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European Journal of Therapeutics
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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Case Reports, Special Editorial, Short Communication, Technical Notes, Letters to the Editor, Reply by Authors, Image, Withdrawn, Erratum, Retraction Notice, Editorial (EIC use only), In memory of (Invited by EIC only) and Book Review (Invited by EIC only) in the fields of medical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).

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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.

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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.

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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:
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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:
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- 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).

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.
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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

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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 October 2024 Issue (Vol: 30, No: 5) 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], Murat Akbaba [12], İlyas Başkonuş [13]

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E-mail: aysebalat@hotmail.com

Dear Colleagues,

We are very pleased to present you with another issue of the *Eur J Ther* (2024; Vol: 30, Issue: 5). This issue contains very valuable articles, and we believe you will read them with great interest.

Another happiness is that the *Eur J Ther* is progressing day by day. In this respect, we would like to share with you that Index Copernicus' application of the *Eur J Ther* has also been approved (ICV 2023: 100.00) this year [1].

Finally, we would like to sincerely thank our esteemed authors who contributed to the preparation of this issue, our referees who meticulously carried out the evaluation process, our team that provides the technical infrastructure of our journal, and our esteemed readers who support us in every issue.

We hope to share more beautiful developments with our regards.

REFERENCES

[1] European Journal of Therapeutics | ICI Journals Master List. https://journals.indexcopernicus.com/search/details?id=54431 Accessed Date 29 Oct 2024

How to Cite;

Balat A, Eren ŞH, Menzilcioğlu MS, Bahşi İ, Doğan İ, Kaplan DS, Karadağ M, Özçelik AA, Sarı F, Yıldız H, Akbaba M, Başkonuş I. (2024) Welcome to the Aug 2024 Issue (Vol: 30, No: 4) and Current News of the European Journal of Therapeutics . Eur J Ther. 30(5):e41-e41. https://doi.org/10.58600/eurjther2537

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Original Research

Comparison of Outpatient and Inpatient Consultations Requested from Child and Adolescent Psychiatry

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ABSTRACT

Objective: This study aims to examine the sociodemographic and clinical characteristics of child and adolescent psychiatry consultations and to compare characteristics of outpatient and inpatient consultations.

Methods: A total of 354 patients who were consulted to child and adolescent psychiatry department of a university hospital were included in the study. Data were collected regarding sociodemographic characteristics of patients, departments requesting consultation, diagnoses, and treatment methods.

Results: The cases included in the study had a mean age of 10.6±4.8 years,with 51.4% being girls. Adolescent girls formed the majority of the inpatient group. The pediatric neurology outpatient clinic had the highest frequency of requested consultations (22.3%), and the most common reason for consultation was a suicide attempt (12.1%). Of the cases for which consultation was requested, 67.8% were diagnosed with at least one psychiatric disorder, and the most common diagnoses were anxiety disorders, with 12.7%. In the inpatient group, impulsive suicide attempts and depressive disorders were more frequent compared to the outpatient group, while intellectual disability was less common. Parent education was provided for 97.2% of the cases, pharmacological treatment was recommended for 44.6% of the cases, and the most commonly prescribed medications were selective serotonin reuptake inhibitors. Psychotherapy and special education were significantly more common in the outpatient group compared to the inpatient treatment group.

Conclusion: The current study revealed significant differences in consultations requested from child and adolescent psychiatry between the outpatient and inpatient groups regarding consultation reasons, diagnoses, and treatment modalities. The findings provide valuable information for clinical practice and service development.

Keywords: Consultation, child, adolescent, psychiatry, inpatients, outpatients

INTRODUCTION

Psychiatric problems may occur frequently in both inpatients and outpatients. and psychiatric disorders or problems can significantly impact managing and treating medical and surgical illnesses [1]. Consultation-liaison (C-L) psychiatry for children and adolescents is a specialized field. It focuses on the assessment and treatment of mental disorders in pediatric patients with concurrent medical or surgical conditions [2].

There are differences in the demographic and clinical characteristics of inpatients and outpatients consulted to child and adolescent psychiatry. Studies focusing on child and adolescent psychiatric consultations requested for inpatients display a higher representation of girls during adolescence [3]. Additionally, it is noteworthy that inpatients tend to have more chronic diseases. A recent study suggested that depression and childhood trauma in chronic diseases may contribute to treatment resistance [4]. Furthermore, most studies examining inpatients indicate that depressive disorder is the most frequently diagnosed condition [3,5], and psychopathology may be more prevalent among inpatients [5,6]. Moreover, a study found that the timing of psychiatric consultation in patients hospitalized for medical or surgical reasons shortened the length of hospital stay and was associated with reductions in total hospital costs [7]. On the other hand, our understanding of child and adolescent psychiatric consultations for outpatients, encompassing various medical and surgical reasons [2,8] and our knowledge regarding the comparison between inpatients and outpatients remains limited.

Main Points;

- The most common reasons for consultation were suicidal attempts and irritability.
- Anxiety disorders were the most common diagnoses.
- Parental education was provided for most cases, and SSRIs were frequently prescribed.
- In the inpatient group, impulsive suicide attempts and depressive disorders were more frequent compared to outpatients.
- There were significant differences in the distribution of recommended therapeutic interventions, such as psychotherapy and special education, between outpatient and inpatient consultations.

The characteristics, reasons for referral, diagnoses, and recommended treatments of child and adolescent psychiatric consultations may vary between outpatient and inpatient settings [9]. Identifying these differences can be beneficial for developing child and adolescent psychiatric services, meeting needs, and resolving problems [2,9].

The aim of the current study is to compare the sociodemographic and clinical characteristics of inpatient and outpatient children and adolescents referred to the child and adolescent psychiatry clinic of a university hospital. The study hypotheses are (i) there will be significant differences in age and gender distribution, and (ii) the departments requesting consultations, reasons for requesting consultations, diagnostic patterns, and treatment recommendations will vary between inpatient and outpatient groups.

MATERIALS AND METHODS

Sample

Between July 2014 and June 2016, medical records of patients aged 0-18 in a univesity hospital were reviewed retrospectively. Cases for whom a consultation was requested from the Department of Child and Adolescent Psychiatry were included in the study. Cases with missing archive files were excluded. The data examined included the sociodemographic characteristics of the referred patients, the departments requesting the consultations, the psychiatric diagnoses given following the evaluations, the treatment methods employed, and the preferred psychiatric agents if pharmacological treatment was administered. Our clinic has implemented a routine practice for diagnostic evaluation, especially for consultation cases. Additionally, cases were evaluated by at least two child and adolescent psychiatrists.

This routine practice involved individual interviews with the patients, family interviews, and the administration of the Kiddie Schedule for Affective Disorders and Schizophrenia Present and Lifetime Version – Turkish version (K-SADS-PL-T), which is a semi-structured psychiatric interview. This interview form has been validated and has demonstrated reliability for psychiatric disorders in children and adolescents. The inter-rater reliability of the form is between 0.625 and 0.875[10]. Diagnoses like pervasive developmental disorders that weren't included in the interview form were excluded by the clinician using appropriate scales during the interview. These scales were not included in our study. The localethics committee approved the research protocol under protocol number 2018/1378 on April 12, 2018.

Statistical Analysis

The data were analyzed using IBM SPSS Statistics version 27.0 (IBM Corp. Released 2020. IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY: IBM Corp.) statistical software package. The normality of the data was assessed using the Kolmogorov-Smirnov test. Descriptive statistics were reported in percentages, and the mean and standard deviation were provided for normally distributed data. For non-normally distributed data, the median and minimum-maximum values were reported. Student's t-test was employed to compare the study groups for normally distributed data. Chi-square analysis was used for categorical variables. A significance level of $p < 0.05$ and a 95% confidence interval were considered statistically significant. The figure was created using RStudio, an integrated development environment for R. Additionally, the "Venn Diagram" R package was used [11,12].

RESULTS

According to the data obtained from the information processing center of the hospital, between July 2014 and June 2016, 14,556 pediatric and adolescent patients under 18 received inpatient treatment, while 171,601 received outpatient treatment. Additionally, 405 children with medical or surgical problems were consulted to child and adolescent psychiatry. Of the child psychiatry consultations, 275 were receiving outpatient treatment whereas 130 were receiving inpatient treatment. However, 51 of the inpatient cases did not have any medical records or had outpatient psychiatric clinic visits. Therefore, our study included a total of 354 children and adolescents, consisting of 130 (36.7%) patients receiving inpatient treatment and 224 (63.2%) patients receiving outpatient treatment.

Table 1. Comparison of sociodemographic characteristics between outpatient and inpatient groups

Variables	Outpatient group (n=224)	Inpatient group (n=130)	*Statistics*	*p*
Age, year, mean±SD	9.43±4.78	12.48±4.29	*t=6.17*	**< 0.001**
	n (%)	**n (%)**		
Sex				
Male	124 (55.4)	48 (36.9)	χ^2=*11.19*	**0.001**
Female	100 (44.6)	82 (63.1)		
Type of birth				
Cesarean section	71 (31.7)	26 (20.0)	χ^2=*5.66*	**0.017**
Vaginal delivery	153 (68.3)	104 (80.0)		
Cronic Disease				
Present	75 (33.5)	44 (33.8)	χ^2=*0.005*	0.944
Absent	149 (66.5)	86 (66.2)		
Mother's employment status				
Employed	58 (25.9)	44 (33.8)	χ^2=*2.54*	0.111
Unemployed	166 (74.1)	86 (66.2)		
Maternal psychiatric history				
Present	19 (8.5)	25 (19.2)	χ^2=*8.73*	**0.003**
Absent	205 (91.5)	105 (80.8)		
Father's employment status				
Employed	214 (95.5)	113 (86.9)	χ^2=*8.66*	**0.003**
Unemployed	10 (4.5)	17 (13.0)		
Paternal psychiatric history				
Present	12 (5.4)	10 (7.7)	χ^2=*0.77*	0.380
Absent	212 (94.6)	120 (92.7)		
Consanguineous marriage				
Present	32 (14.3)	19 (14.6)	χ^2=*0.007*	0.932
Absent	192 (85.7)	111 (85.4)		

The mean age of all cases was 10.6±4.8 years. Among the patients for whom consultation was requested, 20.3% (n=72) were under six years old, 28.8% (n=102) were between 6-12 years old, and 50.8% were 12 years and older. The distribution of cases was 51.4% (n=182) female and 48.6% (n=172) male. There were significant differences in age means and gender distributions between the outpatient and inpatient groups (p<.001; p=.001, respectively). Sociodemographic characteristics are presented in Table 1.

Table 2 includes the clinics from which consultations were requested. 92.1% of all consultations (n=326) were requested from departments related to pediatric health and diseases and pediatric surgery. The rate of requesting child and adolescent psychiatry consultations for outpatient cases was 0.9%, while it was 0.16% for inpatient cases. The clinics with the highest number of consultation requests were the pediatric neurology outpatient clinic (n=79), the general pediatrics outpatient clinic (n=77), and the general pediatrics ward (n=34). Non-pediatric clinics refer to ophthalmology, otolaryngology, plastic surgery, dermatology, orthopedics, and physical therapy and rehabilitation departments.

Suicidal attempts (12.1%; n=43) and irritability (11.3%; n=40) were the most common reasons for consultation (Table 3). The consultation was requested for two or more reasons in 18 cases. Other causes included various complaints such as academic failure, hair pulling, and confusion. Further details regarding the reasons for consultation can be found in Table 3.

According to the consulted cases, 32.2% (n=114) received counseling that consisted of general recommendations only. Among them, 67.8% (n=240) received at least one psychiatric disorder diagnosis. Additionally, 45 cases received two different diagnoses, and 6 cases received three different diagnoses. The most common diagnosis among cases referred for consultation from child and adolescent psychiatry were anxiety disorders (12.7%; n=45) (Table 4). Almost all consulted cases (97.2%; n=344) received parental education. It was found that 87.9% of the cases (n=311) received two or more treatment modalities. No treatment method was applied to 7 cases. The diagnoses are provided in Table 4, and the treatment modalities are visualized in Figure 1.

Pharmacological treatment was recommended for 44.6% (n=158) of the consulted children and adolescents. The most frequently recommended psychopharmacological agent was selective serotonin reuptake inhibitors (SSRIs) (21.8%; n=77). The recommended pharmacological treatments are shown in Table 5.

There were significant differences between the outpatient and inpatient groups in terms of impulsive suicide attempts ($p < .001$), intellectual disabilities ($p = .011$), and depressive disorders ($p = .003$). Counseling, psychotherapy, and special education were significantly more prevalent in the outpatient group ($p = .02$, $p < .001$, and $p < .001$). Of all consultations, 13.6% (n=48) began receiving special education following the consultation. Additionally, 66.9% of the cases (n=237) attended three or more follow-up visits. The comparison of diagnoses and treatment modalities between outpatient and inpatient groups is detailed in Table 6.

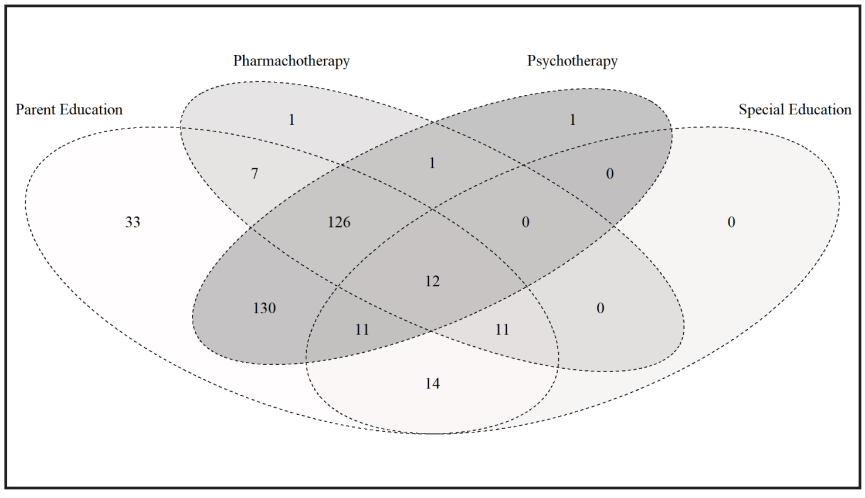

Figure 1. Distribution of treatment methods

Table 2. Clinics requesting consultations from child and adolescent psychiatry

Clinics	Outpatients n=224 (%)	Inpatients n=130 (%)	Total n=354 (%)
General pediatric clinic	77 (34.4)	34 (26.2)	111 (31.4)
Pediatric neurology	79 (35.3)	12 (9.2)	91 (25.7)
Pediatric endocrinology	26 (11.6)	8 (6.2)	33 (9.3)
Pediatric surgery	2 (0.9)	29 (22.3)	31 (8.8)
Pediatric emergency department	-	17 (13.1)	17 (4.8)
Pediatric intensive care unit	-	13 (10.0)	13 (3.7)
Pediatric gastroenterology	11 (4.9)	-	11 (3.1)
Other clinics related to pediatrics	14 (6.3)	4 (3.1)	18 (5.1)
Non-pediatric clinics	15 (6.7)	13 (10.0)	28 (7.9)
Total	**224 (63.3)**	**130 (37.7)**	**354 (100.0)**

