" as a product of the knowledge and cognitive history of humanity as a whole? I think these questions are notable for AI's position as an author and whether that position is acceptable. Again, should AI be considered a tool (is given instrumental purpose) or as a value in itself (has intrinsic value)? As a fundamental question, this is also important for the acceptability or position of AI as an author. Without going into deep discussions for now, for example, how or what is the difference between AI as a tool and a classical data analysis tool? Does such a difference, if any, really warrant the attribution of authorship to AI? Questions like these seem important. Although different ethical discussions seem possible, the concept of responsibility to which the authors refer seems worth discussing. For example, it is also remarkable whether AI, as a responsible subject in itself, can also become a tool for other authors to avoid some responsibilities in research and publication ethics. It is known that such a debate exists in the field of clinical ethics [4].

As a result, as the article authors said, the authorship of artificial intelligence will lead to

important discussions. Therefore, it will be necessary to consider philosophical (and ethical) questions about the position of artificial intelligence.

Yours sincerely,

Author Contributions: Conception: A, Y - Design: A, Y - Supervision: - Fundings: -Materials: - Data Collection and/or Processing: - Analysis and/or Interpretation: A, Y - Literature: A, Y - Review: A, Y - Writing: A, Y - Critical Review: A, Y

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Letter to Editor

Artificial Intelligence Co-Authorship: Perspectives on Scientific Accuracy and Responsibility

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Dear Editors,

Amidst the rapid advancements in artificial intelligence tools, we have read the editorials published in your journal on the subject of "artificial intelligence and academic articles" with great interest [1, 2]. First and foremost, we would like to express our gratitude for fostering an essential platform for discourse on this current topic. Thank you for creating a significant environment for discussion.

The rapid advancements emerging in artificial intelligence tools undoubtedly promise significant contributions not only in various fields but also in the realm of science. However, just as in scientific progress, it is clear that the opportunities supporting science and enabling its advancement are also evolving. For instance, had we sent this letter to your journal thirty years ago, we might have needed to send it through postal mail. Alternatively, if our writing had been published in your journal twenty years ago, we could have read it in hard copy rather than in an online environment. Similarly, had we been practising medicine hundreds of years ago, we wouldn't have had the capability to do anything for patients that we can easily treat today with the aid of ultrasound guidance in the operating room.

It is highly likely that in the future, thanks to artificial intelligence tools, many tasks will become significantly more efficient and practical. From this perspective, we believe that incorporating artificial intelligence tools into the realm of science is a necessity. However, as you have also pointed out in your editorial articles [1, 2], we believe that the inclusion of artificial intelligence tools as authors in academic research is a significant topic of debate. Based on our current knowledge and perspective, we believe this situation may not be entirely appropriate.

We believe that one of the most crucial points of contention regarding the inclusion of artificial intelligence tools as authors in academic research is the concept of "accuracy". Artificial intelligence provides us with information it finds on the internet. Whether these sources are genuinely obtained from reputable journals cannot be definitively determined. This poses

a significant challenge in ensuring the accuracy of such contributions. This also suggests that articles written by artificial intelligence may not be sufficiently reliable. For instance, when we input "the lumbar transforaminal injection method" into ChatGPT, it provides a lot of information on the topic. However, when asked for references, it responds with, "The information I provide is based on a vast dataset of text from a wide range of sources available on the internet, including books, websites, research papers, and more." Indeed, it can also retrieve information from virtual and/or fake accounts. In essence, as of now, artificial intelligence lacks a truth filter similar to that of a human. While artificial intelligence facilitates rapid access to information, the uncertainty arising from data unreliability raises doubts about the information it presents. Furthermore, we believe that artificial intelligence cannot share an equal level of responsibility with human authors for the information it provides. For these reasons, we are of the opinion that the responsibility for confirming the accuracy of information presented by AI applications lies entirely with the human authors, and we believe that artificial intelligence applications should not be listed as authors in articles.

Yours Sincerely,

Keywords: artificial intelligence, ChatGPT, scientific accuracy.

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Letter to Editor

Follow-up of Artificial Intelligence Development and its Controlled Contribution to the Article: Step to the Authorship?

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Dear Editors,

I am excited to see a current discussion in this journal [1]. In another editorial article, the questions and answers directed to Chat Generative Pre-Trained Transformer (ChatGPT), an artificial intelligence (AI) product, about the authorship process contributed to my idea of conducting a similar study [2]. In addition, addressing the editorial question, the second answer partially satisfied me because it was more innovative. Although the answers of ChatGPT in this article are apparent in the text, their contribution is not included in the footnote, explanation, acknowledgment, or reference; in some studies, it is shown in the footnote [3]; in some, it is included in the acknowledgment section [4]. Such contributions or the nature of the support received should also be evaluated and clearly stated. Which section should be included for AI-enabled content such as ChatGPT? Since accessing the previous accesses is impossible, it may be better to copy them to a visual or open access place instead of giving them a reference in the sources.

Although many subjects need to be read further and a detailed examination is required, a brief description of the working mechanism should be made. AI's deep learning is to experiment with a given set of inputs and outputs and make suggestions, and when it encounters a new input, it gives it an appropriate output. As I analyze the book chapters [5] that examine the success of AI programs in the process of inventing, producing art, and doing the work of different professional groups, such as lawyers or doctors in some fields, with appropriate learning algorithms, I think that they may have a promising potential for the future in terms of writing articles. In an environment without prejudice, such as the Turing test, there has been much discussion about the superiority of intelligence only when compared to the machine [5]. In addition, the fact that AI provides a contribution whose authorship cannot be detected by similarity or plagiarism programs, which are different software products, makes this situation difficult to understand.

In one of the studies contributing to this editorial correspondence, various AI examples with different functions and more capabilities are given, apart from ChatGPT. In addition, while drawing attention to the trust problem, margin of error, and differences in the level

of development between programs, it was emphasized that the suitability of using AI applications for general and simple service operations such as article language editing to reduce financial costs should be treated without prejudice [6]. Another article stated that the support to be received from AI after the article was written would be more reliable, controlled, and risk-free [7]. The article that questioned AI's identity on philosophical and ethical grounds was also remarkable [8]. In a different approach, it was stated that with the increase of pseudoscience authors, scientific and unethical situations may be encountered more frequently and different filtering systems should be used as a precaution. Language translation or text editing contributions were seen as an advantage [9]. In these conditions, where ethical problems are not resolved, it is stated that authorship is not correct and that it should be used to increase the quality of the article by making use of its features that facilitate writing [10]. These articles mention general topics about the potential uses of AI in article writing, possible harms, and cautions are mentioned.

The study, which listed suggestions for the practical use of AI in authorship, emphasized the lack of creativity and deep analysis power required for authorship [11]. Another study stated that AI could not be accepted as an author because AI could not take responsibility for its writings, did not comply with research ethics and violated copyright law [12]. As I asked the ChatGPT-3.5 model, another researcher who shared its answer with a similar approach stated that it does not see itself as a researcher and author but that its helpful contributions can be used in the writing and subsequent processes [4]. In another article, which deals with topics such as the areas of use of AI in research, the accuracy of ChatGPT was found to be generally positive [13]. In the article on the opportunities and challenges of AI, which offers comprehensive guidance, the authors expressed their concerns about transparency and explainability of authorship [3]. In a different study, the authorship criteria of The International Committee of Medical Journal Editors (ICMJE) and the Committee on Publication Ethics (COPE) were mentioned and it was explained that AI cannot make a significant contribution with data collection and interpretation, cannot approve the final version of the article, and can only collaborate in writing [14]. Another leading study revealed that AI meets only three of the 14 criteria, namely visualization, drafting, and editing, according to CRediT (Contributor Roles Taxonomy), which is important in terms of authorship criteria. The authors shared the reason why ChatGPT could not meet these criteria and their answers when other criteria were queried with ChatGPT. In parallel with the study, the ChatGPT-3.5 model gave the same answer to my questions and stated that it would not be accepted as an author [15].

General concerns and criticisms focus on the fact that AI cannot take responsibility because of erroneous information and that there are no sanctions in unethical situations. Although there is no such reality now, the debate seems to continue in the coming period, even if AI contributes more than humans and is accepted as an author who is given responsibility and punished. These may depend on where the process will evolve with the contributions of lawyers and software developers and the regulations to be taken according to new developments. Therefore, for now, studies for controlled and comprehensive planning should be followed by authorities from international multidisciplinary fields such as lawyers, professional organizations, publishers, journal editorial boards, and ethics committees. Even if AI is not accepted as an author due to current conditions, the location of AI applications and general criteria, it quickly stepped into the academic studies environment and its authorship has come to the fore and discussions will be held.