Table 3. Reasons for requesting child and adolescent psychiatric consultations

Reasons for requesting consultations	n (%)
Suicide attempts	43 (12.1)
Irritability	40 (11.3)
Non-compliance or resistance to treatment	31 (8.8)
Speech delay	24 (6.8)
Attention deficit/hyperactivity	16 (4.5)
Stuttering / Speech difficulty	11 (3.1)
Enuresis / Encopresis	10 (2.8)
Unhappiness	10 (2.8)
Headache	10 (2.8)
Health council	10 (2.8)
Behavioral problems	9 (2.5)
Suspicion of autism	9 (2.5)
Fears	9 (2.5)
Developmental delay	9 (2.5)
Dizziness	8 (2.3)
Sleep problems	8 (2.3)
Eating problems	8 (2.3)
Crying	8 (2.3)
Syncope	8 (2.3)
Tic	7 (2.0)
Fecal retention	7 (2.0)
Vomiting	7 (2.0)
Abdominal pain	7 (2.0)
Substance use	6 (1.7)
Social withdrawal	6 (1.7)
Obsessions	6 (1.7)
Inability to learn writing and reading	6 (1.7)

Nail biting	6 (1.7)
Chest pain	6 (1.4)
Hallucination	6 (1.4)
Other reasons	37 (10.0)

Table 4. Distribution of psychiatric diagnoses based on evaluation results

Psychiatric disorders	**n (%)**
Anxiety disorders	44 (12.4)
Impulsive suicide attempts	36 (10.2)
Intellectual disabilities	35 (9.9)
Conduct disorder	29 (8.2)
Attention deficit/hyperactivity disorder	29 (8.2)
Depressive disorders	23 (6.5)
Speech and language disorder/delay	19 (5.4)
Enuresis / Encopresis	16 (4.5)
Autism spectrum disorder	13 (3.7)
Adjustment disorder	12 (3.4)
Somatoform disorder	11 (3.1)
Stuttering	6 (1.7)
Sleep disorders	6 (1.7)
Eating disorders	5 (1.4)
Obsessive-compulsive disorder	4 (1.1)
Bipolar disorder	3 (0.8)
Counseling	114 (32.2)

Table 5. Pharmacological treatments recommended as a result of consultation (n=354)

Psychotropic Medications	**n (%)**
Selective Serotonin Reuptake Inhibitors (SSRIs)	59 (16.7)
Antipsychotics	39 (11.0)
Psychostimulants	21 (5.9)
SSRIs + Antipsychotics	17 (4.8)
Tricyclic Antidepressants	16 (4.5)
Anxiolytics	2 (0.6)
Atomoxetine	2 (0.6)
Antipsychotics + Psychostimulants	1 (0.3)
SSRIs + Psychostimulants	1 (0.3)
Total	**158 (44.6)**

Table 6. Comparison of outpatient and inpatient groups in terms of diagnoses and treatments

Variables	Outpatients n=224 (%)	Inpatients n=130 (%)	*Statistics*	*p*
Anxiety disorders	31 (13.8)	14 (10.8)	χ^2=*0.699*	0.403
Impulsive suicide attempts	2 (0.9)	34 (26.2)	χ^2=*57.460*	**< 0.001**
Intellectual disability	29 (12.9)	6 (4.6)	χ^2=*6.408*	**0.011**
Conduct disorder	17 (7.6)	12 (9.2)	χ^2=*0.295*	0.587
Attention deficit/hyperactivity disorder	23 (10.3)	6 (4.6)	χ^2=*3.495*	0.620
Depressive disorders	8 (3.6)	15 (11.5)	χ^2=*8.595*	**0.003**
Counseling	82 (36.6)	32 (24.6)	χ^2=*5.418*	**0.020**
Psychotherapy	166 (74.1)	115 (88.5)	χ^2=*10.36*	**0.001**
Pharmacotherapy	130 (58.0)	66 (50.8)	χ^2=*1.76*	0.185
SSRIs	36 (16.1)	41 (31.6)	χ^2=*2.432*	0.119
Parent education	220 (98.2)	124 (95.4)	χ^2=*2.40*	0.121
Special education	42 (18.8)	6 (4.6)	χ^2=*14.02*	**< 0.001**
Follow-up visits				
<3 visits	71 (31.7)	46 (35.4)	χ^2=*0.506*	0.477
≥3 visits	153 (68.3)	84 (64.6)		

DISCUSSION

In this retrospective study, the sociodemographic and clinical characteristics of outpatient and inpatient treatment groups were compared in consultations requested from child and adolescent psychiatry. Although numerous descriptive studies evaluate consultations requested from child and adolescent psychiatry, studies comparing outpatient and inpatient treatment groups are limited. Our findings revealed several notable differences between the two groups, which have significant implications for clinical practice and service development.

Previous studies revealed that the average age of cases ranged between 10 and 13, with a majority of girls [13-15]. However, only a few studies examining consultations requested fromchild and adolescent psychiatry have shown a predominance of boys [5]. In our current study, there was a higher proportion of adolescent girls. Furthermore, the inpatient group had a much higher proportion of girls than the outpatient group. Previous research found that the majority of child and adolescent psychiatric consults were requested for inpatients [3, 14-16]. In contrast, most patients consulted in this study were outpatients, with more boys as inpatients and girls as outpatients.

According to studies, pediatric clinics request consultations on child and adolescent mental health most often [3, 5] particularly pediatric neurology clinics [14]. Pediatrics was the department that sought the most consultations in our study, with the child neurology outpatient clinic accounting for 22.3% (n=79) of all consultations. Mental and neurological symptoms are known to be inextricably linked and can occasionally overlap [17]. It should be kept in mind that psychiatric symptoms may often accompany neurological symptoms in nervous system diseases, and patients may present to the neurology clinic with psychiatric symptoms.

In studies conducted in our country, the most common reason for requesting consultations from child and adolescent psychiatry were suicide attempts [3, 5, 15, 16, 18]. Suicide risk assessment was the most common reason for referral to child psychiatry in a recent review [2]. In our study, the most common reasons for seeking consultation from child and adolescent psychiatry were suicide attempts and irritability. It is known that suicide attempts are more frequently observed in females and the adolescent and young adult age group [19]. The predominance of female patients and the adolescent group among the consulted cases may have been caused by the predominance of suicide attempts.

Interestingly, despite the high number of consultations related to suicide attempts, emergency department consultations were relatively low (4.8%; n=17). However, previous studies reported

that the emergency department was the most frequent department requesting child and adolescent psychiatry consultations [16, 20]. After the initial intervention of patients who present with a suicide attempt in our emergency service, they are subsequently followed up in either the pediatric ward or the pediatric intensive care unit. After the patients who have attempted suicide achieve stability and are no longer in immediate life-threatening danger, they are referred to child and adolescent psychiatry for consultation Which may have been the primary reason for the lower number of consultations from the emergency department in our study sample.

Two-thirds of the evaluated children and adolescents have been diagnosed with at least one mental disorder. The most common diagnosis is anxiety disorder, with a prevalence rate of 12.7%. Additionally, in the inpatient group, there were significantly higher rates of impulsive suicide attempts and depressive disorders compared to the outpatient group, while intellectual disability was significantly lower. However, there was no significant difference between the two groups regarding anxiety disorders. The rates of obtaining a diagnosis in consultations for child and adolescent psychiatry generally range from 50% to 84% [6, 16, 21]. However, there are variations in the most commonly reported diagnoses across studies. Most studies have reported that depressive disorders are the most frequent diagnosis [5, 15]. Nevertheless, one study identified anxiety disorders as the most common diagnosis in consultation psychiatry [22]. The diagnostic differences can be attributed to our study's combined evaluation of the outpatient and inpatient groups, the higher number of cases in the outpatient group, and the increased likelihood of requesting consultations from the pediatric neurology outpatient clinic.

In our study, at least one pharmacological agent was recommended for 44.6% of the cases, with SSRIs being the most commonly used agents. The outpatient and inpatient groups had no significant difference in pharmacotherapy and SSRI prescription. Significant variations exist in pharmacological agent use in similar studies conducted in our country. Our findings regarding pharmacological agent use are consistent with several recent studies [16, 21]. However, it should be highlighted that some studies show utilization rates higher than 70% [6, 22]and lower than 30% [3, 5], indicating significant variation in practice. In our study, anxiety disorder was the most common diagnosis, influencing the choice of SSRI medication. Medication usage rates varied due to geographical, cultural, and socioeconomic differences. Further research is necessary to assess drug efficacy and safety in child psychiatric consultations.

Finally, there was a higher rate of psychoeducation and special education in the outpatient group than in the inpatient group. Moreover, 13.6% (n=48) of all consultation cases started to receive special education after the consultation. In population-based studies, special education rates are between 10-20% [23]. Notably, a consultation study conducted in our country reported that 28% of the consulted cases were directed to special education [24]. Current findings indicate that cases requiring consultation from child and adolescent psychiatrists require a higher level of special educational support than the general population. Concomitant developmental and mental disorders can significantly affect treatment compliance. Therefore, the high rate of special education in the outpatient group emphasizes the importance of comprehensive evaluation for developmental and mental disorders and subsequent referral to appropriate educational services.

Limitations and Strengths

The most important limitation of the study is its cross-sectional design and retrospective data collection. Some case records were missing or inaccessible. It was also not possible to verify the accuracy and timeliness of the data. Therefore, the generalizability of the research findings is limited. Another limitation is the non-random sampling method. Additionally, the research environment, being a university hospital, may have partially influenced the randomization process. Certain patient groups may be more likely to present to a university hospital, which may lead to selection bias. Therefore, it's essential to acknowledge these potential biases when interpreting the study results. Finally, data should be interpreted with caution due to the lack of inter-rater standardization.

The descriptive and correlational design is a key strength of the study. This design allowed detailed description and comparison of the characteristics and treatment methods of outpatient and inpatient cases referred for psychiatric consultation. Using the K-SADS-PL tool as a data collection tool in our clinic contributed to the objective and standardized diagnosis of the cases. Finally, to the best of our knowledge, another critical aspect of the study is that it is the first comparison of outpatient and inpatient groups and serves as a reference for future research.

CONCLUSION

The current retrospective study examined sociodemographic and clinical characteristics of child and adolescent psychiatry consultations, comparing outpatient and inpatient treatment groups. The findings revealed notable differences between the two groups in terms of age, gender, departments requesting consultation, reasons for seeking consultation, diagnoses, and treatment options. These findings contribute to the understanding of various aspects of child and adolescent psychiatric consultations requested in outpatient and inpatient settings.

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REFERENCES

[1] McBride KE, Solomon MJ, Lambert T, et al. (2021) Surgical experience for patients with serious mental illness: a qualitative study. BMC Psychiatry 21:47. https://doi.org/10.1186/s12888-021-03056-x

[2] Becker JE, Smith JR, Hazen EP (2020) Pediatric Consultation-Liaison Psychiatry: An Update and Review. Psychosomatics 61:467-480. https://doi.org/10.1016/j.psym.2020.04.015

[3] Eraslan AN, Aydın Görücü R, Yılmaz A (2021) Evaluation of Psychiatric Consultations Required for Child and Adolescent Patients Hospitalized in a Training and Research Hospital. Turkish Journal of Pediatric Disease 15:451-458. https://doi.org/10.12956/tchd.778921

[4] Bujoreanu S, White MT, Gerber B, Ibeziako P (2015) Effect of timing of psychiatry consultation on length of pediatric hospitalization and hospital charges. Hospital Pediatrics 5:269-275. https://doi.org/10.1542/hpeds.2014-0079

[5] Ersoy Şimşek EG, Eyüboğlu M, Eyüboğlu D (2019) Evaluation of Child and Adolescent Psychiatric Consultations in A University Hospital. Osmangazi Medical Journal 41:248-256. https://doi.org/10.20515/otd.480686

[6] Alpaslan AH, Kocak U, Cobanoglu C, Gorucu Y (2015) Evaluation of Child and Adolescent Psychiatry Consultations in a University Hospital. New/Yeni Symposium 53:10-16. https://doi.org/10.5455/NYS.20151214020808

[7] Yilgor A, Kurhan F (2024) Is Childhood Trauma a Risk Factor for Resistant Epilepsy? Journal of Interpersonal Violence 39:1228-1244. https://doi.org/10.1177/08862605231203964

[8] Walg M, Avaliani T, Großmeier M, Hapfelmeier G (2021) Reasons for Outpatient Psychiatric Consultations of Unaccompanied Minor Refugees. Deutsches Arzteblatt International 118:597-598. https://doi.org/10.3238/arztebl.m2021.0177

[9] Reinblatt SP, Coble K, Williams JL, Cotton AM, Bettencourt AF (2022) Characteristics of Primary Care Providers' Consultations With a Statewide Child Psychiatry Access Program Regarding Autism Spectrum Disorder. J Acad Consult Liaison Psychiatry 63:463-473. https://doi.org/10.1016/j.jaclp.2022.01.004

[10] Gokler B, Unal F, Pehlivanturk B, et al. (2004) Reliability and Validity of Schedule for Affective Disorders and Schizophrenia for School Age Children-Present and Lifetime Version-Turkish Version (K-SADS-PL-T). Turk J Child Adolesc Ment Health 11:109-116

[11] Chen H (2022) VennDiagram: Generate High-Resolution Venn and Euler Plots. R package version 1.7.3. https://CRAN.R-project.org/package=VennDiagram. Accessed Date Accessed 2022 Accessed

[12] Team RC (2023) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/. Accessed Date Accessed 2023 Accessed

[13] Aktepe E, Kocaman O, Isik A, Eroglu F (2013) An Evaluation of the Child and Adolescent Psychiatry Consultation Services Requested in a University Hospital. TAF Preventive Medicine Bulletin 12:1. https://doi.org/10.5455/pmb.1-1355473983

[14] Dikeç G, Baysan Arabacı L, Uzunoğlu G (2020) Bir Bölge Psikiyatri Hastanesi Çocuk ve Ergen Kliniğinde İstenen Konsültasyonların Değerlendirilmesi. Acibadem Universitesi Saglik Bilimleri Dergisi 11:418-422. https://doi.org/10.31067/0.2019.193

[15] Topal Z, Karadağ M, Çalışgan B, et al. (2021) Characteristics of Child and Adolescent Psychiatry Consultations at a University Hospital and Accuracy Rates of Recognition of Childhood Psychiatric Diseases by Nonpsychiatry Specialists. Middle Black Sea Journal of Health Science 7:88-96. https://doi.org/10.19127/mbsjohs.881342

[16] Özek Erkuran H, Önen Ö (2022) Evaluation of Psychiatric Consultations Requested for Inpatient Children and Adolescents in a Training and Research Hospital. Turkish Journal of Child and Adolescent Mental Health 29:204-209. https://doi.org/10.4274/tjcamh.galenos.2021.36450

[17] Zimmerman M, Morgan TA, Stanton K (2018) The severity of psychiatric disorders. World Psychiatry 17:258-275. https://doi.org/10.1002/wps.20569

[18] Bilginer Ç, Karadeniz S, Aydoğdu S, Bulut Şahin D (2021) Child Mental Health Services in Emergency Department of a University Hospital. Turkish Journal of Child and Adolescent Mental Health 28:12-19. https://doi.org/10.4274/tjcamh.galenos.2020.91300

[19] Miranda-Mendizabal A, Castellví P, Parés-Badell O, et al. (2019) Gender differences in suicidal behavior in adolescents and young adults: systematic review and meta-analysis of longitudinal studies. International Journal of Public Health 64:265-283. https://doi.org/10.1007/s00038-018-1196-1

[20] Çolpan M, Eray Ş, Vural P (2013) Evaluation of Consultations Requested from the Departments of Child and Adolescent Psychiatry of Uludağ University Hospital within the Previous Year. Güncel Pediatri 11:102-106. https://doi.org/10.4274/Jcp.11.63825

[21] Göker Z, Güney E, Dinç G, Üneri Ö (2014) The Evaluation of the Psychiatric Consultations of Children and Adolescents Hospitalised in a Training and Research Hospital. Turkish Journal of Pediatric Disease 8:17-24. https://doi.org/10.12956/tjpd.2014.39

[22] Gokcen C, Celik YI (2011) The Evaluation Of Child And Adolescent Psychiatry Consultations from other Inpatient Clinics in a Training Hospital (The Evaluation Of Child And Adolescent Psychiatry Consultations from other Inpatient Clinics in a Training Hospital). Sakarya Medical Journal 1:140-144. https://doi.org/10.5505/sakaryamj.2011.25744

[23] National Center for Education Statistics. Students With Disabilities. Condition of Education. U.S. Department of Education, Institute of Education Sciences (2023) https://nces.ed.gov/programs/coe/indicator/cgg/students-with-disabilities

[24] Kılıç B, Uslu R, Ayla A (2007) A Preliminary Evaluation of Consultation-Liaison Psychiatry Services For Children at A University Hospital: Lessons Learned to Enhance Efficacy. Yeni Symposium 45:163-169. https://neuropsychiatricinvestigation.org/en/a-preliminary-evaluation-of-consultation-liaison-psychiatry-services-for-children-at-a-university-hospital-lessons-learned-to-enhance-efficacy-13227.

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Original Research

Evaluation of Sella Turcica and Maxilla Morphometry of Individuals with Cleft Lip and Palate on Lateral Cephalometric Radiographs

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ABSTRACT

Objective: The objective of this study was to evaluate the dimensions and the morphology of the sella turcica, as well as maxillary cephalometric landmarks, in patients with and without clefts.

Methods: Lateral cephalometric radiographs of 55 cleft patients and 55 non-cleft (control) patients were included in the study. The morphology of the sella turcica, including its shape, height, width, and diameter was evaluated. Additionally, maxillary cephalometric measurements, comprising four lengths and two angles, were assessed on the radiographs. The chi-squared test was employed to compare sella turcica shapes between the cleft and non-cleft groups. Independent samples t-tests were conducted to analyze dimensional parameters between groups and genders.

Results: Significant relationship was found between groups with cleft and non-cleft for sella shapes ($p=0.032$). There was no statistical association for sella dimensions according to the cleft presence ($p>0.05$). All maxillary cephalometric measurements were significantly greater in individuals of the non-cleft group compared to those in the cleft group (ANS-PNS, A-PNS, S-N-ANS , S-N-A, N-A) except R-PNS.

Conclusion: Patients with clefts more frequently exhibited a flattened sella shape, whereas those without clefts tended to have a round sella shape. Maxillary cephalometric dimensions were lower in the individuals of cleft group.

Keywords: Cleft lip; cleft palate; lateral cephalometric radiography; maxilla

INTRODUCTION

Cleft lip and palate (CLP), a widespread developmental malformation, affecting the craniofacial region [1]. Significant ethnic differences exist in the incidence of CLP, the reported range changes between 1/700 and 1/1000 [2]. Although the etiology of CLP is not known exactly, it is known that many cases are multifactorial [1].

Embryonic development entails intricate interactions among the oral cavity, hypothalamus, and pituitary gland. Any abnormalities during the development of these structures can lead to anatomical and functional disorders [3]. The sella turcica has a shape resembling a saddle, located on the sphenoid bone body and hosts the pituitary gland [3, 4]. The pituitary gland develops prior to the

formation of the sella turcica, so any abnormalities in pituitary gland development could influence the size and morphology of the sella turcica [1,5]. Literature shows some differences in shape and size of the sella in the presence of pathologies, syndromes and anomalies associated with the craniofacial region [1,5-9]. The presence of CLP has been determined to cause abnormal and/or smaller sella turcica [1,3,8-9].

CLP influences the craniofacial development in many different ways as developmental defects, secondary functional disturbances, and iatrogenic factors secondary to surgical treatment [10]. Iatrogenic outcomes such as surgical technique, time of performing the surgery, approach and experience of the surgeon affect maxillary growth to some extent. However, significant interindividual differences were observed in a group of patients operated on by a single surgeon, minimizing iatrogenic variations. This implies that individual-specific intrinsic factors can have a notable impact on maxillary growth potential. Because maxillary growth potential is influenced by multiple factors, ongoing research continues to explore the causes of maxillary hypoplasia and associated occlusion disorders [6,11].

The lateral cephalometric radiographs provide imaging and evaluation of many oral and craniofacial structures. Cephalometric radioraphy is still frequently used in cleft patients [4,12]. In these images, which are already taken for diagnostic reasons, the sella turcica region is visible. Significant changes in both the morphology and dimesion of the sella turcica can be demonstrated on lateral cephalometric radiographs [4,6]. At the same time, lateral cephalometric radiographs are considered clinically valuable to identify growth predictors [12].

Main Points

- CLP influences the craniofacial region in many different ways.
- Cleft presence does not affect the sella dimensions but causes a decrease in maxillary dimensions.
- Maxillary dimensions are lower in cleft group compared to non-cleft group.
- Flattened sella was most common in cleft group, and round sella was most common in non-cleft group.

The objective of the present study was to compare the sella turcica morphology, sella turcica dimensions and maxillary cephalometric dimensions between individuals with nonsyndromic cleft and non-cleft counterparts by using lateral cephalometric radiographs. The null hypothesis of this research proposed that there would be no difference in the morphology and dimensions of the sella turcica, as well as in maxillary cephalometric measurements, between cleft and non-cleft patient groups.

MATERIALS AND METHODS

Ethics committee approval of the study was obtained from the Non-Interventional Ethics Committee of Selcuk University Faculty of Dentistry. (Approval No: 2022/41).

Sample Size Estimation

The sample size of the study was assigned using G*Power (v. 3.1.9.7). Sample size for the independent samples t-test was calculated according to the Cohen's medium effect size (d=0.5) as a minimum 51 individiuals in each group with %80 power and %95 confidence interval (α=0.05). Considering the possibility of data loss, the number of samples for the groups was set to 55.

Study Design

Our retrospective study was conducted on 55 non-syndromic cleft and 55 non-cleft patients, aged 9-24 years, who applied to Selcuk University Department of Orthodontics for diagnosis and treatment. Our study was carried out on radiographs that were taken with the same device (Planmeca ProMax 2D, Planmeca Oy, Helsinki, Finlandiya) in accordance with standard lateral cephalometric radiograph taking rules. The cephalostat also had a reference ruler thus magnification could be measured. All sella turcica and maxillary cephalometric measurements on radiographic images were made with ImageJ (1.52a) (a publicly available software for image analysis). The radiographs of the patients whose anamnesis revealed any surgical intervention in the maxillofacial and pituitary region, syndrome and systemic disease related to the craniofacial region except cleft lip and palate, orthodontic treatment history, hormonal drug usage, and any disease or trauma affecting the craniofacial region were exclusion criteria for the study. Inclusion criteria included images with adequate image quality and no artifacts that would affect the evaluation.

Sella Turcica Dimensional Measurements and Morphology

Size of the sella turcica (length, depth, and diameter) was measured according to method of Silverman [13]. The length was determined as the distance between tuberculum sella and tip of dorsum sella and the depth was measured perpendicular to this line to the deepest point of sellar floor. The diameter of the sella turcica was evaluated as the distance from tuberculum sella to a point on the posterior inner wall of the fossa located at the farthest point from the tuberculum sella [1, 4]. All measurements were converted to milimeter (mm) according to the reference ruler. Morphologies of sella turcica were evaluated by using the method described by Camp [14] (Figure 1). A maxillofacial radiologist with 11 years of experience peformed all the measurements and an orthodontist with 19 years of experience evaluated the morphology of sella turcica seperately.

Maxillary Cephalometric Measurements;

Six lateral cephalometric structures were identified (Sella (S), nasion (N), registration point (R), anterior nasal spine (ANS), posterior nasal spine (PNS) and cephalometric A point (A). Four length and two angle measurements were made over these anatomical structures (Figure 2).

Statistical Analysis;

SPSS 22.0 was used for all statistical analyses. The distribution of data by groups and gender was analyzed with descriptive statistics. Interobserver reliability was calculated by using kappa (κ) statistics. Shapiro-wilk test was used to test the normality of data. A chi-squared test was applied to determine the relationship between cleft presence and sella morphology. To analyze the difference between cleft group and non-cleft group in terms

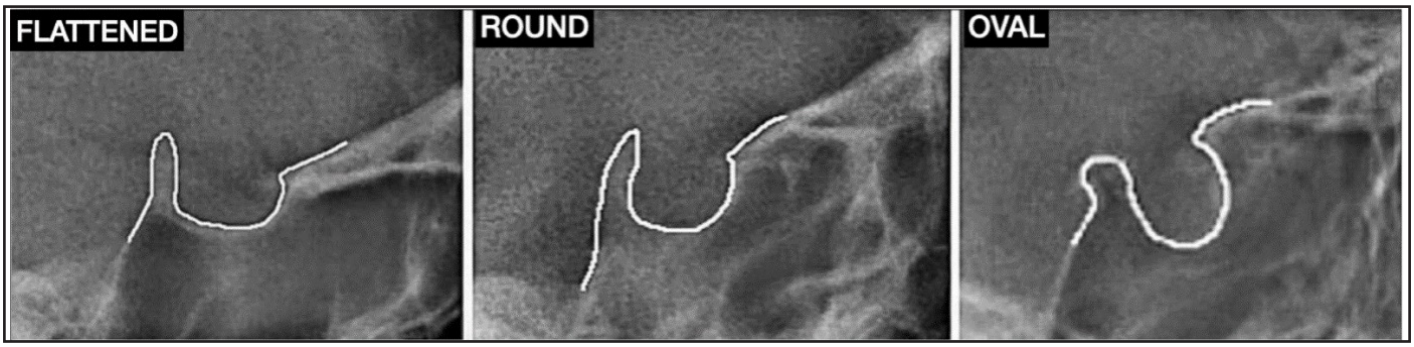

Figure 1. The basic classification method of sella turcica morphology as flattened, round and oval.

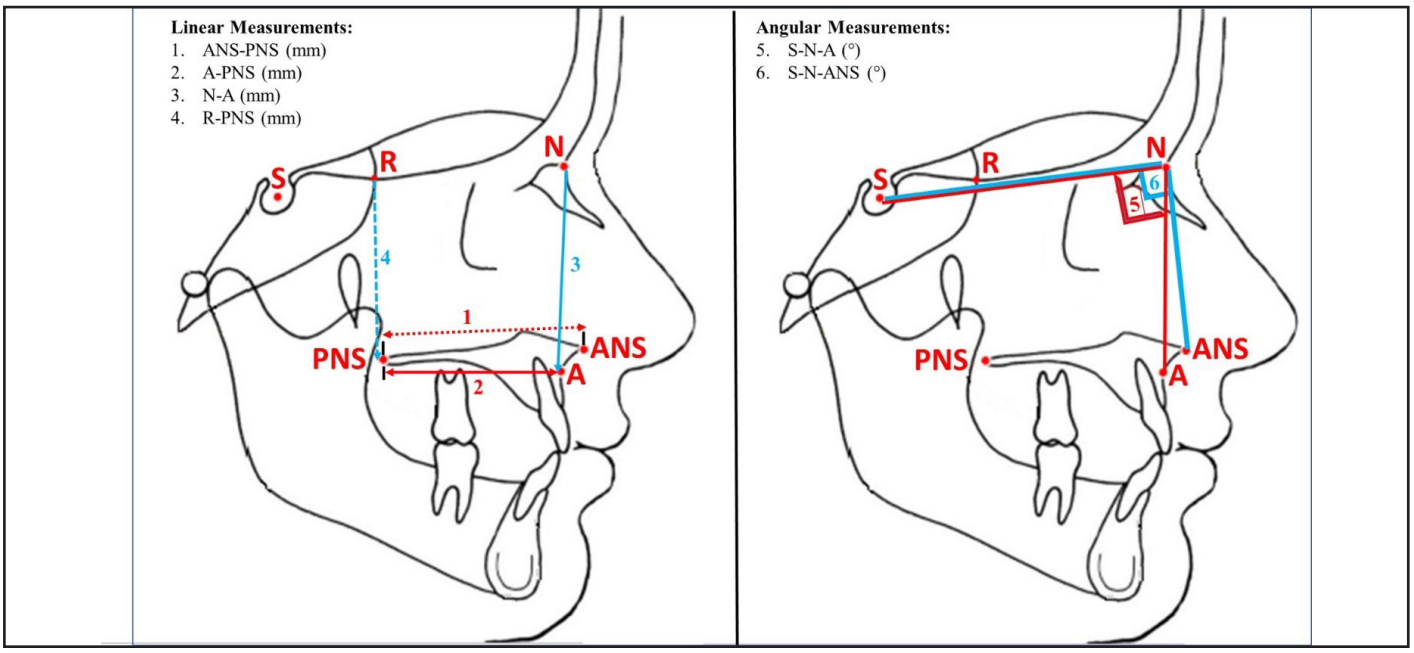

Figure 2. Tracing of a lateral cephalometic radiography showing landmarks and measurements.

of sella turcica and maxillary cephalometric measurements independent sample t-test was used.

RESULTS

The average age of patients with cleft palate was 14.53 years (±3.99), while for the non-cleft group it was 13.93 years (±2.97). Among the participants, 61 (55.5%) were under 15 years old, and 49 (44.5%) were 15 years or older. Demographic data of the patients are shown in Table 1.

There was a very high agreement between the two observers in the evaluation of sella tucica morphologies (κ-value=0.945). The different observations were re-evaluated by the two observers and the final decision was made. The predominant sella shape in the non-cleft group was round, whereas in the cleft group, it was flattened. The difference in sella shapes between the groups was statistically significant (p=0.032). The distribution of the sella shapes according to the cleft presence was seen in Table 2. The shapes of the sella turcica based on gender in both groups are given in Table 3 and there was no statistical difference between the genders for both groups (p>0.05).

When comparing the linear measurements of sella turcica no statistically significant difference was observed between cleft and non-cleft groups. When we compared the maxillary cephalometric measurements between the groups, it was seen that all dimensions were higher in the non-cleft group and this difference was statistically significant for all measurements except R-PNS (Table 4). The distribution of dimensional measurements in cleft and non-cleft groups according to gender and age groups is shown in Table 5.

Table 1. Demographic data of the patients.

		Cleft Presence		
		Non-cleft	**Cleft**	**Total**
Gender n (%)	**Female**	23 (20.9%)	37 (33.6%)	60 (54.5%)
	Male	32 (29.1%)	18 (16.4%)	50 (44.5%)
	Total	55 (50%)	55 (50%)	110 (100%)
Age	**Min.**	9	9	9
	Max.	19	24	24
	Mean±SD	13.93±2.97	14.53±3.99	14.23±3.51

Table 2. The distribution of different shapes of sella turcica in cleft and non-cleft groups.

	Cleft group n(%)	**Non-cleft group n(%)**	**p**
Round	29 (52.7%)	17 (30.9%)	0.032
Oval	7 (12.7%)	16 (29.1%)	
Flattened	19 (34.5%)	22 (40%)	
Total	55 (100%)	55 (100%)	

Table 3. The distribution of different shapes of sella turcica according to genders in the groups.