Best Regards,

Acknowledgments: My questions mentioned in the text were answered with my account using the ChatGPT-3.5 model on https://chat.openai.com/. Based on ChatGPT's recommendation, instead of thanking ChatGPT directly, I would like to thank its developers and researchers, the OpenAI company responsible for creating and maintaining the AI application, and the AI developer responsible team.

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Letter to Editor

The Horrible Scenario in Cath Lab: Percutaneous Management of Guide Wire Entrapment During Coronary Intervention

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Dear Editor,

Advancements in invasive coronary angiography and accumulated experience have improved the success of interventions in challenging coronary artery lesions and associated complications. However, the approach and success in managing rare complications such as guide wire entrapment depend on the patient's hemodynamic status, continuity of coronary flow, capabilities of the angiography laboratory and the operator's expertise. In this letter, we present a case of guide wire entrapment during coronary intervention, the difficulties encountered during percutaneous removal attempts, and the finally applied conservative approach.

Patient Information

A 56-year-old male, known for active smoking and a history of three-vessel coronary bypass surgery four years ago, presented with pressing chest pain. The patient had undergone coronary angiography (CAG) a year ago, and medical follow-up was recommended. Due to the diagnosis of unstable angina pectoris, the patient underwent another angiography. Following the stent implantation for significant stenosis after the anastomosis in the saphenous-LAD graft, attempts to retrieve the guidewire resulted in stent deformation (Figure 1) and entrapment. Despite efforts to retract the guidewire, it was unsuccessful. Subsequently, the case was urgently taken over, maintaining the catheter and guidewire in a sterile manner (Figure 1). After obtaining cardiovascular surgical consultations, a decision was made to reattempt the procedure through percutaneous coronary intervention.

After ensuring proper field cleanliness, the procedure began by confirming the absence of catheter thrombus. It was observed that there was no distal flow in the first images (Figure 2). Attempts to enter the stent with a 1.0x12 mm Artimes balloon (Brosmed) were unsuccessful, and after the balloon's deformation, a second attempt was made with another balloon but was also unsuccessful. Microcatheters were used to enter the stent, but they got trapped, and only after various manipulations, the microcatheter could be retracted. Subsequent attempts with PT-2 and Fielder XT-A Guidewires for the buddy wire technique were unsuccessful due to entrapment between stent struts (Figure 1). Considering the thinness of the distal vessel and the chronic near 99% stenosis similar to previous CAG images, it was decided to attempt distal

wire detachment due to the high surgical risk in this patient. However, despite attempts, the wire did not detach. During the wire retraction, the heart shadow on fluoroscopy moved, and the patient experienced severe pain. Since repeated pull-backs were unsuccessful, consecutive and prolonged torques were applied to the wire, resulting in distal wire fracture (Figure 2). Echocardiographic control showed no effusion. The patient was transferred to the coronary intensive care unit. Following one day in the intensive care unit and two days in the cardiology service without symptoms, the patient was discharged with dual antiplatelet therapy. No anginal symptoms were reported during one-year follow-ups.

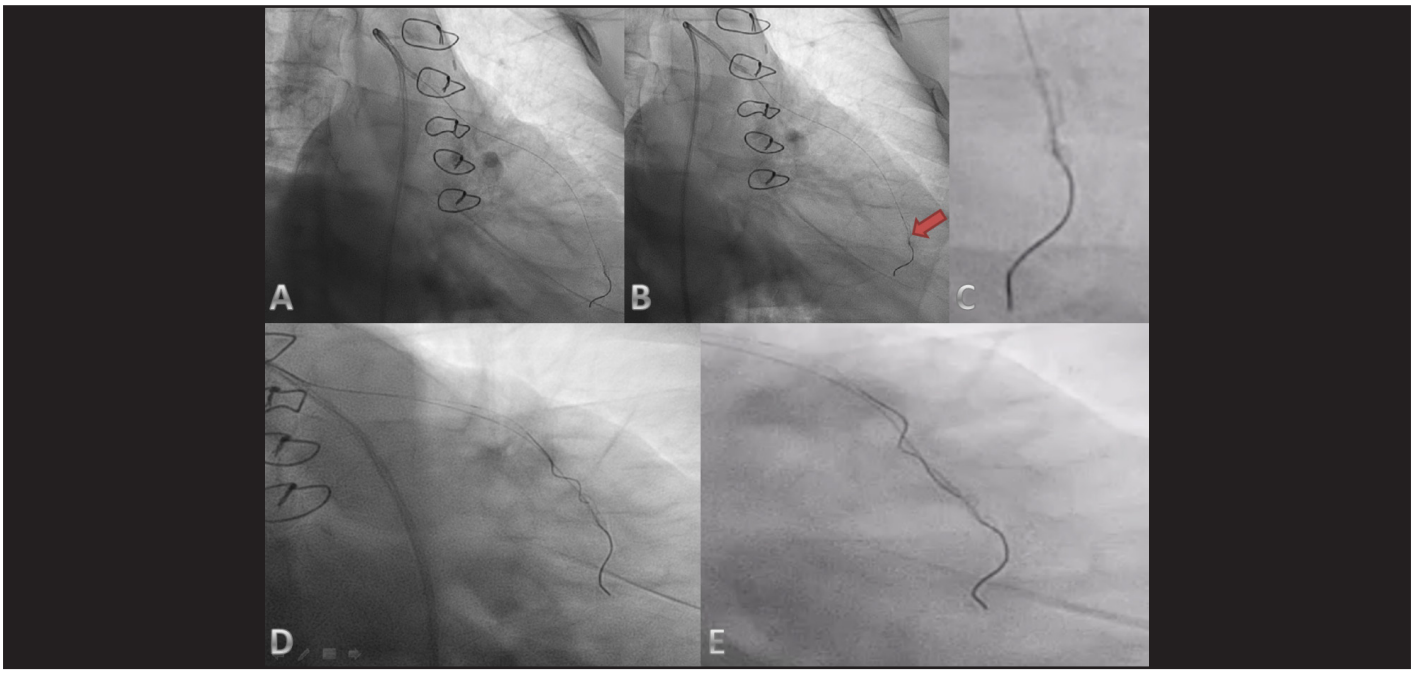

Figure 1. A: First image after transfer, B: Deformed stent view marked with red arrow, C: Zoomed image of deformed stent, D: Failure to send the second guide wire, E: Failure to send the second guide wire zoomed in

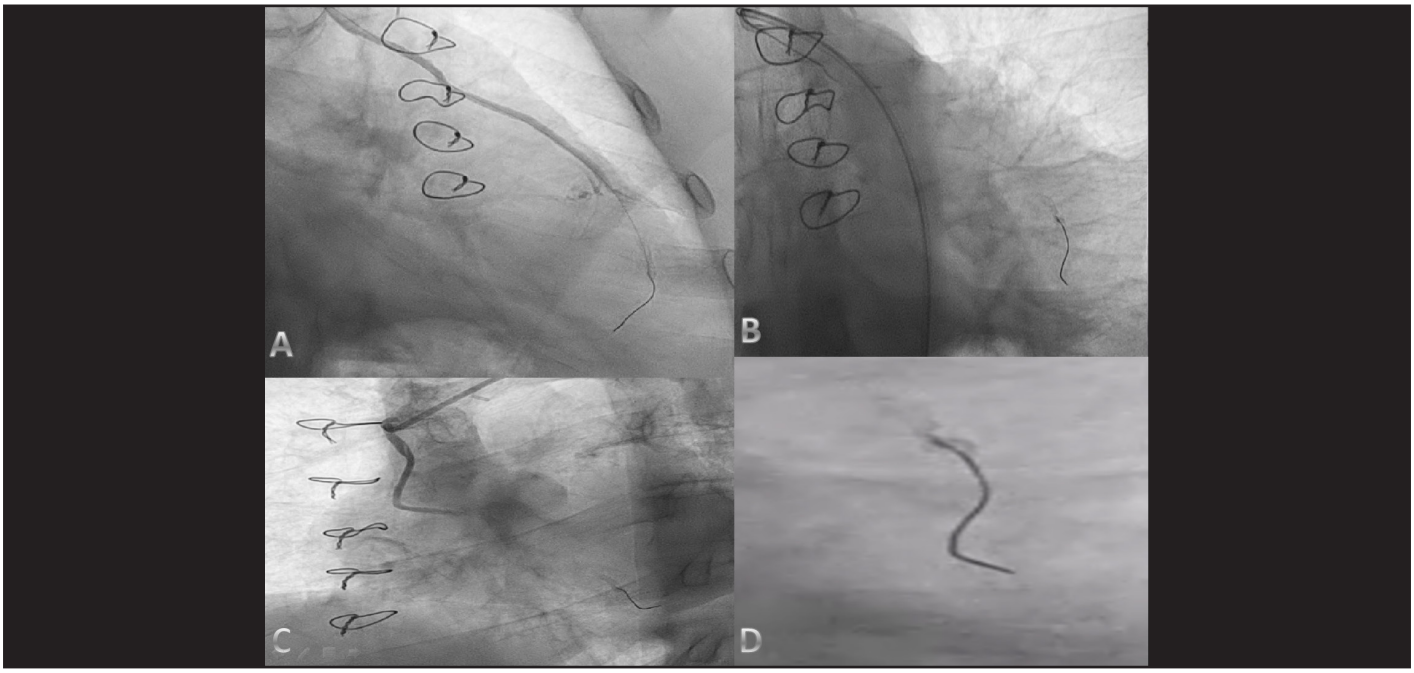

Figure 2. A: There is no distal flow in the coronary where the guide wire is located, B: Appearance of the broken wire after manipulation, C: Dissection line in saphenous RCA graft, D: Zoomed-in view of the broken distal part of the wire

DISCUSSION

Prior to coronary intervention, determining the appropriate strategy based on coronary anatomy and lesion characteristics, along with selecting the appropriate guide wire, constitutes the initial step in preventing complications related to the guide wire. Guide wire entrapment is rare, with an incidence of approximately 0.1-0.2% [1]. The localization of the entrapped wire, the patient's hemodynamic status, and the continuity of coronary blood flow determine the approach to the complication. In a review of 48 reports involving 67 patients, guide wire entrapment was treated surgically in 29 cases (43.3%), percutaneously in 28 cases (41.8%) and conservatively in 10 cases (14.9%) [1]. Techniques such as the multiwire technique, snare loop capture, microcatheter support, and balloon inflation can be applied percutaneously to release the trapped wire [2]. Various approaches have been developed over the years for managing a broken guide wire, given concerns about thrombosis, dissection, distal or systemic embolization caused by a broken piece of the system.

Potential causes for guide wire breakage include aggressive manipulation, cutting with an atherectomy device, entrapment between stent struts, and wire deformation. Apart from percutaneous wire removal, surgical removal or conservative approaches may be considered depending on the patient's condition [3]. In cases where surgical decisions are made for additional reasons, surgical removal of the wire should be considered [4]. Complications such as hemodynamic deterioration and loss of coronary flow may necessitate urgent intervention. In hemodynamically stable patients, a conservative approach may be considered for wire fragments that do not affect coronary flow, especially those located distally or in insignificant side branches.

In our case, it was believed that the wire broke from the region where it was entrapped due to excessive manipulation. Applying torque to the wire while it was still inside the microcatheter during the wire-breaking stage seemed to be a more suitable approach as it was thought to cause less damage to the surrounding structures.

Evaluating the localization of the broken piece and its relationship with vessel and stent structures through intracoronary imaging (IVUS/OCT) is crucial for observation. In our case, the procedure was performed under urgent conditions, and we did not have a ready-to-use intracoronary imaging device. Due to the patient's stable hemodynamics after the distal wire manipulation and the wire's thin location in the distal vessel with chronic stenosis, we opted for a conservative approach. However, it is evident that our patient and we were fortunate due to the thin structure of the distal vessel and the small area affected by the flow. Complications would likely have a more fatal course in cases affecting larger feeding areas.

The patient was discharged with dual antiplatelet therapy due to stent implantation. However, even if a stent had not been placed, it would be appropriate to provide dual antiaggregant therapy in the first six months of follow-up to prevent platelet activation caused by the broken guide wire [5]. No additional intervention was considered during the one-year follow-up due to the absence of active complaints. While experience and treatment methods for guide wire-related complications vary, further research is necessary.

Yours sincerely,

Keywords: Coronary Angiography, Guide-wire Fracture, Guide wire Entrapment

Funding: This study received no funding.

Conflict of Interest: The authors report no conflict of interest.

Each author takes responsibility for all aspects of there liability and freedom from bias of the data presented and their discussed interpretation.

Written informed consent was obtained from patient.

In our article, no support was received from artificial intelligence-based assistive technologies.

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Letter to Editor

Optimising Rehabilitation Strategies for Postpartum Elderly Gravida with In Vitro Fertilisation Conception

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Dear Editor,

As per World Health Organization (WHO) data, 5-15% of couples of reproductive age experience infertility. In vitro fertilization-embryo transfer (IVF-ET), which initially appeared at the end of the twenty-first century, is not only a core component of assisted reproductive technology but also an important way to treat infertile patients in modern medicine, giving the majority of infertile patients fertility hope [1]. Advanced maternal age (AMA) is a major clinical and social problem. At present, there is a significant increase in the percentage of women who delay pregnancy until their late third or early fourth decade of life [2]. Many elderly women prefer to use IVF to have children. However, it has been observed that the age of the female was one of the key determinants limiting fertility and reproductive results [3]. The International Council of Obstetricians and Gynaecologists introduced the term "elderly primigravida" in 1958 to describe women over the age of 35 who were embarking on their first pregnancy. Pre-eclampsia, gestational diabetes, foetal abnormalities, and premature birth have all been identified to carry an increased risk of maternal and foetal morbidity during the same time [4]. Women of advanced maternal age are frequently considered as if they need the level of care required for any high-risk pregnancy, and they are given special attention even when there is no scientific basis for it and no medical issues are evident. However, because of pre-existing and pregnancy-related morbidity, as well as high maternal expectations, these women require more intervention throughout pregnancy and delivery [5]. Advanced maternal age is linked to several financial, social, and physical problems for the mother as well as for the foetus [2]. Some studies discovered that elderly gravida were more likely to have a child with Down syndrome, as well as a higher chance of miscarriage and hypertension. However, the chances of requiring a Caesarean section, having a preterm or low-birth-weight baby, having a stillbirth, or having multiple births were not as well determined [5,6]. Intense physical change occurs during pregnancy, and many women experience significant emotional upheaval during this time. While improving the chances of favourable maternal and newborn outcomes during pregnancy remains the major objective of prenatal care, emphasis should also be given to how pregnancy-related conditions might influence a woman's life [7]. The loading and position of the vertebral column, as well as the muscular forces along it and in the

weight-bearing joints, alter throughout pregnancy. Physiotherapy is vital in obstetrics, both during pregnancy and after delivery [8,9]improper gait and difficulty in carrying out activities of daily living since the patient did not show any concern initially. Assessment, medical history and treatment have been discussed in this case study. Conclusion: The case study concluded that there is a significant effect of the given therapeutic intervention on the muscle strength, muscle re-education and Activities of daily living (ADL. Hence, we present this letter to the editor of post-partum elderly gravida with IVF conception with gestational hypertension and gestational diabetes mellitus with cervical stitch in situ with its structured physiotherapy management.

Patient Information

A 51-year-old woman and her 57-year-old husband opted for IVF trials because of advancing age, intending to conceive a child. The couple initiated infertility treatment in 2019. The menstrual history was regular, with a 30-day cycle lasting four 4 days, and the flow was of moderate intensity. Hysteroscopy revealed bilateral tubal blockage. IVF trials were carried out, resulting in conception during the third attempt; however, miscarriage occurred during the second month of pregnancy. Additional trials were pursued, leading to the successful conception of the fifth attempt. During pregnancy, the patient developed gestational diabetes and hypertension. As a result, a cervical stitch was placed at the 20th week of gestation. At 33.5 weeks of pregnancy, the patient underwent an elective lower segment caesarean section, giving birth to a baby girl weighing 2.3 kg. Two days after delivery, physiotherapy was recommended. She reported experiencing pain at the suture site, as well as upper and lower backaches, along with urinary incontinence.

Clinical Findings

The patient exhibited a well-nourished physique with a mesomorphic body build. Her hemodynamic condition was stable. Upon observation, the patient displayed a forward head posture, thoracic spine extension, anterior tilt of the pelvis, and increased curvature of the lower back. Waddling gait was also observed. Palpation revealed grade 2 tenderness and pain level of 6/10 at the suture site (Pfannenstiel incision) on the NPRS scale. During the general examination, chest expansion was limited, diastasis recti measured 3 cm in width, and pelvic floor strength was assessed as Grade 1. The strength of the upper abdominal muscles was graded as Fair + (6), whereas the strength of the lower abdominal muscles was graded as Fair (5).

Therapeutic Intervention

Medical Management

The medical treatment strategy featured a combination of injections and medications to meet particular health concerns. The following drugs were administered to the patient as injections: tax, metro, pan, tramadol, amikacin, augmentin, and lomoh. The patient's treatment regimen included multiple medications in addition to injections. Metformin, Augmentin, Metro, Pan, Chymorol Forte, and Limcee were among these medications. Every medication had a distinct function that helped to manage the patient's medical condition and aid in her recovery. A Jonac Suppository was also used as part of the therapy plan to improve medical management approach.