	Cleft group n (%)			**Non-cleft group n (%)**		
	Female	**Male**	**Total**	**Female**	**Male**	**Total**
Round	4 (7.3%)	13 (23.6%)	17 (30.9%)	19 (34.5%)	10 (18.2%)	29 (52.7%)
Oval	6 (10.9%)	10 (18.2%)	16 (29.1%)	2 (3.6%)	5 (9.1%)	7 (12.7%)
Flattened	8 (14.5%)	14 (25.5%)	22 (40%)	11 (20%)	8 (14.5%)	19 (34.5%)
Total	18 (32.7%)	37 (67.3%)	55 (100%)	32 (58.2%)	23 (41.8%)	55 (100%)
χ^2	0.622			0.206		

Table 4. Dimensions of the sella turcica and cephalometric landmarks according to the cleft presence. (Independent samples t test; p<0.05 is statisticallly significant)

	Cleft group n=55	Non-cleft group n=55	p
Diameter (mm)	8.41±1.86	8.36±1.68	0.897
Depth (mm)	7.53±1.88	7.35±1.59	0.598
Length (mm)	8.44±2.18	8.62±2.19	0.667
ANS-PNS (mm)	45.57±4.34	48.31±3.95	**0.001**
A-PNS (mm)	40.91±4.9	43.09±3.62	**0.009**
S-N-ANS (º)	82.32±4.8	84.96±3.59	**0.001**
S-N-A (º)	77.49±5.41	79.23±3.47	**0.047**
N-A (mm)	50.05±4.55	52.43±3.66	**0.003**
R-PNS (mm)	44.1±5.14	45.84±4.28	0.055

Table 5. Dimensions of the sella turcica and cephalometric landmarks in the subjects according to gender and age in cleft and non-cleft groups. (Independent samples t test; p<0.05 is statisticallly significant)

	Cleft group (mean±SD)			Non-cleft group (mean±SD)		
	Female n=18	**Male n=37**	**p**	**Female n=32**	**Male n=23**	**p**
Diameter (mm)	8.11±2.43	8.6±2.08	0.907	8.16±1.2	8.64±2.17	0.344
Depth (mm)	7.96±1.93	7.31±1.84	0.235	7.17±1.32	7.61±1.89	0.311
Length (mm)	8.44±1.79	8.38±1.93	0.441	8.17±1.66	9.24±2.68	0.098
ANS-PNS (mm)	45.48±3.47	45.61±4.76	0.918	47.47±3.18	49.48±4.66	0.062
A-PNS (mm)	40.88±4.58	40.93±5.11	0.976	42.18±2.81	44.35±4.28	**0.028**
S-N-ANS (º)	83.32±5.65	81.82±4.33	0.280	84.68±3.72	85.35±3.46	0.501
S-N-A (º)	78.38±6.37	77.06±4.93	0.400	78.81±3.59	79.82±3.29	0.292
N-A (mm)	49.27±3.27	50.43±5.06	0.379	52.43±3.35	52.41±4.12	0.987
R-PNS (mm)	42.26±3.19	44.99±5.69	0.064	45.14±2.9	46.82±5.6	0.195
	<15 n=29	**≥15 n=26**	**p**	**<15 n=23**	**≥15 n=32**	**p**
Diameter (mm)	8.05±2.01	8.81±1.62	0.132	7.92±1.73	8.99±1.39	**0.018**
Depth (mm)	7.19±1.75	7.9±1.98	0.161	6.89±1.38	8±1.65	**0.009**
Length (mm)	7.95±2.19	8.98±2.09	0.081	8.46±2.18	8.83±2.23	0.548
ANS-PNS (mm)	44.96±4.04	46.24±4.65	0.278	49.83±3.93	47.22±3.64	**0.014**
A-PNS (mm)	40.97±4.4	40.85±5.5	0.929	44.03±3.94	42.42±3.28	0.105
S-N-ANS (º)	83.1±4.96	81.44±4.56	0.205	84.61±3.59	85.45±3.62	0.394
S-N-A (º)	78.69±5.37	76.15±5.22	0.081	79.06±3.32	79.47±3.72	0.673
N-A (mm)	47.47±2.91	52.93±4.35	**0.000**	51.85±3.6	53.23±3.66	0.169
R-PNS (mm)	41.33±4.29	47.17±4.22	**0.000**	44.25±3.74	48.07±4.04	**0.001**

DISCUSSION

In this study the morphology, sella dimensions, and maxillary cephalometric measurements on lateral cephalometric images were assessed and compared between CLP patients and non-cleft patients. The present study results showed no significant difference in the sella dimensional measurements between cleft and non-cleft groups. In the literature, it is seen that there are different results in studies evaluating the interrelation between the cleft presence and the sella dimensions [1,3,8,15-21]. Yalcin [1] reported that, only the difference for length was statistically significant, dimensions of the sella turcica was smaller in the cleft group compared to non-cleft indviduals. On the contrary, Yasa et al [3] conducted larger depth, length and diameter for the sella turcica in cleft group according to the non-cleft group. Alike our results, Canıgür Bavbek et al [9] told that, the difference between the cleft and non-cleft groups was not statistically significant as to sella dimensions. Similar to this study, no statistically significant difference was found between the groups in our study. In a recent three-dimensional study conducted by El Tabakh et al [19] on cone beam computed tomography images, no relationship was found between sella dimensions and the presence of cleft. Van der Plas et al [22] analyzed the difference in pituitary volume between isolated cleft and non-cleft patients, and reported no significant difference in average pituitary volumes between groups. The significant differences between study results may be due to the study population size, imaging methods, age distribution and the study group's cleft phenotypes.

There were no gender-based differences observed in sella turcica dimensions within both groups like the studies of Yalcin [1], Yasa et al [3] and Shah et al [23]. Kumar and Govindraju [4] noticed that the sella length was higher in men, depth was higher in women and there was no difference in mean diameter in both genders.

In point of the age of the individuals although sella dimensions were not statistically significant in cleft group, depth and diameter was longer in patients ≥15 years of age in the non-cleft group. Similarly to our results, Yalcin [1], stated that diameter and depth of sella were found to be longer in the older non cleft age group. Yasa et al [3] conducted that the length of the sella turcica in the cleft group and the depth of the sella turcica in the non-cleft group are affected by age. In the general evaluation, it was observed that age increases the sella depth and diameter. Alkofide et al [8] found a significant increase in all sella turcica dimensions in both cleft and non-cleft subjects. Shresta et al [24] assessed the interrelation between sella dimensions and age, in the group of 18-30 years old individuals, and the authors found no statistically significant relation. Choi et al [25] deduced that the sella turcica size increase continues up to 25 years old and over the age of 26, no significant difference is seen. Considering all these findings, it is seen that age has an effect on the sella dimensions of non-cleft individuals.

According to our study, morphology of sella was evaluated according to the basic classification method (round, oval, flattened). Flattened sella turcica was more common in cleft group. In the studies by Yalcin [1] and Yasa et al [3], in which the shape of the sella turcica was evaluated with a similar classification method, they found that the flattened shape was more common in the cleft group, similar to our study. Since the objectivity and reproducibility of the morphological evaluations were controversial, the two observers were evaluated all radiographs separately and then the different ones were re-evaluated together.

Cleft also affects craniofacial development for different reasons such as intrinsic developmental deficiencies, functional distortions, and iatrogenic consequences [10]. According to the cephalometric studies spatial relationships between maxilla and mandible varies in children with and without cleft [10, 26]. In the present study, all ANS-PNS (mm), A-PNS (mm), S-N-ANS (°), S-N-A (°), N-A (mm) values were statistically higher in the non-cleft group. According to Khanna et al [10] observations reflect the posterior positioning of the premaxilla with respect to cranial base in surgically operated cleft patients. Similarly, in this study, the S-N-A measurement was significantly reduced in patients belonging to the cleft group. In a study it is reported that in all age groups of the study population S-N-ANS angle was decreased in un-operated cleft patients according to non-cleft group [27]. This has been interpreted as supporting the possibility of maxillary hypoplasia. In the same study, it was concluded that dimensional parameters related to facial height also varied between cleft and non-cleftgroups, and maxillary hypoplasia was observed even in the absence of surgical intervention [27].

The limitations of this study are that a two-dimensional imaging method was used, although it still maintains its importance in the orthodontic treatment process. Apart from this, the presence of clefts was not separated as unilateral and bilateral. It is thought that these subgroups to be formed in larger sample groups will contribute to the literature.

CONCLUSION

In our study on cephalometric radiographs, which are still indispensable in orthodontic diagnosis and treatment, it was shown that cleft did not affect the sellar dimensions, but caused a decrease in maxillary dimensions and maxillary hypoplasia. Also the study demonstrated flattened sella was most common in cleft group, and round sella was most common in non-cleft group.

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Author Contributions: Conception: Dİ - Design: Dİ, HK - Supervision: HK - Data Collection and/or Processing: Dİ, HK - Analysis and/or Interpretation: Dİ - Literature Review: Dİ, HK - Writing: Dİ- Critical Review: HK.

REFERENCES

[1] Yalcin ED (2020) Morphometric Analysis of Sella Turcica Using Cone-Beam Computed Tomography in Patients With Cleft Lip and Palate. J Craniofac Surg. 31:306-309. https://doi.org/10.1097/scs.0000000000005881

[2] Eslamian L, Latifi F, Hejazi M, Aslani F, Rakhshan V (2019) Lateral cephalometric measurements of Iranians with surgically repaired unilateral cleft lips and palates. Int Orthod. 17:304-311. https://doi.org/10.1016/j.ortho.2019.03.013

[3] Yasa Y, Bayrakdar IS, Ocak A, Duman SB, Dedeoglu N (2017) Evaluation of Sella Turcica Shape and Dimensions in Cleft Subjects Using Cone-Beam Computed Tomography. Med Princ Pract. 26:280-285. https://doi.org/10.1159/000453526

[4] Kumar TSM, Govindraju P (2017) Relationship between the morphological variation of sella turcica with age and gender: A digital radiographic study. J Indian Acad Oral Med Radiol. 29:164-169 https://doi.org/10.4103/jiaomr.JIAOMR_146_16

[5] Kjær I (2015) Sella turcica morphology and the pituitary gland—a new contribution to craniofacial diagnostics based on histology and neuroradiology. Eur J Orthod. 37:28-36 https://doi.org/10.1093/ejo/cjs091

[6] Antonarakis GS, Huanca Ghislanzoni L, Fisher DM (2022) Sella turcica dimensions and maxillary growth in patients with unilateral cleft lip and palate. J Stomatol Oral Maxillofac Surg. 123:e916-e921. https://doi.org/10.1016/j.jormas.2022.06.008

[7] Tekiner H, Acer N, Kelestimur F (2015) Sella turcica: an anatomical, endocrinological, and historical perspective. Pituitary. 18:575-578. https://doi.org/10.1007/s11102-014-0609-2

[8] Alkofide EA (2008) Sella turcica morphology and dimensions in cleft subjects. Cleft Palate Craniofac J. 45:647-653. https://doi.org/10.1597/07-058.1

[9] Bavbek NC, Kaymaz FT, Türköz Ç (2019) Evaluation of the cranial base and sella turcica morphology in patients with unilateral cleft lip and palate. Acta Odontol Turc. 36:33-40 https://doi.org/10.17214/gaziaot.493495

[10] Khanna R, Tikku T, Verma SL, Verma G, Dwivedi S (2020) Comparison of maxillofacial growth characteristics in patients with and without cleft lip and palate. J Cleft Lip Palate Craniofacial Anomalies. 7(1):30-42 https://doi.org/7:30-42 10.4103/jclpca.jclpca_22_19

[11] Costanza Meazzini M, Tortora C, Morabito A, Garattini G, Brusati R (2011) Factors that affect variability in impairment of maxillary growth in patients with cleft lip and palate treated using the same surgical protocol. J Plast Surg Hand Su. 45:188-193 https://doi.org/10.3109/2000656X.2011.583493

[12] Doğan E, Çınarcık H, Doğan S, Dindaroğlu F (2020) Is Cephalometric Analysis Reliable in Cases with Cleft Lip and Palate? EÜ Dishek Fak Derg. 41(1):27-37 https://doi.org/10.5505/eudfd.2020.71463

[13] Silverman FN (1957) Roentgen standards for size of the pituitary fossa from infancy through adolescence. Am J Roentgenol. 78:451-460

[14] Camp JD (1924) The normal and pathologic anatomy of the sella turcica as revealed by roentgenograms. Am J Roentgenol Radium Ther. 12:143-156 https://doi.

org/10.1148/1.2.65

[15] Antonarakis GS, Huanca Ghislanzoni L, La Scala GC, Fisher DM (2020) Sella turcica morphometrics in children with unilateral cleft lip and palate. Orthod Craniofac Res. 23:398-403 https://doi.org/10.1111/ocr.12380

[16] Cesur E, Altug A, Toygar-Memikoglu U, Gumru-Celikel D, Tagrikulu B, Erbay E (2018) Assessment of sella turcica area and skeletal maturation patterns of children with unilateral cleft lip and palate. Orthod Craniofac Res. 21:78-83 https://doi.org/10.1111/ocr.12219

[17] Sundareswaran S, Nipun C (2015) Bridging the gap: sella turcica in unilateral cleft lip and palate patients. Cleft Palate Craniofac J. 52:597-604 https://doi.org/10.1597/13-258.

[18] Paknahad M, Shahidi S, Khaleghi I (2017) A cone beam computed tomographic evaluation of the size of the sella turcica in patients with cleft lip and palate. J Orthod. 44:164-168 https://doi.org/10.1080/14653125.2017.1343221

[19] Eltabakh AG, Gemeay E, El Sayed W, El Rawdy AM, Ramadan AAEF (2023) Shape and Dimensions of Sella Turcica in Cleft And Non-Cleft Palate Subjects: A Radiographic Study. Dent Sci Update. 4:115-122 https://doi.org/10.21608/dsu.2023.142037.1127

[20] Zawiślak A, Jankowska A, Grocholewicz K, Janiszewska-Olszowska J (2023) Morphological Variations and Anomalies of the Sella Turcica on Lateral Cephalograms of Cleft-Palate-Only (CPO) Patients. Diagnostics. 13:2510 https://doi.org/10.3390/diagnostics13152510

[21] Mosavat F, Sarmadi S, Amini A, Asgari M (2023) Evaluation of dimension and bridging of sella turcica and presence of ponticulus posticus in individuals with and without cleft: a comparative study. Cleft Palate Craniofac J. 60:695-700 https://doi.org/10.1177/10556656221075935

[22] van der Plas E, Caspell CJ, Aerts AM, Tsalikian E, Richman LC, Dawson JD, Nopoulos P (2012) Height, BMI, and pituitary volume in individuals with and without isolated cleft lip and/or palate. Pediatr Res.71:612-618 https://doi.org/10.1038/pr.2012.12

[23] Shah A, Bashir U, Ilyas T (2011) The shape and size of the sella turcica in skeletal class I, II & III in patients presenting at Islamic International Dental Hospital, Islamabad. Pakistan Oral and Dental journal. 31(1):104-110 https://doi.org/10.1093/ejo/cjm049

[24] Shrestha GK, Pokharel PR, Gyawali R, Bhattarai B, Giri J (2018) The morphology and bridging of the sella turcica in adult orthodontic patients. BMC Oral Health. 18:1-8 https://doi.org/10.1186/s12903-018-0499-1

[25] Choi WJ, Hwang EH, Lee SR (2001) The study of shape and size of normal sella turcica in cephalometric radiographs. Korean J Oral Maxillofac Radiol. 31:43-49

[26] Bishara SE, Jakobsen JR, Krause JC, Sosa-Martinez R (1986) Cephalometric comparisons of individuals from India and Mexico with unoperated cleft lip and palate. Cleft Palate J. 23:116-125

[27] Jain P, Agarwal A, Srivastava A (2009) Lateral cephalometric evaluation in cleft palate patients. Pol J Surg.1:23-27 https://doi.org/10.2478/v10035-009-0004-2

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Original Research

Uncommon HLA Alleles Observed in a Population of Istanbul Province

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ABSTRACT

Objective: New polymorphisms are formed in human leucocyte antigen (HLA) genes with point mutations, gene conversions, and duplication, and the diversity continues to increase. Various new HLA alleles have significant roles in transplantation, and epidemiologic and population studies. The aim of our study was to determine the status of HLA alleles in the Turkish population, which is uncommon, well-defined, and non-defined in the world population according to the international ImMunoGeneTics information system® (IMGT) database.