Physiotherapy Management

Patient Counselling

The patient was given information about the altered physiological changes that occur in the female body after pregnancy, as well as age-related transitions. The physiotherapist conducted a discussion about the value of regular exercise and how it affects mental and physical health. The patient was given practical advice on nursing practices as well as instructions on splinting measures to alleviate pain at the suture site. In addition, the family received education on postpartum depression, which provided them with the knowledge to assist the patient in making these life-changing changes.

Management

Day 1 to Week 1

The patient was given guidance on multiple techniques to help with her rehabilitation throughout. These included teaching the splinting technique, forced expiratory technique, and applying an ice pack to the suture site for 10 minutes three times a day for pain relief. Correct breastfeeding demonstrations were given. Static back and abdominal exercises with 10 repetitions twice a day were included in the patient's exercise program to enhance muscle strength. Additionally, rhomboid stretching (10 repetitions) was used to alleviate the upper back pain. Cervical range of motion exercises (10 repetitions per set) were also performed. The patient was advised to avoid rotational and side flexion movements to prevent strain on the diastasis recti abdominis muscle. Instruction regarding pelvic floor contractions was also provided. Breathing exercises, including thoracic expansion (Figure 1) and deep breathing, were included (10 repetitions for two sets). Ankle-toe movements (20 repetitions

twice daily) were introduced to prevent complications and postural correction exercises were initiated.

Figure 1. Patient performing thoracic expansion exercise

Figure 2. Patient performing pelvic bridging

Week 2 – Week 4

Continuing the patient's progression, deeper breathing exercises, including deep breathing and diaphragmatic breathing (10 repetitions for three sets daily) were introduced. Kegel exercises (the hold relaxation technique) were taught for pelvic floor strengthening. Pelvic tilting, hip adductor and abductor rolls, and pelvic bridging (Figure 2) were included, each with a 5-second hold for 10 repetitions once a day. For diastasis recti, transverse abdominis contractions with a 5-second hold for 20 repetitions were incorporated. Bilateral upper- and lower-limb mobility exercises were introduced, accompanied by stretching exercises and strength training. Postural correction exercises remained constant.

Week 4 – Week 6

Progression was seen in the Kegel exercises, with the addition of pelvic bridging with hip roll, a combination of hip adductor and abductor rolls, and hook-lying hip rolls, each with a 10-second hold for 10 repetitions. Core strengthening was heightened along with ongoing breathing exercises. Stretching exercises were maintained with a 10-second hold for three repetitions twice daily. Gait and balance training was continued.

Week 6 – Week 8

Aerobic exercises commenced, preceded by a 10-minute warm-up and cool-down session. Core strengthening, postural correction, and Kegel exercises were also performed. Quadruped, opposite arm and leg raises, side bends, leg lowers, neck stretches, and cat and camel exercises were introduced.

Home Exercise Program

For the patient's home exercise regimen, warm-up and cool-down sessions included low-impact aerobics and modified yoga or pilates. Additionally, running/jogging and resistance training were incorporated for at least 20 minutes, three days per week.

Follow-up and Outcomes

The patient underwent assessment for postnatal depression using the Edinburgh Postnatal Depression Scale, and functional activity was graded using the FIM Scale. Pelvic floor grading, mid-stream-stop flow test, and abdominal Manual Muscle Testing (MMT) were used to evaluate pelvic floor and abdominal muscle strength, respectively. The Numeric Pain Rating Scale (NPRS) was used to gauge overall pain levels. A manual technique was applied to grade the diastasis recti. In summary, there was a noticeable moderate improvement following treatment. The patient was advised to return to the rehabilitation clinic over a follow-up period of three weeks. However, since the patient had relocated to another city, a home exercise program was prescribed. A detailed breakdown of the scores for each outcome before and after the treatment is shown in Table 1.

Table 1. Scoring of Outcome Measures

Serial number	Outcome Measure	Pre-treatment	Post-treatment
1.	Edinburgh postnatal depression scale	8	2
2.	FIM	70	122
3.	Pelvic floor grading	1	4
4.	NPRS	6	1
5.	Diastasis recti	3cm	1cm
6.	Upper Abdominal MMT	Fair + (6)	Good (8)
	Lower Abdominal MMT	Fair (5)	Good + (9)
7.	Mid-stream stop flow test	1	3

DISCUSSION

Physical activity during and after pregnancy is an important aspect as many physical and emotion changes taken place in women body, Ana Victoria Montoya Arizabaleta et al. conducted a randomized trial on 64 pregnant women to study the effects of a 3-month supervised exercise program and came to the conclusion that primarily aerobic exercise during pregnancy enhances health-related quality of life [7]. After a caesarian section, twenty women participated in a pilot study by Qurat Ul Ain et al. to evaluate the pain relief and functional activities following the procedure. The results of the study showed that postnatal exercises increase mobility and alleviate pain in post-natal period [10]. Diastasis recti abdominis (DRA) is more common in pregnant and postpartum women. However, there is a paucity of knowledge about this condition among women. Menaka Radhakrishnan and Karthik Ramamurthy concluded in a scoping review on efficacy and challenges in the treatment of diastasis recti abdominis that recently minimally invasive surgery has been created to reduce IRD. However, it is not always applicable. Exercise treatment is recommended for women, even during pregnancy. Various research on exercise treatment for DRA patients have indicated considerable outcomes, even though the exercise program for DRA has to be thoroughly standardized [11].

According to Kaj Wedenberg et al. prospective ‘s randomized study on 60 pregnant women, which compared acupuncture with physiotherapy for the treatment of low-back and pelvic pain, acupuncture provided better pain relief and reduced disability as compared to physiotherapy [12]. Using an only one exercise and advice-based physical therapy intervention in early pregnancy, Moffatt, M. et al. conducted a pilot study on the prevention of pregnancy-related lumbo-pelvic pain and noted that several protocol modifications would be necessary to ensure the satisfactory conclusion of a larger-scale study [13].

CONCLUSION

This presentation posed a challenge because the patient was an elderly gravida. The therapy was customized to accommodate both pregnancy-related adjustments and geriatric changes along with their accompanying complications. The results after treatment indicated a moderate improvement in the outcome measures. This letter has the potential to assist other therapists in devising more effective rehabilitation plans.

Sincerely yours,

Keywords: Elderly, Post-partum, rehabilitation, Advanced maternal age, IVF

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Conflicts of interest

The authors declare no conflicts of interest.

Informed Consent

Consent was obtained from the patient.

Ethical Statement

This study does not require ethics committee approval.

Author Contribution

All authors contributed equally to the final manuscripts.

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Letter to Editor

Alternative Approach in Colorectal Anastomotic Stricture: Bougie Dilatation

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Dear Editor,

Anostomotic stricture (AS) is seen in 2-30% of patients after colorectal surgery. Various factors such as tissue ischaemia, anastomotic leakage and radiotherapy have been suggested in its pathogenesis [1,2]. Endoscopic methods (balloon dilatation, bougie dilatation, stents, endoscopic electroincision), digital dilatation, surgical methods (stapler stricturoplasty, transanal circular stapler resection, transabdominal redo-anastomosis) and corticosteroids are used in AS [3,4]. Surgery is generally preferred in complete/near complete AS [1]. Stents; the benefit of stents in AS after oncological surgery has not been shown [3]. However, Philip BC Pangg et al. treated near-total AS non-operatively with the colonic/rectal endoscopic ultrasound (EUS) anastomosis technique and a hot lumen metallic stent [5]. We applied 3-stage bougie dilation to the patient with near complete anastomotic stricture.

A 59-year-old female patient who underwent laparoscopic anterior resection due to sigmoid colon tumor was followed up with complaints of abdominal swelling, intermittent abdominal pain and difficulty in defecation. One month later, when colonoscopy was performed, near complete anastomotic stricture was observed. Bougie dilatation was performed with maloney flexible bougie dilators under wire guidance. Bougie dilatation was performed 3 times with fifteen days intervals. After the first (33, 36 and 42 F) and the second bougie dilatation (36, 42 F), the upper segment of the anastomotic stricture was reached by gastroscopy. After dilatation with a bougie (42, 45 F) for the third time, the colonoscope was easily passed through the anastomosis line to the upper segment. Six months later, colonoscopy was performed and the proximal part of the anastomosis was easily passed without the use of bougie dilators.

Balloon dilatation is the first method used in AS. However, several repetitions are necessary for the success of the procedure. In addition, the risk of perforation increases when the stricture diameter is <5 mm and length >1 cm. The chances of success in AS are lower compared to bougie dilatation. Endoscopic electroincision is recommended in failure of balloon dilatation. Digital dilatation: used in distal anorectal anostamotic strictures. Corticosteroid application: very large studies are not available. Bougie dilatation in AS provides tactile feedback, allowing the amount of resistance to the passage of the dilator to be estimated and perforation to be avoided. Bougie dilatation method is simple, inexpensive and low risk of complications. Especially Maloney flexible silicone bougie minimise the risk of complications. Bougie dilatators can remain intact for many years and can be reused. But balloon dilatators are not reused. Surgical methods are used in 3-4% (complete/near complete AS) in the failure of endoscopic methods. But mortality risk is high [1,4-6].

Therefore, the alternative method of Philip BC Pang et al. can be applied [5]. However, due to limited endoscopic ultrasonography (EUS) centers, gradual dilatation can be performed with flexible bougie dilators in case of anastomosis stricture.

Yours Sincerely,

Keywords: Anastomotic stricture, colorectal surgery, bougie dilatation.

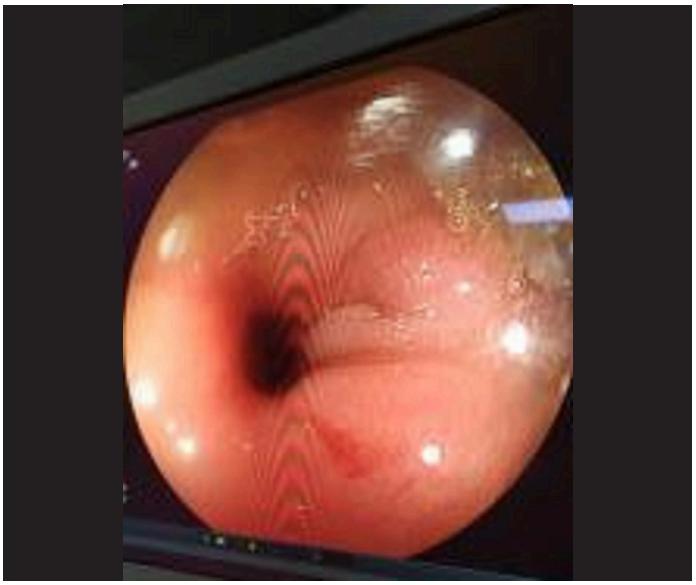

Figure 1. Before bougie dilatation

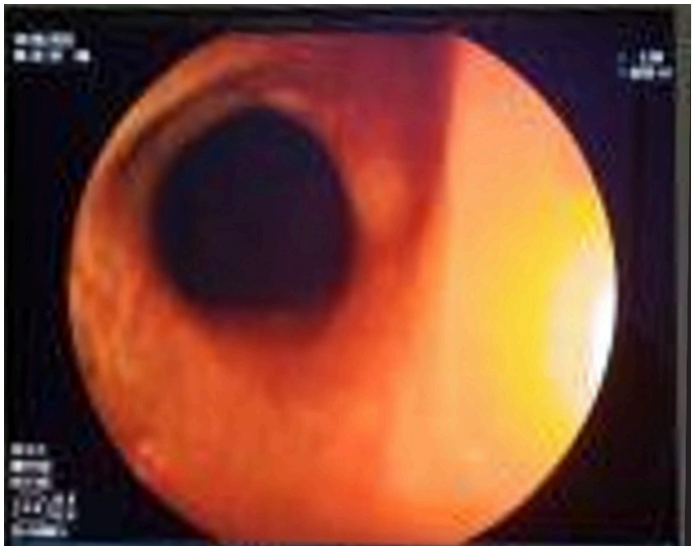

Figure 2. 6 months after bougie dilation

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Letter to Editor

The Role of Artificial Intelligence in Academic Paper Writing and Its Potential as a Co-Author: Letter to the Editor

Response: May Artificial Intelligence Be a Co-Author on an Academic Paper?

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Dear Editors,

I read your editorial content with great interest [1]. As a young academic in the spring of my career, I would like to share my views, suggestions, and experiences regarding the use of artificial intelligence in academic papers. Like any individual from Generation Y, I also grew up watching the adventures of the Jetsons family. The talking service robot, automated production lines, flying cars, and, most importantly for us now, robot doctors were all products of artificial intelligence, although I didn't know their name back then. My interest in artificial intelligence and researching its applicability in the field of healthcare may be attributed to these early experiences, but who knows for sure? I believe this is where my first encounter with artificial intelligence began.

After the COVID-19 pandemic, there has been a rapid development in artificial intelligence technologies. Whether the timing was purely coincidental or influenced by the quarantines and lockdowns, we do not know. ChatGPT, it seems, has become one of the most well-known advancements, both among academics and the general public. This chatbot talks with us, answers our questions, conducts research on our behalf, and even writes articles [2]. But can ChatGPT really be used for writing academic papers?

In my experience, using ChatGPT for academic paper writing is quite risky. It can generate a draft that an academic might spend weeks or even months trying to write, in a very short amount of time. This aspect is undoubtedly enticing. However, caution must be exercised when using it. The database on which ChatGPT is built consists not only of academic information but also includes information from any website. You never know which information ChatGPT is using to generate the text. When you ask it to provide references for the generated sentences, it can produce fake DOI numbers or give you the DOI of an unrelated article. The only way to verify the accuracy of the generated information is for authors to manually fact-check it.

High-impact scientific journals such as Springer-Nature and Science currently do not accept ChatGPT as a co-author [3,4]. Taylor & Francis journals have indicated that they will review this

situation, while many Elsevier journals have already included ChatGPT as a co-author [5]. The underlying issue that journals have with this is determining who takes responsibility for the information in the articles. Additionally, the fact that ChatGPT does not possess a completely independent thought process and generates information based on the web can lead to plagiarism concerns.

So, is ChatGPT the only chatbot that can be used in the medical field? In fact, there are chatbots that can generate more superior information in the medical field than ChatGPT. Some of these models include BioLinkBERT, DRAGON, Galactica, PubMed GPT (now known as BioMedLM), and the upcoming Med-PALM 2. However, running these models requires at least some coding knowledge. According to Google's claims, Med-PALM 2 achieved an 86.5% success rate in the United States Medical License Exams (USMLE), while its closest competitor, PubmedGPT, achieved only a 50.3% success rate [6]. Med-PALM 2 could be an important chatbot for the medical field, or, more technically, a Large Language Model (LLM), but we will have to wait a little longer to see it in action.

Given the current situation, how can we benefit from these LLMs in academic paper writing? My recommendation is to use them to enhance the meaning of texts you have written rather than having them write the entire text from scratch. This way, the main context of the sentences remains the same, and the overall accuracy of the generated information does not change significantly. Additionally, ChatGPT is a valuable tool for translating your original text into different languages or for grammar corrections. While professional language editing services can cost between $100 and $500, ChatGPT is a free and faster alternative. However, it is important to read and check the translated or grammar-corrected text after using the chatbot. Sometimes it can generate sentences that are unrelated to your original ones. If you alert the chatbot to this issue, it will correct its responses, or you can simply open a new tab and write what you need from scratch, which I recommend the second option. Another useful feature of ChatGPT for article writing could be generating abstracts. Journals often have restrictive rules regarding word limits and abstract structures, and ChatGPT can facilitate solving these challenges.

In conclusion, whether it's ChatGPT or other LLMs, I believe that they are currently not entirely suitable for writing academic papers from scratch or being listed as co-authors. We need to closely follow developments in this field. Only when an LLM model is created that relies solely on academic databases and provides genuine references for each sentence it generates, can it be used for writing academic papers from scratch or being listed as a co-author. However, at that point, plagiarism issues should be carefully examined and discussed. We should not be prejudiced against LLMs and should explore new ways of using them while awaiting technological advancements.

Yours sincerely,

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Letter to Editor

Concerns About Co-Authoring AI Tools in Academic Papers

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Dear Editors

With great attention and interest, I read the editors' short brief yet thought-provoking editorials [1,2] and it has helped me combine valuable information with my research and experiences. Today, artificial intelligence has become an application that we can use in all areas of our lives, being versatile, and able to analyze, collect and interpret. Writing ChatGPT that we can barely bring together for weeks or even months of work, and other AI applications can be used in minutes or even. We seconds can see that it produces original writings and offers a wide range of information. It is obvious that the time-saving experience provided by artificial intelligence provides convenience in most areas of our lives. But that's human researchers and artificial intelligence it may cause us to not understand some points about certain differences between the two. For example, when we look at the difference between an article written with artificial intelligence and an article written with human intelligence, it is undoubtedly almost understandable at first glance impossible.

Because of life's developing and changing conditions, no field wanted to be left behind and turned to itself to build its essence, one of which is undoubtedly artificial Intelligence. With the rapid progression of the COVID-19 pandemic and swiftly evolving political decisions, technology has become exceedingly practical and adaptive, undergoing continuous transformation.