Methods: We performed HLA-A, -B, -C, -DQB1, and DRB1 loci at the four-field resolution level, using Sanger- sequence-based typing (SBT) for 5592 healthy, unrelated bone marrow donor volunteers from Istanbul Province. The uncommon alleles were also confirmed using high-throughput next-generation sequencing (NGS).

Results: Uncommon alleles were determined at five loci as follows: HLA-A*01:155, 02:66, 02:90, 02:110, 02:343, 03:82, 24:28, 24:146, 24:276, 24:356, 31:23,33:33, 68:38; HLA-B *07:240, 18:19, 35:193, 40:303, 51:69, 51:169; HLA-C*04:39, 06:40, 07:93, 12:149, 15:73; HLA-DRB1*11:149, 13:14:02 and HLA-DQB1*03:27. All alleles were arranged according to the common and well-documented (CWD) 3.0.0 catalog.

Conclusion: This is the first study to show uncommon alleles in our population. These reported data increase the knowledge of HLA polymorphisms in the Turkish population and provide a basis for further studies in population genetics. This information may also be useful in determining whether a matched, unrelated donor is unlikely to be found so that a mismatch strategy, an extended family search, or alternate therapy, can be pursued, thus saving time and cost for patients.

Keywords: HLA; uncommon HLA alleles; next-generation sequencing; sequencing-based typing; HLA catalog

INTRODUCTION

Highly polymorphic human leucocyte antigen (HLA) class I and class II molecules are the key molecules for controlling the specificity of antigen presentation. They work with other sets of molecules located within the HLA region to process the antigen into peptides or other fragments [1-4]. HLA typing, such as sequence-specific oligonucleotide probe polymerase chain reaction (PCR-SSOP), sequence-specific primer (PCR-SSP), and sequence-based typing (SBT) are traditional "gold standard" assays are labor-intensive, costly, and relatively low throughput [5-7].

The next-generation sequencing (NGS) used as the routine clinical work of HLAs has led to the development of population-specific HLA typing data pools (Allele Frequency Net Database (AFND)) and better assessment of regional HLA specificity [8]. The introduction of NGS technologies, which provide clonal sequence information and may be used to determine phase over long stretches of DNA, has the potential to overcome many of the limitations of SBT, and 38,909 HLA and related alleles have been described [9].

Significant differences in the frequency of common and well-documented alleles (CWD) recently identified in Europe have been demonstrated. This number continues to increase with the discovery of new HLA alleles. In addition, new polymorphisms are occurring in HLA genes through point mutations, gene transformations, and fragment changes. New alleles may probably be detected at a low frequency in the population. HLA studies associated with viral agents suggest that rare HLA alleles confer a 'selective advantage' to the host against the virus [10,11]. Three catalogs for HLA alleles have been organized to date. The first catalog was prepared by ASHI in 2007 and revised by EFI in 2012 and updated to version 2.0.0. With these updates, alleles have been categorized as CWD. Accordingly, common alleles were defined as alleles with a frequency of over 0.001 observed in a population of at least 1500 individuals. Well-documented alleles are less frequent than common alleles, detected at least five times in the population or three times in a shared haplotype. Apart from these, an allele group is alleles that have been detected 1-3 times in the population after identification. The frequencies of these alleles are extremely low and have been termed non-CWD alleles. The latest updated version of the catalog was published by Hurley et al. It has been updated to version CWD 3.0.0 with the article published in January 2000 [12].

Main Points;

- Uncommon alleles detected in a population of Istanbul Province.
- The NGS technique will bring new changes and alleles.
- All alleles were arranged according to the CWD 3.0.0 catalog.

Türkiye has a genetically diverse population due to its geographic location and historical migration routes. However, comprehensive and high-resolution data on how this diversity is reflected in HLA allele distribution are limited. In this study, we aimed to identify rare HLA alleles in the Turkish population classified as uncommon, well-defined, and non-defined in the world population according to the international ImMunoGeneTics information system® (IMGT) database. Also, to provide detailed information on the frequency, genetic variation, and potential clinical significance of these alleles. An important lesson learnt from genetic studies in HIV is that viral replication is significantly inhibited by the immune system with less common or even rare HLA alleles.

The study was conducted retrospectively and included the typing results of 3 consecutive years (2016-2019). The data of different groups in the Turkish population : the first group used as a data source were unrelated volunteer donors from the Istanbul Bone Marrow Donor Registry and the second group was the healthy individuals.

The study includes the following objectives:
HLA allele diversity: To determine the diversity and frequency of rare HLA alleles belonging to HLA-A, HLA-B, HLA-C, HLA-DRB1 and HLA-DQB1 loci in the Turkish population.
Genetic Analysis: To perform comparative genetic analysis of the obtained HLA sequences with other known HLA alleles worldwide and to examine the genetic similarities and differences of the HLA profile of the Turkish population with other populations.
Creating a Database: In light of the data obtained, to create a database containing HLA alleles specific to the Turkish population and integrate these data into global HLA databases.

MATERIALS AND METHODS

Participants and Sample Collection
healthy individuals (n=5592) who voluntarily participated in

Istanbul University Istanbul Faculty of Medicine between 2016-2019 and gave informed consent were included in the study. Genomic DNA was isolated from blood samples obtained from the participants. DNA isolation was performed using a Qiagen EZ1 Advanced XL instrument and the isolated DNA samples were stored at -20°C.

HLA Typing

Sanger sequencing-based typing (Sanger-SBT) was used to determine HLA alleles. HLA typing was performed in the tissue typing laboratory at Istanbul Medical Faculty. This laboratory is accredited by the European Federation of Immunogenetics (EFI) for clinical HLA typing. The HLA loci and exons typed were as follows: HLA-A and HLA-B: Exons 1-5, HLA-C: Exon 1-7, HLA-DR Exon 2-3, HLA-DQ Exon 2-3.

Allele Detection and Data Analysis

In total, 11,184 HLA alleles were detected. Twenty-seven of these alleles were detected for the first time in the Turkish population. These rare alleles were evaluated using the IMGT database (version 3.55.0, 2024-01).

Verification with NGS

Confirmation of rare alleles was performed using NGS methods. Multiple long-range PCR primers were designed to amplify HLA-A, HLA-B, HLA-C, DRB1, and DQB1 genes from the promoter region to the 3'-UTR region for NGS typing. Omixon Holotype HLA™ kit was used for NGS and the One Lambda Secore Locus Sequencing Kit was used for Sanger sequencing.

Statistical Analysis

Descriptive statistics are given as mean±standard deviation for numerical variables and number for categorical variables. The SPSS for Windows version 21.0 package software was used for statistical analysis. The IMGT-HLA database system was used for the detection of uncommon alleles.

The uncommon alleles detected were analyzed in the IMGT database, aligned to the closest available allele sequence. This database enables the validation of newly identified HLA alleles by aligning them with the closest allele sequences already known and catalogued. This alignment is done to confirm the accuracy of the identified alleles and understand the genetic variation of the new alleles. In this way, it is indicated that the rare HLA alleles obtained in the study have been compared and validated against the closest similar allele sequences in the IMGT database.

RESULTS

Five thousand five hundred ninety-two volunteers were included in our study (F/M: 3256/2336; age range; 19-46 years; mean age 37.79 ± 9.2 years). Among the 11,184 alleles detected, the uncommon, well-defined, and non-CWD allele distributions were as follows: HLA-A*01:155, 02:66, 02:90, 02:110, 02:343, 03:82, 24:28, 24:146, 24:276, 24:356, 31:23,33:33, 68:38; HLA-B *07:240, 18:19, 35:193, 40:303, 51:69, 51:169; HLA-C*04:39, 06:40, 07:93, 12:149, 15:73; HLA-DRB1*11:149, 13:14:02, and HLA-DQB1*03:27.

When the whole HLA gene was amplified, we also detected nucleotide positions (Table 1). All alleles were arranged according to the CWD 3.0.0 catalog. When these alleles were also checked against the HLA-IMGT database, we found that most were confirmed by only one laboratory. The table contains the IMGT/HLA accession numbers, version number, the most closely matched allele information and nucleotide change, and exon numbers in which the change was detected for these alleles. All this information was obtained using the IMGT-HLA database (Table 1).

In our study, among the HLA alleles detected using Sanger and NGS methods, we identified alleles that were rarely detected in the Turkish population and in most populations. Although the separation force of some tools is more limited in Sanger sequencing, the allele can be precisely distinguished using NGS. According to the IMGT database, the ethnic groups of 10 alleles were determined, but nine were not determined (Table 2).

The characteristics of variation in HLA class I loci, possibly caused by single point mutations, are described in detail based on the sequences of alleles submitted to the IPD-IMGT/HLA database. We know that the frequency of HLA alleles in different populations reflects the evolutionary history of the population, and this effect is manifested in CWD allele frequencies.

The comparison of the 27 alleles we identified in our population with the information in the IMGT-HLA database showed that the well-defined alleles were generally identified in the Middle East and North Africa (MENA) and/or European (EURO) populations. Other alleles were those that have not been observed in other populations since they were first identified (Table 2). The name A*01:155 was officially assigned by the World Health Organization (WHO) Nomenclature Committee for Factors of the HLA System in March 2014. This follows the agreed policy that,

subject to the conditions stated in the most recent Nomenclature Report, names will be assigned to new sequences as they are identified. In August 2023, our Confirmatory HLA sequence (delivery number HWS10061798) was officially named by the WHO Nomenclature Committee for factors of the HLA System.

Table 1. IMGT/HLA accession numbers, version number, the most closely matched allele information and nucleotide change, exon numbers

	IMGT/HLA	IMGT/HLA 3.45.0			
Locus	**IPD Accession**	**Alel name**	**Closest match**	**Position/Base change**	**Exon**
A	HLA11389	A*01:155	A*01:01:01:01/ A*01:155	810 G>A	4
	HLA01781	A*02:66	A*02:01:01:01 / A*02:66	750 C>T	4
	HLA02374	A*02:90	A*02:01:01:01 / A*02:90	292 C>G	2
	HLA02776	A*02:110	A*02:01:01:01 / A*02:110	355 G>A	3
				362 G>T	3
				368 A>T	3
	HLA04893	A*02:243	A*02:01:01:01 / A*02:243	97 T>C	2
				98 T>C	2
	HLA05359	A*03:82	A*03:01:01:01 / A*03:82	411 C>T	3
				412 C>G	3
				413 G>A	3
				414 G>A	3
				418 G>C	3
				527 A>T	3
				538 T>C	3
				539 T>A	3
	HLA01268	A*24:28	A*24:02:01:01 / A*24:28	292 G>C	2
				299 A>G	2
				301 A>G	2
				307 C>G	2
				311 T>C	2
				313 G>C	2
				314 C>T	2
				317 T>G	2
				319 C>G	2
	HLA05673	A*24:146	A*24:02:01:01 / A*24:146	411 C>T	3
				412 C>A	3
	HLA10976	A*24:276	A*24:02:01:01 / A*24:276	209 A>G	2
	HLA15818	A*24:356	A*24:02:01:01 / A*24:356	349 C>T	3
	HLA03417	A*31:23	A*31:01:02:01 / A*31:23	811 G>A	4
	HLA05633	A*33:33	A*33:01:01:01 / A*33:33	463 C>A	3
				468 T>C	3

				583 C>T	3
	HLA02859	A*68:38	A*68:01:01:01 / A*68:38	102 C>T	2
				376 G>A	3
B	HLA12761	B*07:240	B*07:02:01:01 / B*07:240	301 A>G	2
				302 G>A	2
	HLA01782	B*18:19	B*18:01:01:01 / B*08:19	527 T>A	3
				538 C>T	3
				539 T>G	3
	HLA07692	B*35:193	B*35:01:01:01 / B*35:193	134 G>C	2
				419 C>T	3
	HLA13250	B*40:303	B*40:01:02:01 / B*40:303	103 G>T	2
				106 A>G	2
				363 G>C	3
				499 T>A	3
				512 T>G	3
				603 C>G	3
				605 A>C	3
				610 G>C	3
				618 T>G	3
				693 T>C	4
				959 T>C	5
				1008 T>C	5
	HLA03716	B*51:69	B*51:01:01:01 / B*51:69	475 G>A	3
	HLA11052	B*51:169	B*51:01:01:01 / B*51:169	670 A>T	4
C	HLA03735	C*04:39	C*04:01:01:01 / C*04:39	568 G>C	3
	HLA05184	C*06:40	C*06:02:01:01 / C*06:40	544 G>A	3
	HLA04399	C*07:93	C*07:01:01:01 / C*07:93	445 G>A	3
				912 C>T	5
	HLA13043	C*12:149	C*12:03:01:01/ C*12:149	173 T>C	2
		C*15:73	C*15:02:01:01 / C*15:73	454 G>C	3
DRB1	HLA10205	DRB1*11:149	DRB1*11:01:01:01 / DRB1*11:149	181 T>C	2
				189 A>G	2
				197 A>T	2
				429 C>G	3
	HLA01154	DRB1*13:14:02	DRB1*13:01:01:01/DRB1*13:14:02	181 C>T	2
				189 G>A	2
				196 A>T	2
				258 T>C	2
				261 C>T	2
				286 A>T	2

				298 G>A	2
				299 A>G	2
				344 T>G	3
				345 G>T	3
DQB1		DQB1*03:27	DQB1*03:01:01:01 / DQB1*03:27	343 T>C	3

Table 2. According to IMGT database alleles etnic group.

A*01:155	
A*02:66	
A*02:90	WD (NOT MENA)
A*02:110	WD (MENA, EURO)
A*02:243	
A*03:82	NONE
A*24:28	WD (EURO, HIS)
A*24:146	WD (MENA)
A*24:276	NONE
A*24:356	NONE
A*31:23	
A*33:33	WD (MENA)
A*68:38	WD (MENA)
B*07:240	NONE
B*18:19	WD (EURO,MENA)
B*35:193	WD (MENA)
B*40:303	NONE
B*51:69	WD (EURO, MENA)
B*51:169	NONE
C*04:39	WD (MENA)
C*06:40	NONE
C*07:93	WD (EURO, MENA)
C*12:149	WD (NOT MENA)
C*15:73	
DRB1*11:149	NONE
DRB1*13:14:02	WD
DQB1*03:27	NONE

MENA: Middle east and North Africa, EURO: Europian Population, HIS: Hispanic

DISCUSSION

The literature related to this subject give us an idea about how NGS will bring new changes and alleles to the field of HLA typing. Our data show that the diversity of HLA alleles and haplotypes is a result of the mixing of different populations in Türkiye. The coexistence of people from different populations will further increase the diversity of HLA alleles and haplotypes in the Turkish population in the coming years. As a result, it will be difficult to find suitable donors for human stem cell, tissue, and solid organ transplantation. Therefore, continuous updating of allele and haplotype frequency information will increase the accuracy and reliability of information about the immunogenic profile of the population. Thus, suitable donors can be found quickly, waiting lists for transplants can be shortened, and survival rates among recipients may be increased.

Today, using molecular methods, it has been shown to be possible to identify hundreds of different HLA alleles that differ in one or more nucleotides. Even single amino acid differences are known to cause immunological responses to donor antigens after haematopoietic stem cell transplantation. [12]. In our population, we found 27 well-defined and non CWD allele such as HLA-A*01:155, 02:66, 02:90, 02:110, 02:343, 03:82, 24:28, 24:146, 24:276, 24:356, 31:23, 33:33, 68:38; HLA-B *07:240, 18:19, 35:193, 40:303, 51:69, 51:169; HLA-C*04:39, 06:40, 07:93, 12:149, 15:73; HLA-DRB1*11:149, 13:14:02 and HLA-DQB1*03:27.

Kamenaric et al. identified six new HLA alleles (HLA-A*01:200, A*02:836, A*11:01:01:44, B*08:251, B*18:169 and C*05:46:01:02 and very rare (HLA-B*08:78, DRB1*12:39, DRB1*13:23:02 and DQB1*06:09:04) or rare (HLA-A*24:41, B*39:40:01N, B*51:78:01, DRB1*01:31 and DRB1*14: 111) in the Croatian population [13]. In another study conducted in the Croatian population, it was reported that eight HLA alleles from the ‘rare’ and ‘very rare’ categories were in the process of being sent to the rare allele database; A*02:11, one of these alleles, was also present among the rare alleles identified in our study. In a study conducted with donors registered in the DKMS (Deutsche Knochenmarkspenderdatei) database, it was reported that the 02:11 allele was also observed in Romanian, Bosnian,

Polish, Greek, Croatian, Turkish, and United States Hispanic populations [14]. The alleles A*02:11, A*68:38, B*18:19, B*51:69, C*04:39, C*07:93, C*15:73, DRB1*13:14, which were reported to be detected in Turkish donors in the allele frequency analysis of the same study, were similar to the rare alleles detected in our study [14].

The A*68:38 and DRB1*13:14 alleles detected in a study conducted with HLA-A, HLA-B, HLA-C, HLA-DRB1, HLA-DQB1 and HLA-DPB1 allele frequencies of Saudi stem cell donors were similar to the rare alleles detected in our study [15]. Analysis of the Buddhist Tzu Chi Stem Cell Center's (BTCSCC) HLA database of 291,677 bone marrow volunteer donors from 1993 to 2008, showed that 27 rare alleles were identified and six new alleles were discovered in Taiwan population. Rare alleles were confirmed using an SSP typing protocol and/or an SBT method [16]. The rare alleles detected in these studies have differences from the alleles detected in our study. Creating such databases by uncovering new/rare HLA alleles and haplotypes in each population will facilitate donor search and speed up the turnaround time of the procedure, as well as help transplant centers to quickly decide whether to search for an HLA-matched unrelated donor or cord blood unit or to proceed with a haploidentical donor [17]. In our study, we identified 11,184 alleles from 5592 volunteer donors. Of these alleles, we identified 13 HLA-A, six HLA-B, five HLA-C, three HLA-DR, and one HLA-DQ allele as well-defined or not yet categorized according to the IMGT database and CWD3.0.0 catalog.

Limitations

The characteristic of the study population, which is restricted to only the Marmara region, is the most important limitation.

CONCLUSIONS

Similar studies based on next-generation sequencing techniques, as in our study, are important in terms of documenting and recording possible new and rare alleles. These reported data increase the knowledge of HLA polymorphisms in the Turkish population and provide a basis for further studies in population genetics. This information may also be useful in determining that a matched unrelated donor is unlikely to be found so that a mismatch strategy, an extended family search or alternate therapy, can be pursued thus saving time and cost for the patient. The results of this study will contribute to a better understanding of the genetic structure of the Turkish population and the development of personalized medicine practices by providing important information in terms of both scientific and clinical applications. Furthermore, the identification of uncommon HLA alleles may significantly guide future genetic research and transplantation medicine.

Conflict of Interest: The authors report no conflicts of interest related to this study.

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Ethical Approval: This study was approved by the Ethics Committee of the Istanbul University Faculty of Medicine (Ethics no: 470) and in accordance with the standards of the Declaration of Helsinki.

Author Contributions: Yeliz Ogret provided technical support and materials and interpretation of the data, drafting of the paper. Suleyman Rustu Oguz provided the concept, design, interpretation of the data, drafting of the paper, and gave final approval. Hayriye Senturk Ciftci contributed to the concept and design, assembled the data, and helped with the data analysis. Sedat Karadeniz provided the data and statistical input. Demet Kivanc help with assembly of data with technical support. Fatma Savran Oğuz provided the concept drafting of the paper, and gave final approval.

REFERENCES

[1] Kelly A, Trowsdale J (2019) Genetics of antigen processing and presentation. Immunogenetics. 71:161-170. https://doi.org/10.1007/s00251-018-1082-2

[2] Robinson J, Halliwell JA, Hayhurst JD, Flicek P, Parham P, Marsh SGE (2015). The IPD and IMGT/HLA database: allele variant databases. Nucleic Acids Res. 43:423–31. https://doi.org/10.1093/nar/gku1161

[3] Daly AK (2013). Pharmacogenomics of adverse drug reactions. Genome Med. 5:5. https://doi.org/10.1186/gm409

[4] Gough SCL, Simmonds MJ (2007). The HLA region and autoimmune disease: associations and mechanisms of action. Curr Genomics. 8:453–65. https://doi.org/10.2174/138920207783591690

[5] Erlich H (2012). HLA DNA, typing: past, present, and future. Tissue Antigens 80:1–11. https://doi.org/10.1111/

j.1399-0039.2012.01881.x

[6] Dunn PPJ (2011). Human leucocyte antigen typing: techniques and technology, a critical appraisal. Int J Immunogenet. 38:463–73. https://doi.org/10.1111/j.1744-313X.2011.01040.x

[7] Seyhun Y, Kekik C, Karahan G, Oguz SF, Carin MN (2008). Undetected HLA-A,B Antigens by serological method and application of molecular methods. Gaziantep Med. Journal. 14:41-45. https://doi.org/10.58600/eurjther.2008-14-1-1357-arch

[8] Gonzalez-Galarza FF, McCabe A, Melo Dos Santos EJ, Takeshita L, Ghattaoraya G, Jones AR, Middleton D (2018). Allele Frequency Net Database.Methods Mol Biol. 1802:49-62. https://doi.org/10.1007/978-1-4939-8546-3_4.

[9] Robinson J, Barker DJ, Georgiou X, Cooper MA, Flicek P, Marsh SGE (2020). IPD-IMGT/HLA Database. Nucleic Acids Res. 8:948-955. https://doi.org/10.1093/nar/gkz950

[10] Kaur G, Mehra N. (2009). Genetic determinants of HIV-1 infection and progression to AIDS: immune response genes. Review Tissue Antigens.74:373-85.https://doi.org/10.1111/j.1399-0039.2009.01337.x

[11] Hurley CK, Kempenich J, Wadsworth K, Sauter J, Hofmann JA, Schefzyk D, Schmidt AH, Galarza P, Cardozo MBR, Dudkiewicz M, Houdova L, Jindra P, Sorensen BS, Jagannathan L, Mathur A, Linjama T, Torosian T, Freudenberger R, Manolis A, Mavrommatis J, Cereb N, Manor S, Shriki N, Sacchi N, Ameen R, Fisher R, Dunckley H, Andersen I, Alaskar A, Alzahrani M, Hajeer A, Jawdat D, Nicoloso G, Kupatawintu P, Cho L, Kaur A, Bengtsson M, Dehn J (2020). Common, intermediate and well-documented HLA alleles in world populations: CIWD version 3.0.0. HLA. 95:516-531. https://doi.org/10.1111/tan.13811

[12] Schreuder GM, Hurley CK, Marsh SG, Lau M, Fernandez-Vina M, Noreen HJ, et al. (2005). World Marrow Donor Associations Quality Assurance and IT Working Groups; WHO Nomenclature Committee for Factors of the HLA system; 13th International Histocompatibility Workshop Serology Component; International Cell Exchange, UCLA; US National Marrow Donor Program. The HLA Dictionary 2004: a summary of HLA-A, -B, -C, -DRB1/3/4/5 and -DQB1 alleles and their association with serologically defined HLA-A, -B, -C, -DR and -DQ antigens. Tissue Antigens. 65:1-55. https://doi.org/10.1111/j.1399-0039.2005.00350.x

[13] Burek Kamenaric M, Maskalan M, Grubic Z, Stingl Jankovic K, Zunec R (2020). Detection of novel and confirmation of very rare and rare HLA alleles by next generation sequencing in Croatia. HLA. 96:70-75. https://doi.org/10.1111/tan.13905

[14] Pingel J, Solloch UV, Hofmann JA, Lange V, Ehninger G, Schmidt AH. (2013). High-resolution HLA haplotype frequencies of stem cell donors in Germany with foreign parentage: how can they be used to improve unrelated donor searches? Hum Immunol. 74:330-40. https://doi.org/10.1016/j.humimm.2012.10.029

[15] Jawdat D, Uyar FA, Alaskar A, Müller CR, Hajeer A. (2020). HLA-A, -B, -C, -DRB1, -DQB1, and -DPB1 Allele and Haplotype Frequencies of 28,927 Saudi Stem Cell Donors Typed by Next-Generation Sequencing. Front Immunol. 22:11:544768. https://doi.org/10.3389/fimmu.2020.544768

[16] Yang KL, Chen MJ, Shyr MH, Lin CL, Lin PY. (2011). Rare HLA alleles and their predicted haplotypes in Tzu Chi Taiwanese marrow donor registry. Int J Immunogenet. 38(3):263-7. https://doi.org/10.1111/j.1744-313X.2010.00992.x

[17] Petersdorf EW (2013). The major histocompatibility complex: a model for understanding graft-versus-host disease. Blood. 12;122:1863-72. https://doi.org/10.1182/blood-2013-05-355982

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Original Research

Evaluating Dentists' Knowledge and Practices in the Use of Local Anesthetics and Unintended Effects in Pediatric Patients

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ABSTRACT

Objective: This cross-sectional study aimed to evaluate a group of Turkish dentists' awareness, preparedness, and competence regarding the unintended effects of LAs in pediatric patients.

Methods: In this study, the questionnaire form titled ' Evaluation of the Knowledge Level of Dentists Regarding the Use of Local Anesthesia and its Unintended Effects in Pediatric Patients' prepared in a digital environment was sent to dentists via Google survey application. The study investigated dentists' awareness and knowledge of the maximum dose of local anesthetic (LA) drugs. The most commonly used LA drugs and the most common complications related to these anesthetics were also determined.

Results: According to the results obtained, the dentists' most frequently preferred LA substances were Articaine+Adrenaline (A+A) and Lidocaine+Adrenaline (L+A). It was found that 91% of the participants performed aspiration before LA applications. It was found that 74% of the dentists participating in the study did not calculate the maximum dose per kilogram when performing local anesthesia in pediatric patients. The three complications encountered by the participating dentists during local anesthesia were found to be anesthetic failure (73%), facial paralysis (26%), and syncope (19%), respectively. In addition, 90% of the dentists who were asked about the first drug they would prefer in anaphylaxis answered adrenaline. When asked about the route of adrenaline injection, the majority of the dentists (64%) responded intramuscularly.

Conclusion: Although the occurrence of anaphylaxis during dental procedures is rare, when it does occur, it can lead to severe complications that may result in death. Dentists should be familiar with the signs of systemic complications that may arise from using LAs. When these findings are encountered after anesthesia, it should be considered that a systemic complication may have occurred, and urgent intervention should be performed. Any delay may cause consequences that may threaten the patient's life. This subject, which is of critical importance in dentistry, should be considered more in undergraduate and postgraduate education, and the level of knowledge should be increased by providing further training courses to update the information.

Keywords: survey, local anesthesia, complications, maximum dose

INTRODUCTION

Local anesthetic (LA) drugs have been primary agents for pain control in dentistry for year. There have been some concerns associated with them, including anesthesia-related complications. Therefore, dentists must precisely understand LA drugs' application method and dosage to minimize complications [1].

LA drugs primarily act by reducing the permeability of ion channels to Na+ ions on the nerve membrane [2]. The nerve cell membrane consists of lipid layers, which are hydrophobic barriers, and drugs with high lipid solubility have longer durations of action, potential, and rapid onset compared to those with low lipid solubility [3]. The concentration of anesthetic agents in nerve fibers also affects anesthesia efficacy. LA drugs are primarily classified as ester and amide according to their chemical structure. In the field of dentistry, amide-based anesthetics (Lidocaine, Mepivacaine, Bupivacaine, Articaine, and Prilocaine) are used extensively [4]. Lidocaine is particularly notable for its safety profile and high tolerability, and dentists also prefer articaine in Türkiye. Having comprehensive knowledge of the usage, pharmacokinetics, contraindications, and possible side effects of local anesthetics is crucial. It is equally important to keep this information up-to-date.

As with any invasive procedure, side effects can occur during local anesthesia applications [5]. These reactions can vary from local blanching to severe reactions such as anaphylactic shock and systemic toxicity [6]. Life-threatening hypersensitivity reactions are infrequent and are known to occur in less than 1% of cases [7]. In dentistry, LA drugs are primarily used with the addition of a vasoconstrictor. Severe and life-threatening toxic reactions result due to relatively high doses of LAs or the vasoconstrictor agent. Such reactions can be prevented with proper patient assessment and following dosage protocols before administering the LA agent [8]. LAs used in dentistry are minimal in dosage, and systemic effects after absorption are rare.

Main Points;

- Local anesthetics are the most used drugs in dentistry.
- Dentists may encounter various complications related to the use of local anaesthetics.
- Dentists should have adequate knowledge of local anesthetics, appropriate dose calculation, and management of complications.
- This subject is critical to dentistry and should be emphasized more in undergraduate and postgraduate education, with further training courses provided to update dentists' knowledge.

However, toxic effects may occur due to incorrect vascular injection and rapid increases in blood levels, especially in the pediatric population [9]. For this reason, the correct use of LA agents in dental clinics is crucial. Any dentist using an anesthetic agent must have sufficient knowledge about the dosage and content of the anesthetic used [8]. It is crucial for dentists to be aware of the possible permanent side effects of local anesthesia and have the necessary equipment to handle them [10].

Therefore, this cross-sectional study aimed to evaluate a group of Turkish dentists' awareness, preparedness, and competence regarding the unintended effects of LAs in pediatric patients. This study aims to assess dentists' knowledge of LA treatment protocols (e.g. dosage, complications), LA preference, experience with adverse events, and proficiency in managing anaphylactic attacks in pediatric patients.

MATERIAL AND METHODS

The ethical approval for the research was obtained from Selcuk University Faculty of Dentistry (Decision No: 2020/02, dated 13.02.2020). The digitally prepared questionnaire titled "Evaluation of the Knowledge Level of Dentists Regarding the Use of Local Anesthesia and its Unintended Effects in Pediatric Patients" was sent to participants via the Google Forms application. The volunteers participating in the study were evaluated anonymously, and no fee was requested from the participants. The questionnaire consisted of 16 questions. The first three questions represent "demographic" data, such as the age, gender, and title of the individuals filling out the questionnaire. The continuation of the questionnaire covers 13 questions related to local anesthesia application methods and complications (Table 1). The questions were prepared in multiple-choice or yes/no format. The survey was piloted on six dentists. Their feedback helped us to change some questions. Individuals who graduated from the dental faculty and voluntarily agreed to participate were included in the study. The participation period of the study was between 15.02.2020 and 03.11.2020.