Many research studies have begun to be conducted around the world, with the need for individuals to conduct faster and more extensive research to bring together new and diverse resources.

While the utilization of artificial intelligence (AI) appears as one of the most promising options for this purpose, we must inquire whether its inclusion as a co-author adheres to ethical and technical standards or if it occasionally neglects these principles.

In my opinion, involving AI tools like ChatGPT as a co-author can potentially lead to ethical complexities, especially in terms of responsibility and accountability.

Language models powered by artificial intelligence lack consciousness, autonomy, and the

ability to claim ownership of their contributions. Ascribing authorship to these models blurs lines of responsibility and weakens the ethical obligations inherent in scholarly authorship. Simultaneously, the essence of scholarly authorship lies in the generation of hypotheses, experimentation, data analysis, and interpretation, attributes ascribed to individuals who actively contribute. In this context, even though ChatGPT and other artificial intelligence models expeditiously furnish us with desired information through rapid interactions, it is fundamentally derived from existing human input sources. In essence, these AI systems do not so much transform or recreate a wellspring of knowledge as they present it in its preexisting state. Introducing ChatGPT as a co-author could evoke the assumption of its active engagement, potentially blurring the distinction between the assistance offered by researchers and that by the AI, rendering it challenging for observers to distinctly discern their respective contributions.

Consequently, artificial intelligence's contributions, evident when examining scientific articles and many other sources we seek, are undeniably substantial. While the knowledge it presents may introduce entirely novel perspectives, rather than accrediting artificial intelligence as an author, we should confine its recognition to the acknowledgment section solely for its contributions. This approach allows us to acknowledge the collaborative efforts of both human and artificial intelligence, upholding transparency while respecting and adhering to traditional authorship norms.

Yours sincerely,

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Letter to Editor

Artificial Intelligence and Article Writing

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Dear Editors,

I was very interested in your editorial [1]. Technological advancements are so rapid that humans are unable to keep up. And we eagerly await the outcomes of technological advancement. The study of artificial intelligence is one of the topics that has recently gained popularity. If someone had predicted a hundred years ago, "A technology will come that will detect the commands you receive, act accordingly, and write scientific articles," we would laugh at her. Many artificial intelligence dreams that appear weird to us now, I believe, will come true shortly. The Generative Pre-Trained Transformer 3 (GPT3) was developed a few years ago, in line with improvements in artificial intelligence. With this model, artificial intelligence was used to generate content that resembled human-written documents. A more advanced version of ChatGPT was produced a few years later. In March 2023, the most recent GPT4 version was launched. Errors in article writing have been reduced using this and comparable applications such as artificial intelligence. In fact, it has become nearly impossible to detect the difference between publications written by scientists and articles written by artificial intelligence. Some journals have begun to accept artificial intelligence apps as co-authors [2].

Article authoring is made considerably easier by artificial intelligence. In terms of time savings, using artificial intelligence products such as ChatGPT and GPT 4 may make sense. However, there are some risks associated with this circumstance. For example, because disseminating personal data is a felony, authors may feel compelled to conceal some information in order to preserve personal data. Artificial intelligence programs can readily perpetrate crimes involving personal data by releasing secret facts. This situation presents numerous legal issues. Furthermore, the language to be used in article writing may alter depending on the topic of study. In this instance, it is vital to go check the words chosen by artificial intelligence programs in the article. As a result, my recommendation is to use tools such as ChatGPT, GPT 4, or similar basic jobs once the user has written the article themselves. Leaving all of the work to these apps may cause more harm than good.

Kind Regards

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Letter to Editor

The Use of Controlled Artificial Intelligence as a Co-Author in Academic Article Writing

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Dear Editors,

We have read with interest your very valuable editorials on the use of artificial intelligence (AI) applications, which is a very current topic, in academic writing [1,2]. The opinions and thoughts of the authors about writing articles with the help of AI have been the focus of attention recently [1-3]. First of all, thank you very much for creating a discussion platform for the most used and mentioned development of the century (ChatGPT). Today, technology is indispensable in our lives, and in the last ten years, AI and the products obtained with this technology are an indication that Al will take place in all areas of our lives in the future. The one of the most important areas affected by technological developments is undoubtedly the world of science. Scientific articles, which are a product of scientific research, evolve depending on constantly renewed technological developments. Endnote, Zotero, Mendeley, which are frequently used in article writing; Plagiarism programs such as Turnitin, Ithenticate, SmallSEOTools etc. have taken their places among the indispensables of academics [4].

The most up-to-date technology that will help academics and scientific applications is the ChatGPT application, which is a product of AI, which is rapidly advancing in the world and is appreciated by millions of users [5]. Recently, one of the most discussed topics in the academic world is the use of AI as a co-author in academic articles [1-3,6]. Many authors argue that AI cannot be co-authors in article writing [3,6,7], some authors argue that it will be impossible to avoid the benefits that technology can provide us [8,9], while some authors argue that it is useful but needs to be developed [10].

In article writing, taking advantage of the conveniences that AI will offer to academicians shortens the duration of the work and provides ease of access. We think that the use of AI will be beneficial in many stages such as the literature review for a study, the creation of references in article writing, the preparation of the article according to the format of the journal to which it is planned to be sent, etc.

Bahşi and Küçükbingöz [3] stated that the most important point is accuracy which may pose a problem in using AI programs such as ChatGPT or GPT4 in writing an article. AI serves

information that it scans on the internet, and we do not know whether its source is always correct. Lee [6] emphasized that the inability of AI to take place as an author in article writing is not ethical in terms of not being able to take responsibility. However, there are researchers who emphasize that the main responsibility for article writing lies with the author [9]. When we evaluate the perspectives of the authors on the subject, we think that the parts of ChatGPT that need to be improved in terms of accuracy in citation and lack of ethical controller. Artificial intelligence can be a co-author of articles if a scientific dataset is used, which consists of data that is safer, and controlled and ethical principles are not ignored. However, the final reliability of the articles should be checked with a human brain. Technology should be used absolutely, but it should not be based entirely on it.

As a result, it is imperative to keep up with science in the developing and changing world. We believe that AI being the co-author of our articles, provided that it is limited and supervised, will provide academics with the opportunity to save time and reach results faster.

Regards,

Keywords: Artificial Intelligence, Academic Article Writing, ChatGPT.

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Letter to Editor

Artificial Intelligence Tools in Academic Article Writing: Is it a Tool or a Co-Author?

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Dear Editors,

I have read your editorials on the use of artificial intelligence in academic articles with great attention and enthusiasm [1,2]. In addition, in the comments made to your articles, I reviewed the ethical problems that may arise from the use of artificial intelligence in scientific articles and the contributions that the article will provide in the writing process [3-6].
Although technological developments and advances in artificial intelligence have gained great momentum in recent years, I believe they should be accepted as an accumulation of all humanity. As a matter of fact, in very old sources, there is information that the machines known as robots and automatons at that time were used for entertainment purposes in the centuries before Christ. Furthermore, sophisticated machines, water clocks, and programmable humanoid automatons invented by **İsmâil bin er-Rezzâz el-Cezerî** in the 12th century, which have an important position in our scientific history, have played a significant role in the development of today's robot technology and mechanical sciences.

Artificial intelligence applications are progressively being employed in agriculture, industry, military activities, health, art, and numerous other disciplines. Today, when we type "artificial intelligence" into the Google Scholar, we get 5,410,000 results, demonstrating how these developments have affected the academic world. As indicated in previous comments, I believe that applications such as ChatGPT in academic writings can be used for grammar corrections and abstract editing. Furthermore, these apps might be employed in the introduction section, where broad information about the topic under investigation is provided in the articles. However, since these applications do not only use academic databases during the literature review, the final version of the article should be evaluated by the relevant author. The primary ethical issue with these practices is that they are unable to accept responsibility in proportion to their authority. As a result, regardless of their contribution to the design of the paper, I think that these apps should not be deemed co-authors. However, it should be noted that these applications were used in the article.

In conclusion, I believe that in the not-too-distant future, artificial intelligence applications will make significant contributions to the writing of the article, particularly in academic studies

involving quantitative data. We should use these technologies as a tool to contribute more to academic advancement.