The IBM SPSS 20.0 package program was used for statistical data analysis. Descriptive statistics were presented in terms of frequency and percentage values. Pearson's Chi-square Test or

Table 1. Survey Questions and Answers

QUESTIONS	ANSWERS
1-Gender?	**o Male** **o Female**
2-Age?	**o 23-30** **o 31-40** **o 41-50** **o 51 and over**
3-Title?	**o General Dentist** **o Pediatric Dentistry Resident** **o Pediatric Dentistry Specialist**
4-How many anesthetic syringes do you use maximum for local anesthesia in pediatric patients??	**o 1** **o 2** **o 3** **o 4**
5- Which local anesthetic do you most frequently use in the clinic?	**o Lidocaine+ Adrenaline** **o Articaine+ Adrenaline** **o Prilocaine** **o Others**
6-Do you aspirate before administering local anesthesia?	**o Yes** **o No**
7- Do you calculate the maximum local anesthetic dose/kg before administering in pediatric patients?	**o Yes** **o No**
8- Have you experienced any complications during local anesthetic applications?	**o Syncope** **o Needle breakage** **o Hematoma** **o Anaphylaxis** **o Facial Paralysis** **o Anesthetic insufficiency** **o Trismus** **o Others**
9- Do you inquire about any medication allergies your patient may have? *	**o Yes** **o No**
10- Do you ask your patient if she/he has had local anesthesia administered before?	**o Yes** **o No**
11- Do you perform a test dose of local anesthetic in your routine procedures?	**o Yes** **o No**
12- Have you experienced systemic toxicity due to local anesthetic administration?	**o Yes** **o No**
13- Which of the following symptoms would suggest systemic toxicity of local anesthetic?	**o Nausea-vomiting** **o Skin rash** **o Sweating** **o Hypotension** **o Respiratory distress**
14- Which of the following medications are available in your clinic?	**o Adrenaline** **o Antihistaminics** **o Steroids** **o Glucagon** **o Salbutamol**

15- Which is the first-choice medication for anaphylaxis?	**o Adrenaline** **o Antihistaminics** **o Steroids** **o Glucagon** **o Salbutamol**
16- Which route do you use for adrenaline injection?	**o Intramuscular** **o Intravenous** **o Subcutaneous** **o I do not know.**

Fisher's Exact Chi-square Test was used to compare variable groups. The significance level was accepted as α=0.05.

RESULTS

214 volunteer dentists, comprising 48 men and 166 women, participated in the study. 56% of the participants were general dentists (GD) (n = 120), 27% were pediatric dentistry residents (PDR) (n = 58), and 17% were pediatric dentistry specialists (PDS) (n = 36). The number of female participants in all groups was statistically significantly higher (GD: %68.3/31.7, PDR: %94.8/5.2, PDS: %80.6/19.4). The participants were divided into four age groups: 23-30, 31-40, 41-50, and ≥51. Their tenure was also categorized into 1-5 years, 6-15 years, and more than 16 years.

The comparison between groups regarding age and tenure revealed statistically significant differences ($p < 0.001$). The tenure of pediatric dentist specialists differed from that of other groups. Approximately 80% of specialists had a tenure of 6 years or more, while 87.5% of general dentists and 82.8% of residents had a tenure of at most five years.

Based on the survey results, the majority of participants preferred Articaine+Adrenaline (A+A) (64%) or Lidocaine+Adrenaline (L+A) (33%) for local anesthesia. (Figure 1). It was observed that there were proportional differences in the A+A preferences of dentists with different titles. Specifically, 82.8% of PDRs, 69.4% of PDSs, and 52.5% of GDs preferred A+A. Furthermore, the difference between the A+A preference of PDRs and GDs was found to be statistically significant ($p = 0.001$).

Most participants (60%) stated they used a maximum (max) of two ampoules of LAs for pediatric patients. This answer is not statistically significant between titles and groups. When asked about aspiration before local anesthesia, more than 90% of the participants reported that they aspirated. Again, the differences between titles and age groups regarding whether aspiration was performed before local anesthesia were not statistically significant.

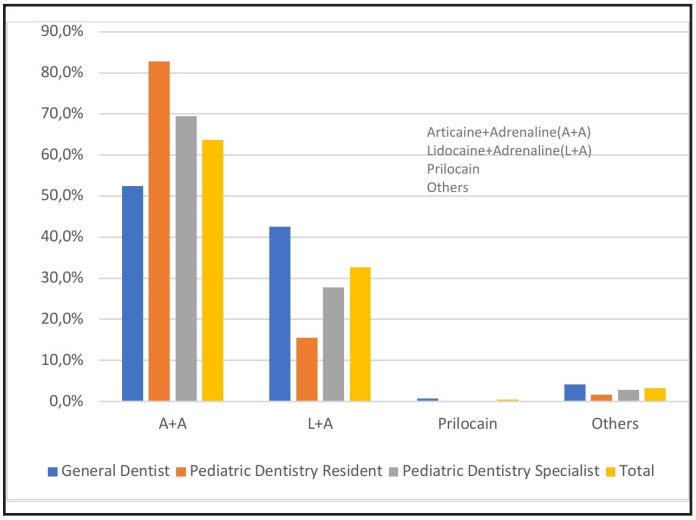

Figure 1. Local Anesthetics preference according to the titles

Most participants (74%) stated they did not calculate the dose per kilogram when performing local anesthesia. Response differences between all title groups were statistically insignificant ($p=0.138$). However, when we reduce the title groups to "PDS" and "Other Dentists," the difference between these two new groups becomes statistically significant, although it is very close to the limit value ($p = 0.047$). Accordingly, 39% of PDS state that they calculate the max dose per kilogram when applying local anesthesia to a pediatric patient, while this rate is 23% for other participants (Figure 2).

Most clinicians (96%) reported inquiring about drug allergies in patients before treatment. Participants were asked whether they question patients' previous local anesthesia experience, and 82% responded positively. The difference between dentists who asked their patients about their previous experience with local anesthesia was found to be statistically significant, with a p-value of 0.048. Interestingly, general dentists showed a 23% "No" response to this question, which was different from the responses

Table 2. Answers to the complications during local anesthesia applications.

Complications experienced during local anesthesia applications	The Whole Answers	PDS	PDR	GD
Anesthesia Failure	%73,0	%69,4	%86,2	%68,3
Facial paralysis	%25,6	%36,1	%36,2	%17,5
Syncope	%19,1	%25,0	%15,5	%19,2
Hematoma	%10,7	%25,0	%5,2	%9,2
Trismus	%5,1	%8,3	%5,2	%4,2
Emphysema	%0,9	-	-	%1,7
Angioedema on the lip	%0,5	%2,8	-	-
Urticaria	%0,5	%2,8	-	-
Short-term epileptic seizure-style convulsions	%0,5	%2,8	-	-
Redness	%0,5	-	%1,7	-
Paresthesia	%0,5	-	-	%0,8
Epileptic seizure	%0,5	-	-	%0,8
I did not experience any complications	%7,9	-	%3,4	%12,5

Pediatric Dentistry Specialists (PDS), Pediatric Dentistry Residents (PDR), General Dentists (GD)

of other groups (Figure 3).

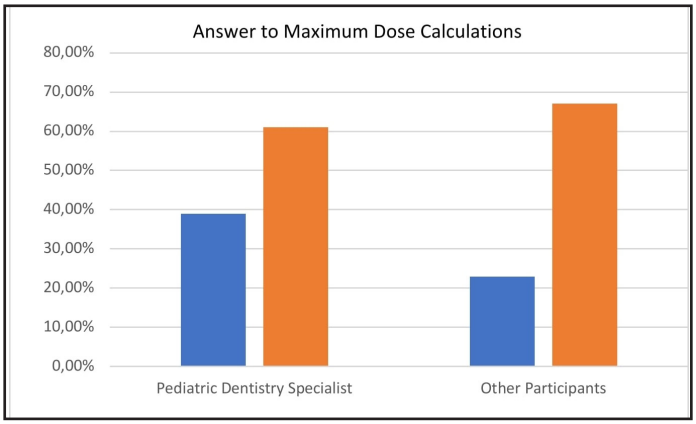

Figure 2. Answer to Maximum dose calculation rates for local anesthesia (p=0.047)

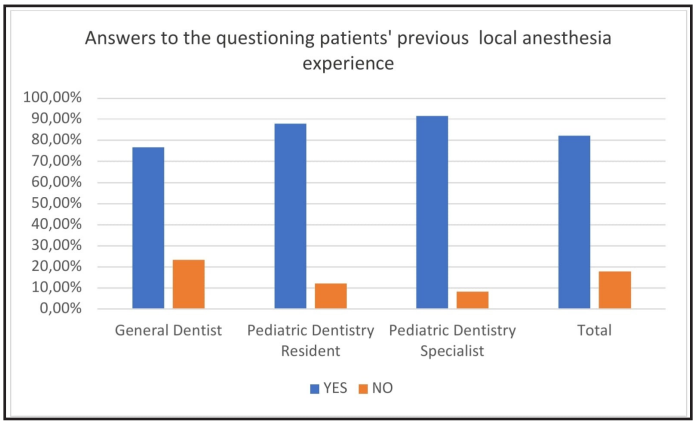

Figure 3. Answers to the questioning patients' previous local anesthesia experience (p=0.048).

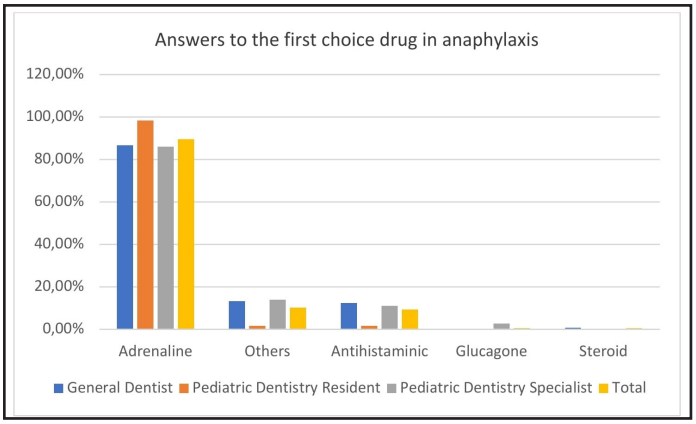

Figure 4. Answers to the first-choice drug in anaphylaxis (p=0.022)

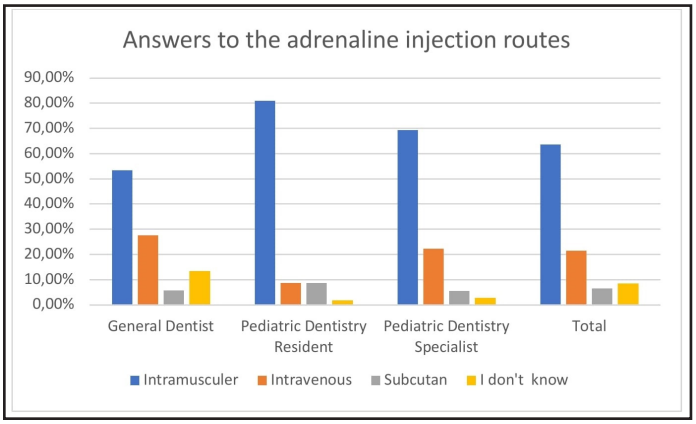

Figure 5. Answers to the adrenaline injection routes

Almost all (98%) participants answered 'No' to whether they performed LA test doses in their routine practice. Nearly all participants (99%) answered 'No' to the question about experiences with systemic toxicity due to LAs.

When asked about the first- line drug of choice for participants in anaphylaxis, the primary (90%) answer was 'adrenaline.' 10% of participants prefer different drugs as the first choice in anaphylaxis (9% Antihistaminic, 0.5% Glucagon, 0.5% Steroid). The preference for adrenaline was almost unanimous (98%) among PDR dentists, leading to a statistically significant difference in drug preference (p=0.022) (Figure 4).

When the question of what route they use for adrenaline, 63.6 % intramuscular (IM), 21.5 % intravenous (IV), and 6.5 % subcutaneous (SC), 8.4 of the participants marked the option of 'I do not know.'. Despite the IM response of 53.3 %of GD, PDR is 81.0 %, and PDS is 69.4 %. The difference between the answers given by dentists in different titles regarding the route used for adrenaline injection is statistically significant (p = 0.002). (Figure 5).

Participants were asked about the complications they encountered during local anesthesia applications. As a result of the answers, the three most common complications are anesthesia failure (73%), facial paralysis (26%) and syncope (19%). (Table 2) Another question asked what the symptoms suggestive of LA systemic toxicity were, and the answers were as follows: respiratory distress (72%), nausea-vomiting (69%), sweating (55%), hypotension (54%), and skin rash (40%). General dentists preferred the 'respiratory distress' most (76%), PDS preferred nausea and vomiting the most (72%), and likewise, PDR preferred nausea and vomiting the most (83%) (Figure 6).

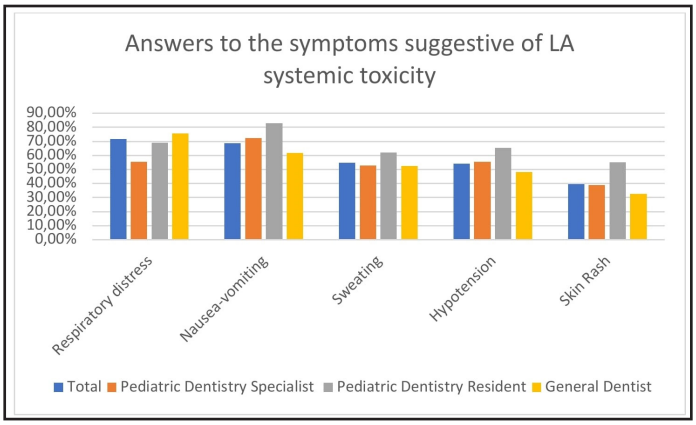

Figure 6. Answers to the symptoms suggestive of local anesthetic systemic toxicity

DISCUSSION

This survey study examined a group of Turkish dentists' knowledge of LA dosage, side effects, and managing side effects in pediatric patients.

A total of 214 volunteer dentists who are treating pediatric patients participated in our study. Bani-Hani et al. conducted a survey study to evaluate the use of LAs in pediatric dentistry. The study collected answers from 72 PDSs. The results showed that Lidocaine with 2% Adrenaline was the most used LA drug among the participants, with 72.2% of them using it. The second most frequently used drug was Articaine with 4% Adrenaline, used by 54.2% of the participants [11]. Ezzeldin et al [12], another survey study collected from 61 PDS, found that the most frequently used LAs were Lidocaine and Articaine. The reason for having more participants in our study might be due to the relatively fewer questions in our survey and the fact that we sent the survey to PDSs and dentists treating pediatric patients. In many studies, Articaine was found to be more effective than lidocaine in terms of anesthetic efficacy in dentistry [13, 14]. When the results found in our study are compared with the literature, it is like the results that Articaine is preferred more than Lidocaine. A reported study has found that newly qualified dentists more commonly use Articaine [15]. This may be due to the recent adoption of Articaine during undergraduate education in universities in Türkiye, as well as the fact that 82% of the participants in the study were between the ages of 23 and 30. Based on these results, it is seen that the differences in the guidelines adopted in different dental schools and in the professional periods between the dentists are effective in the selection of LAs. In a survey study, the maximum number of cartridges used by dentists was questioned, and it was reported that most dentists (87%) could not calculate the correct anesthetic dose [16]. In a survey conducted by Ngan et al., almost half of the respondents (49%) reported using full body weight to determine LA dosage. 44% of respondents reported using the estimated size of the patient, 2% the age of the patient, and 5% other methods such as 'one cartridge anesthetic dose to all patients [17]. In our study, 74% of the dentists surveyed do not calculate the dose per kilogram. The proportion of dentists who calculated the dose was 39% among PDS and 23% among other dentists. These results show similar results to other survey studies, revealing that dentists have insufficient knowledge about dose calculation when applying local anesthesia.

Baluga et al. reviewed 5018 case reports of dental treatment under local anesthesia. The study reported that local anesthesia-

related side effects were observed in only 25 cases (0.5%), and allergic reactions were observed in only two of these cases [18]. Cağıran et al. [19] reported that only one of 30 patients in whom allergy consultation was requested before intervention because of a history of atopic disease or drug allergy had a positive test result. Although rare in the literature, allergic reactions following LA injections may develop due to preservatives (methylparaben) or antioxidants (sulfites) in the solution [4]. If a patient describes a reaction that is at least clinically consistent with allergy, the dentist should refrain from using the offending substance until an allergist has assessed it. In this study, 96% of dentists stated that they asked their patients whether they had drug allergies before treatment. All PDS (100%), 97% of PDR, and 94% of GD responded positively to this question. Although cases of allergy due to local anesthesia are rare, it is essential to ask about allergy status during anamnesis before treatment, considering that complications that may develop may be fatal.