Kind regards,

Keywords: Artificial Intelligence, ChatGPT, academic article writing

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Letter to Editor

Navigating the Impact of Artificial Intelligence on Scholarly Authorship: Transparency and Responsibility in the Technological Era

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Dear Editors,

We are writing in response to your recent editorials regarding the captivating subject of employing artificial intelligence (AI) in the composition of scholarly documents, with a specific focus on the notion of co-authoring with artificial intelligence [1,2]. We would like to express my appreciation to the European Journal of Therapeutics for its diligent commitment to upholding the ethical standards and academic integrity of scholarly publications. In the context of the swiftly progressing technological era, it is important to exercise caution in the utilization of AI in order to uphold our established academic and scientific customs. We concur with the perspective that the incorporation of AI in the production of scholarly papers ought to be explicitly disclosed within the methodology section, in light of its escalating significance in the composition procedure. Ensuring transparency is crucial, as it facilitates a comprehensive understanding of the impact that AI may have on output, including both good and negative implications [3].

Nevertheless, while we acknowledge the utility of AI, we respectfully hold a dissenting viewpoint about the proposition of attributing co-authorship to an AI system such as ChatGPT. The act of being an author entails a level of responsibility that beyond the capabilities of even the most capable AI tool. The AI system lacks the ability to comprehend, analyze, or morally assess the subtleties inherent in the work it contributed to, therefore cannot be held responsible for the accuracy and implications of the work produced. AI serves as a valuable tool for researchers, enhancing both their efficiency and the overall quality of their work [4]. Sophisticated laboratory equipment and complicated statistical software are not regarded as co-authors. The same logic applies to AI. The recognition of AI's significance in academia is crucial, but only to the extent of AI's essence and constraints. A tool serves as a supplementary resource to expedite and enhance the processes of research and writing, although it should not be regarded as an autonomous contributor.

As the dialogue around this topic continues to evolve, we look forward to seeing how

international organizations such as ICMJE and COPE will adapt to this development [5]. With their solid criteria and careful tuning, they can guide us towards a future where we use AI effectively and ethically. Thank you for initiating this important conversation.

Sincerely yours,

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Letter to Editor

Discussion on the Artificial Intelligence (AI) Tools Usage in the Scientific World

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Dear Editors,

We have been reading with great interest your editorial discussion on "Artificial Intelligence and Co-Authorship" which you initiated some time ago [1]. In the current era, the vast amount of data generated from routine applications, scientific research, and the resulting outcomes has surpassed what the human mind can read and evaluate. Therefore, there has been a need to summarize data and develop information processing-based applications for easy access, leading to the design of automated - artificial intelligence-based - tools. Nowadays, these tools are used in various processes, from data collection and analysis to hypothesis generation, experimentation, and simulation.

The use of Artificial Intelligence (AI) tools is highly beneficial in conducting and reporting scientific research. Particularly, for tasks such as literature reviews, identifying research gaps, and learning about collaborations among researchers/institutions, a wide range of AI-based tools has been developed, making it easier for researchers to accomplish these tasks. However, researchers are still seeking solutions to expedite the time-consuming aspects of writing their research.

AI can automate repetitive tasks efficiently and with minimal errors, allowing humans to focus on more creative and strategic tasks. They can make better decisions by forecasting the future based on evaluating various types of existing data. After analysing similar content, they can generate purposeful creative content. They can answer questions on topics that humans may not understand comprehensively and informatively. And of course, they can translate text and speeches accurately and fluently into other languages.

Misuse of AI tools or misinterpretation of results obtained from these applications can have significantly adverse consequences. One notable example of this is the unchecked preparation of academic papers by AI-based software. In fact, ChatGPT has been listed as a co-author in at least four articles in the literature, but corrections have been made in some cases due to its inaccuracies. When the Web of Science is searched, it is seen that ChatGPT was removed from authorship by making corrections in 1 article in which ChatGPT was previously mentioned as a co-author [2], and in two articles in the British Journalism Review and in three articles about ChatGPT in different journals, it was mentioned as a group author.

It has been observed that while AI models like ChatGPT can generate text that appears human-like, there can be issues with interpretation and the presentation of false references, as highlighted in studies in the literature. Therefore, AI-based software like ChatGPT should not be used as co-authors without control but should be used as tools like other software, with the written text going through human oversight. As a result, the full responsibility for what these AI tools produce should rest with the author(s) submitting the article and cannot be attributed to the AI [3].

Organizations such as the Committee on Publication Ethics (COPE), the World Association of Medical Editors (WAME), and the JAMA Network are important regulatory bodies concerning the content and quality of academic publications. They emphasize that individuals who cannot fulfil authorship requirements, such as declaring conflicts of interest, managing publication rights, and licensing agreements because AI tools cannot fulfil these duties, cannot be authors of a paper [4-6]. In line with our recommendations above, these organizations also state that authors must bear full responsibility for everything the AI tool does within the manuscript and for the article's adherence to ethical standards.

In conclusion, AI-based applications contribute significantly to academic research, just as they do in many other fields, and serve as important tools for researchers in academic writing. With long-term development and improvements, we believe that they will gain the ability to write a substantial portion of academic papers as their literature review capabilities expand. However, the accuracy and originality of the written information must always be subject to human oversight to make new contributions to the literature. At this point, AI-based applications come into play again, claiming to detect the difference between AI-generated and human-created content with approximately 99% accuracy. Cases perceived as AI-generated content have been corrected through legal action or appeals to higher authorities [7]. Ultimately, the use of AI-based tools like ChatGPT and AI-generated content in academic studies, like other features of academic work, should be regulated with ethical considerations.

Yours Sincerely,

Conflict of interest: There is no funding or conflict of interest to report.

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Letter to Editor

Immunoglobulin-G4 Related Disease with Multiple Organ Involvement

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Dear Editor,

Immunoglobulin G4-related disease (IgG4-RD) is an immune-mediated fibroinflammatory disease affecting multiple organ systems. The clinical findings may range due to the affected organ. The main distinguishing histopathological features of IgG4-RD are lymphoplasmacytic infiltration, storiform fibrosis, obliterative phlebitis, and mild or moderate tissue eosinophilia. Rarely, it may affect the lungs, hearts, pituitary, meninges, skin, prostate, breast, and thyroid gland [1–3]. In this article, we present a case diagnosed with IgG4-related disease involving multiple organs, including the pituitary, lymphatic system, kidney, and heart, and the patient responded well to immunosuppressive treatment.

A fifty-five-year-old female patient applied with complaints of fatigue, polydipsia, polyuria, widespread body pain, and 20 kg weight loss in the last year. After excluding other possibilities, with a prediagnosis of diabetes insipidus (DI) pituitary MRI was performed which showed an increase in size and heterogeneous patchy contrast enhancement in the adenohypophysis (Figure 1a). Based on the current clinical and imaging findings, the patient's laboratory results were evaluated, and central DI was diagnosed. In computerized thorax tomography, multiple lymph nodes in the mediastinum were detected, the largest of which was 21x17 mm. Tissue sampling was performed with the guidance of EBUS, pathological examination showed no diagnostic findings. Transesophageal Echocardiography (TEE) revealed an appearance consistent with a 10-15 mm thick thrombus surrounding the left atrium wall and narrowing the cavity. Increased thickness narrowing the left atrial lumen was reported in thorax computed tomography (CT) (Figure 2). Further, a cardiac MRI was performed and reported to be consistent with lymphoproliferative-inflammatory involvement rather than thrombus. Abdominopelvic CT was performed and a lesion of 54x28 mm in size, less contrast enhancing than the surrounding parenchyma, in the middle part posterior of the left kidney was detected (Figure 3). Histopathological findings were consistent with inflammatory processes, and no findings in favor of a neoplastic lymphoproliferative process were detected in the samples. Since the patient was presented with pituitary involvement, mediastinal lymphadenopathy, renal and cardiac mass, the IgG4 level was ordered and resulted as 299 mg/dl (3-201). IgG4 staining could not be performed in the current biopsy specimen;

for confirming the diagnosis of IgG4-related disease, a re-biopsy was performed on the kidney mass. Histopathological findings were consistent with IGG4-RD (Figure 4). Due to multisystemic involvement, the patient received 0.6 mg/kg/day oral corticosteroid and mycophenolate mofetil 3x1000 mg/day. The pituitary MRI that was performed in the first month of treatment was normal (Figure 1b). Desmopressin treatment was stopped. Also, control TEE in the first-month follow-up visit showed a significant reduction in the mass image in the left atrium. After three months of follow-up, there was a significant improvement in the patient's symptoms and acute phase response. The corticosteroid was tapered and maintenance treatment with mycophenolate mofetil was continued.

IgG4-related disease is rare and difficult to diagnose, though its presentation may be in a wide variety of clinical features. It is crucial to make an early diagnosis and start treatment early in these patients to prevent morbidity and mortality. In cases with mass lesions and especially with multiple organ involvement, as in our case, IgG4-related disease should be kept in mind, and IgG4 staining should always be kept in mind.

Yours sincerely,

Keywords: Immunoglobulin-G4 Related Disease; Heart; Kidney; hypophysis; multiple organ involvement

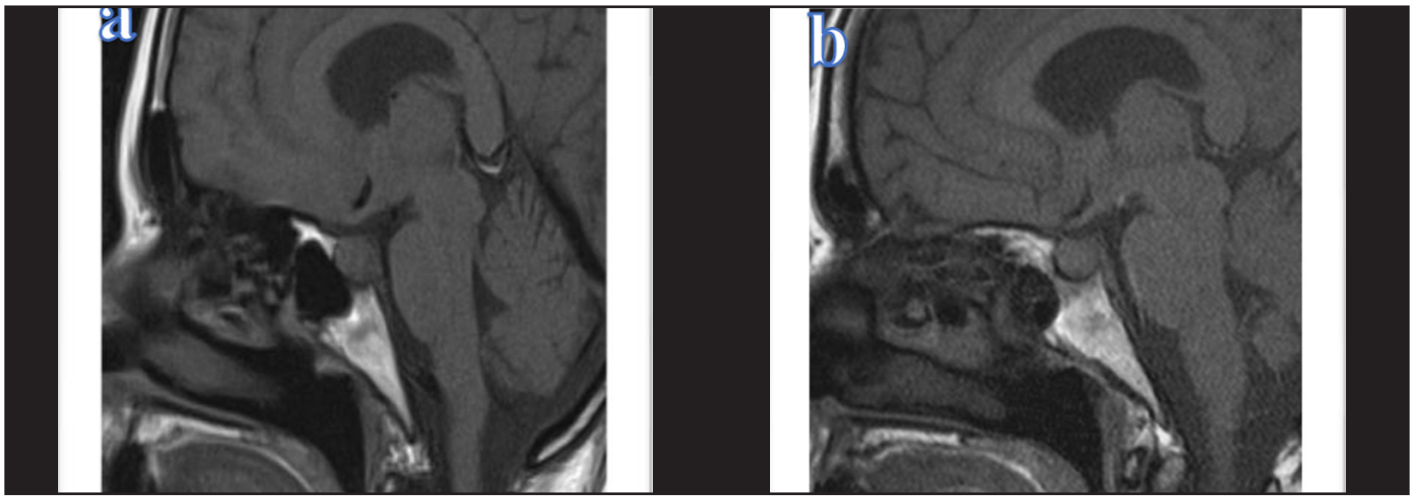

Figure 1.a: Before treatment, sagittal section T1 contrast-enhanced MRI **b:** Control MRI image, reported as normal, (1th month follow-up visit)

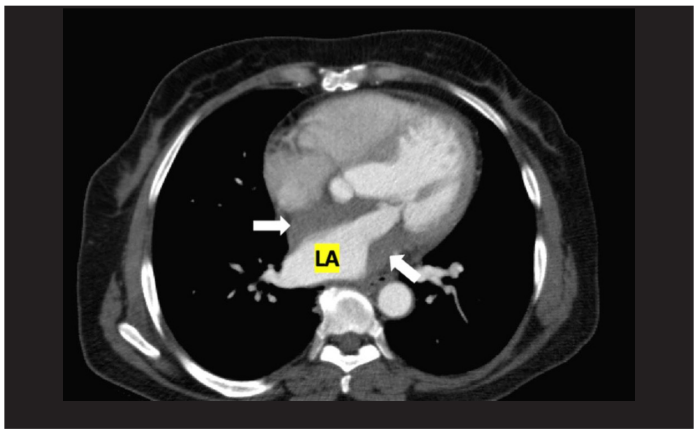

Figure 2. Increased thickness narrowing the left atrial lumen was observed in thorax computed tomography (white arrow) (LA: Left Atrium)

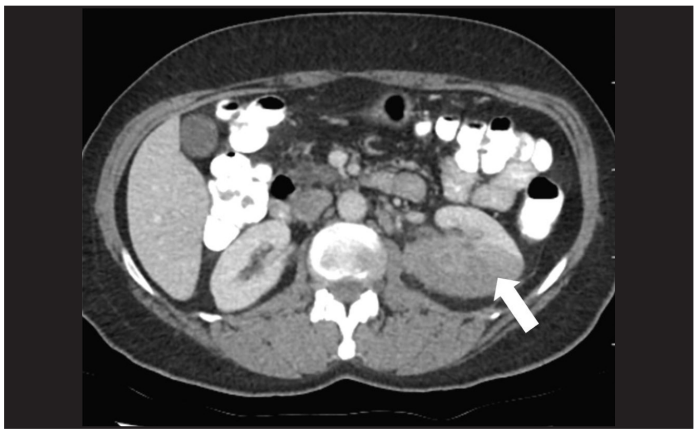

Figure 3. A 54x28 mm lesion with less contrast than the surrounding parenchyma was observed in the middle posterior part of the left kidney in the abdominal computed tomography (white arrow)

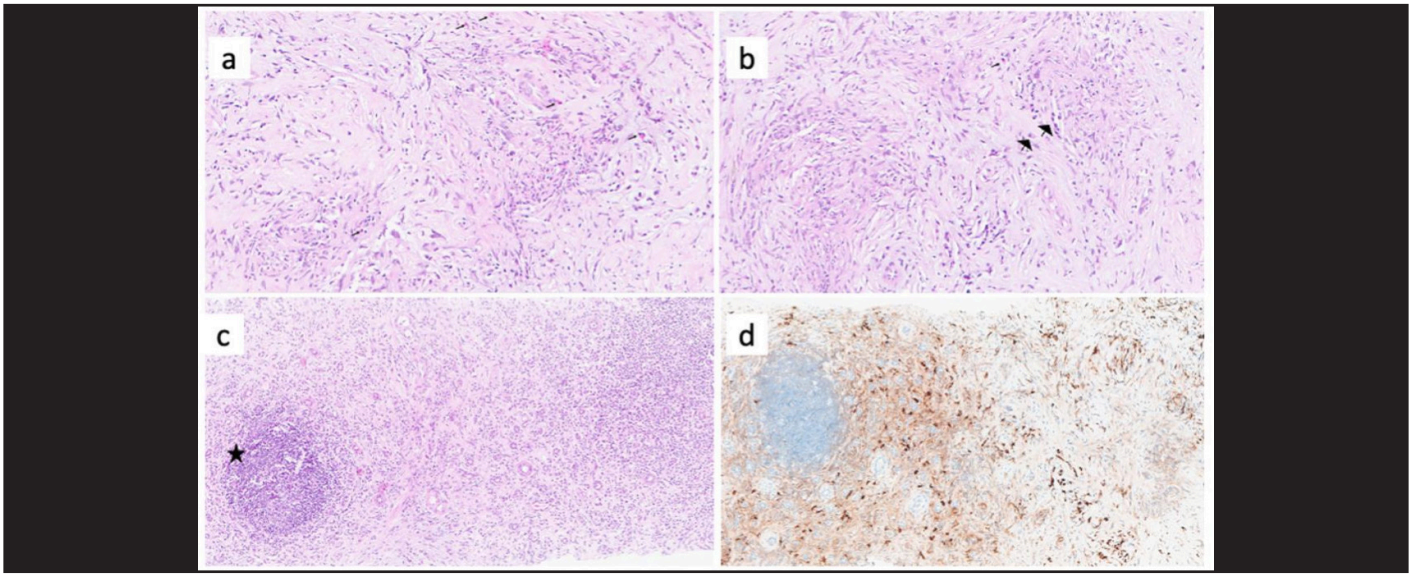

Figure 4.a,b. There is marked fibrosis around the vessels, inflammation rich in plasma cells (arrow head) and eosinophil leukocytes (arrow) (H&E, x100). **c.** Marked fibrosis, inflammation and lymphoid follicle formations around the renal tubules (Star) (H&E, x100). D. In the immunohistochemical study, intense IgG4 expression in plasma cells accompanying inflammation with IgG4 (>25 plasma cells/HPF) was noted (IgG4, x100).

Ethical Approval: Not applicable.

Informed Consent: The patient provided informed written consent before including her data in this report.

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Correction

Correction to: Correlation of Diffusion-weighted MR imaging and FDG PET/CT in the Diagnosis of Metastatic Lymph Nodes of Head and Neck Malignant Tumors

Şamil Şahin[1], Yasar Kemal Duymaz[2], Burak Erkmen[3], Burak Karabulut[4], İldem Deveci[5], Mehmet Sürmeli[5], Aslı Şahin Yılmaz[6], Aslıhan Semiz Oysu[7], Çağatay Oysu[8]

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The original version of this article [1], unfortunately contained an error. The name of *Aslıhan Semiz Oysu*, who is one of the co-authors and took part in every stage of the study, was not inadvertently added to the author list by the corresponding author. The author apologizes for this confusion. Given in this article are the correct author names.

Publisher's Note: The original article was corrected, and a correction note was added.

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