Aspiration before administration reduces the incidence of side effects of LAs. Accidental IV injection may cause acute overdose reactions [20]. According to Malamed, the inferior alveolar nerve block is the technique with the highest risk of positive aspiration [21]. Al-Wattar et al. [22] reported that 90% of the dentists participating in their survey study did not perform aspiration. Lipp et al. showed in their study that more than 60% of dentists were unaware of the toxicity that may occur due to positive aspiration [23]. This study found that 91% of dentists performed aspiration before LA applications. This higher aspiration rate than previous studies is due to dentists' increased knowledge and awareness.

In this study, the most common complication encountered by dentists was an anesthetic failure (73%). The causes of anesthesia failure include anesthetic technique and patient-specific factors. Failure to detect symptoms within 10-15 minutes after administration is considered anesthesia failure [24]. A double or bifid inferior alveolar nerve may be a possible cause of anesthetic failure [25]. Pulpitis or apical periodontitis can lead to anesthetic failure [26]. Infection lowers pH levels, affecting anesthetic dissociation, whereas inflammation heightens patient sensitivity by triggering a primary region of hyperesthesia [27]. According to Potonick and Bajrovic, inflammation causes anesthetic failure in 30-45% of cases, even when the technique is performed correctly [28]. Repeated anesthesia should be avoided in cases of inflammation and infection; repeated anesthetic applications may cause tachyphylaxis [29]. Many authors attribute the failure of LA to the need for more knowledge or experience from dentists. This situation can be prevented by correct anatomical knowledge and learning application techniques. In this study, most participants (73%) reported anesthetic failure. To solve this problem, dentists in Türkiye should be trained in anatomy and the correct application of LAs during their undergraduate and specialty education, and in-service training should be provided regularly throughout their careers.

LAs, frequently used in dentistry, account for most medical emergencies in the clinic. 70% of these are due to fear and stress, and 50% of the reported emergencies are syncope [21]. Das et al. [30] found 49% hematoma, 38% syncope, and 16% anesthetic failure as the most common complications after LA injection. According to the results of a study by Girdler and Smith, the most common emergency encountered by dentists was vasovagal syncope. Among the respondents, 63% reported that their patients had syncope in the last year [31]. In our study, syncope ranked third among the most common complications in the responses of PDS and PDR.GD reported syncope as the second most common complication. Although these results show a lower rate than previous studies, they clearly show that syncope is one of the most common complications.

Another complication frequently encountered by dentists in this study was facial paralysis. Temporary facial paralysis is usually caused by LA entering the capsule of the parotid gland at the posterior border of the mandibular ramus. Temporary facial paralysis after local anesthesia in this area will equal the duration of drug-related soft tissue anesthesia [32]. In this study, 26% of all dentists who participated encountered facial paralysis. The fact that facial paralysis, a preventable complication, occurs so frequently suggests that it is due to a lack of knowledge and attention.

In this study, dentists found trismus to be the fifth most common complication. The leading cause of trismus is trauma to muscles or blood vessels in the infratemporal space following dental anesthetic injections. This complication can be prevented by using short needles for posterior maxillary injections and avoiding multiple injections. Once acute trismus has developed, progression to chronic hypomobility can be prevented by a rapid treatment combination of heat, analgesics, muscle relaxants, and vigorous physiotherapy [33].

Adrenaline is the most essential drug in the treatment of

anaphylaxis. According to the guidelines published by the European Resuscitation Council (ERC) in 2015, side effects are rare when correct IM doses are used [34]. If the patient's condition does not improve within 5 minutes, the IM dose of adrenaline should be repeated. Dentists should be able to recognize and initiate the treatment of anaphylaxis. However, numerous studies in different countries show that most dentists cannot adequately identify and treat anaphylaxis [35]. In developed countries, the incidence of anaphylactic reactions to local anesthesia following dental procedures ranges from 1 in 3,500 to 1 in 13,000 [36]. Although rare, dentists should have the necessary knowledge and equipment to manage allergic reactions, as the consequences can be severe. In a study conducted by Krishnamurthy et al., it was learned that only 62% of dentists had emergency medicine kits in their clinics. According to the results, although 68% of dentists knew that epinephrine was the preferred treatment option for anaphylaxis, only 28% were aware of the route of administration [37]. In our study, 90% of the dentists who were asked about the first drug they would prefer in anaphylaxis answered adrenaline. When asked about the route they used for adrenaline injection, the majority (64%) responded intramuscularly. These results are also compatible with the literature.

The responses to the symptoms suggestive of LA systemic toxicity in our study were respiratory distress (72%), nausea and vomiting (69%), sweating (55%), hypotension (54%), and skin rash (40%). In a study of 593 cases of anaphylaxis, the most common symptoms were urticaria and angioedema (87%), shortness of breath/wheezing (59%), and hypotension (33%) [38]. Mortality in anaphylaxis most commonly occurs due to respiratory failure or cardiovascular collapse [39].

CONCLUSION

Although anaphylaxis during dental procedures is rare, it can lead to severe complications that may even result in death. Therefore, it is crucial for dentists to be familiar with the signs of systemic complications that may arise from using LAs. If such findings are encountered after anesthesia, it should be considered that a systemic complication may have occurred, and urgent intervention should be performed without any delay to prevent consequences that may threaten the patient's life. This subject is of critical importance in dentistry and should be emphasized more in undergraduate and postgraduate education, with further training courses provided to update the knowledge of dentists.

This study was presented as an oral presentation at the 2nd NEU International Dentistry Congress held in Konya on 1-3 October 2022 (https://www.erbakan.edu.tr/en/dishekimligi/haber/500254/2-uluslararasi-dis-hekimligi-kongresi-ve-fuari).

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Informed Consent: Informed consent form was obtained from the participants.

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Author Contributions:

Şükran ÖZ: Data Collection (%40) Data Analysis (%40).

Funda ARUN: Design of the Study | Study Design (%100) Data Collection (%60) Data Analysis (%60) Writing the Article | Writing (%50) / Article Submission and Revision (%50).

Enes Mustafa AŞAR: Writing the Article | Writing (%50) Submission and Editing (%50) .

REFERENCES

[1] Mathison M, Pepper T (2024) Local Anesthesia Techniques in Dentistry and Oral Surgery. StatPearls. StatPearls Publishing Copyright © Treasure Island (FL) https://www.ncbi.nlm.nih.gov/books/NBK580480/

[2] de Jong RH, Wagman IH (1963) Physiological mechanisms of peripheral nerve block by local anesthetics. Anesthesiology 24(5):684-695. https://doi.org/10.1097/00000542-196309000-00019

[3] Taylor A, McLeod G (2020) Basic pharmacology of local anaesthetics. BJA Education 20(2):34-41. https://doi.org/10.1016/j.bjae.2019.10.002

[4] Becker DE, Reed KL (2012) Local anesthetics: review of pharmacological considerations. Anesth Prog 59(2):90-102. http://dx.doi.org/10.2344/0003-3006-59.2.90

[5] Haas DA (2002) An update on local anesthetics in dentistry. J Can Dent Assoc 68(9):546-552. https://pubmed.ncbi.nlm.nih.gov/12366885/JCanDentAssoc.2002;68:546

[6] Finder RL, Moore PA (2002) Adverse drug reactions to local anesthesia. Dent Clin 46(4):747-757. https://doi.org/10.1016/S0011-8532(02)00018-6

[7] Cherobin ACFP, Tavares GT (2020) Safety of local anesthetics. An Bras Dermatol 95(1):82-90. https://doi.org/10.1016/j.abd.2019.09.025

[8] Moore PA, Hersh EV (2010) Local anesthetics: pharmacology and toxicity. Dent Clin 54(4):587-599. https://doi.org/10.1016/j.cden.2010.06.015

[9] Dontukurthy S, Tobias JD (2021) Update on Local Anesthetic Toxicity, Prevention and Treatment During Regional Anesthesia in Infants and Children. J Pediatr Pharmacol Ther 26(5):445-454. https://doi.org/10.5863/1551-6776-26.5.445

[10] Ho J-PT, van Riet TC, Afrian Y, Sem KTCJ, Spijker R, de Lange J, Lindeboom JA (2021) Adverse effects following dental local anesthesia: a literature review. J Dent Anesth Pain Med 21(6):507. https://doi.org/10.17245/jdapm.2021.21.6.507

[11] Bani-Hani T, Al-Fodeh R, Tabnjh A, Leith R (2024) The Use of Local Anesthesia in Pediatric Dentistry: A Survey of Specialists' Current Practices in Children and Attitudes in Relation to Articaine. Int J Dent 2024. https://doi.org/10.1155/2024/2468502

[12] Ezzeldin M, Hanks G, Collard M (2020) United Kingdom pediatric dentistry specialist views on the administration of articaine in children. J Dent Anesth Pain Med 20(5):303-312. https://doi.org/10.17245/jdapm.2020.20.5.303

[13] Nagendrababu V, Pulikkotil S, Suresh A, Veettil S, Bhatia S, Setzer F (2019) Efficacy of local anaesthetic solutions on the success of inferior alveolar nerve block in patients with irreversible pulpitis: a systematic review and network meta-analysis of randomized clinical trials. Int Endod J 52(6):779-789. https://doi.org/10.1111/iej.13072

[14] St George G, Morgan A, Meechan J, Moles DR, Needleman I, Ng YL, Petrie A (2018) Injectable local anaesthetic agents for dental anaesthesia. Cochrane Database Syst Rev(7). https://doi.org/10.1002/14651858.CD006487.pub2

[15] Corbett I, Ramacciato J, Groppo F, Meechan J (2005) A survey of local anaesthetic use among general dental practitioners in the UK attending postgraduate courses on pain control. Br Dent J 199(12):784-787. http://dx.doi.org/10.1038/sj.bdj.4813028

[16] Khalil H (2014) Local anesthetics dosage still a problem for most dentists: A survey of current knowledge and awareness. Saudi J Dent Res 5(1):49-53. https://doi.org/10.1016/j.ksujds.2013.08.002

[17] Ngan K, Richard Crout D, Linscott P (2001) A survey of local and topical anesthesia use by pediatric dentists in the United States. Pediatr Dent 23(3):265-269. https://www.aapd.org/globalassets/media/publications/archives/kohli-23-03.pdf

[18] Baluga JC (2003) Allergy to local anesthetics in dentistry. Myth or reality? Rev Alerg Mex 50(5):176-181. https://pubmed.ncbi.nlm.nih.gov/14631588/

[19] Cagiran E, Efeoglu C, Koca H, Balcıoğlu T (2013) Alergic reactions to local anaesthetics in dentistry. Cumhuriyet Dent J 16(1):1-7. https://doi.org/10.7126/cdj.2012.1030

[20] do Egito Vasconcelos BC, de Miranda Freitas KC, Canuto MR (2008) Frequency of positive aspirations in anesthesia of the inferior alveolar nerve by the direct technique. CEP 54753901. https://core.ac.uk/download/pdf/93038433.pdf

[21] Malamed SF (2004) Handbook of local anesthesia. Elsevier Brasil

[22] Al-Wattar WT, HAMID RS, FATHIE WK (2002) Aspiration before local anesthetic deposition: Its importance in dental practice. Al-Rafidain Dent J 2(1):108-111. http://dx.doi.org/10.33899/rden.2002.165923

[23] Lipp M, Fuder H, Dick W, Stanton-Hicks M, Daubländer M (1993) Exogenous and endogenous plasma levels of epinephrine during dental treatment under local anesthesia. Reg Anesth Pain Med 18(1):6-12. https://pubmed.ncbi.nlm.nih.gov/8448101/

[24] Parirokh M, Abbott PV (2022) Present status and future directions—Mechanisms and management of local anaesthetic failures. Int Endod J 55(S4):951-994. https://doi.org/10.1111/iej.13697

[25] Wolf KT, Brokaw EJ, Bell A, Joy A (2016) Variant inferior

alveolar nerves and implications for local anesthesia. Anesth Prog 63(2):84-90. http://dx.doi.org/10.2344/0003-3006-63.2.84

[26] Fleury A (1990) Local anesthesia failure in endodontic therapy: the acute inflammation factor. Compendium 11(4):210, 212, 214 passim-210, 212, 214 passim. https://pubmed.ncbi.nlm.nih.gov/2201444/

[27] Djoric J, Djinic Krasavcevic A, Barac M, Kuzmanovic Pficer J, Brkovic B, Nikolic-Jakoba N (2023) Efficacy of intraseptal anesthesia obtained by computer-controlled articaine with epinephrine delivery in scaling and root planing. Clin Oral Investig 27(6):2913-2922. https://doi.org/10.21203/rs.3.rs-2201327/v1

[28] Potočnik I, Bajrović F (1999) Failure of inferior alveolar nerve block in endodontics. Dent Traumatol 15(6):247-251. https://doi.org/10.1111/j.1600-9657.1999.tb00782.x

[29] Vandermeulen E (2000) Pain perception, mechanisms of action of local anesthetics and possible causes of failure. Rev Belge Med Dent 55(1):29-40. https://pubmed.ncbi.nlm.nih.gov/11039281/

[30] Das S, Govind S, Jena A (2019) Knowledge and Attitude Regarding Local Anesthesia among Dental Professionals and Awareness in General Population. Indian J Public Health 10(11):831. http://dx.doi.org/10.5958/0976-5506.2019.03591.5

[31] Girdler N, Smith D (1999) Prevalence of emergency events in British dental practice and emergency management skills of British dentists. Resusc 41(2):159-167. https://doi.org/10.1016/S0300-9572(99)00054-4

[32] Sisk AL, Hammer WB, Shelton DW, Joy ED (1986) Complications following removal of impacted third molars: the role of the experience of the surgeon. J Oral Maxillofac Surg 44(11):855-859. https://doi.org/10.1016/0278-2391(86)90221-1

[33] Decloux D, Ouanounou A (2021) Local anaesthesia in dentistry: a review. Int Dent J 71(2):87-95. https://doi.org/10.1111/idj.12615

[34] Truhlář A, Deakin CD, Soar J, Khalifa GEA, Alfonzo A, Bierens JJ, Brattebø G, Brugger H, Dunning J, Hunyadi-Antičević S (2015) European resuscitation council guidelines for resuscitation 2015: section 4. Cardiac arrest in special circumstances. Resusc 95148-201. https://doi.org/10.1016/j.resuscitation.2015.07.017

[35] Maher N, De Looze J, Hoffman G (2014) Anaphylaxis: an update for dental practitioners. Aust Dent J 59(2):142-148. https://doi.org/10.1111/adj.12161

[36] Vervloet D, Magnan A, Birnbaum J, Pradal M (1999) Allergic emergencies seen in surgical suites. Clin Rev Allergy Immunol 17459-467. http://dx.doi.org/10.1007/bf02737650

[37] Krishnamurthy M, Venugopal NK, Leburu A, Kasiswamy Elangovan S, Nehrudhas P (2018) Knowledge and attitude toward anaphylaxis during local anesthesia among dental practitioners in Chennai–a cross-sectional study. Clin Cosmet Investig Dent 10(2018):117-121. https://doi.org/10.2147/CCIDE.S159341

[38] Webb L, Greene E, Lieberman P (2004) Anaphylaxis: a review of 593 cases. J Allergy Clin Immunol 113(2):S240. https://doi.org/10.1016/j.jaci.2004.01.324

[39] Oswalt ML, Kemp SF (2007) Anaphylaxis: office management and prevention. Immunol Allergy Clin North Am 27(2):177-191. https://doi.org/10.1016/j.iac.2007.03.004

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Original Research

Comorbidity of Body Dysmorphic Disorder and Obsessive-Compulsive Disorder in Orthognathic Surgery Patients

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ABSTRACT

Objective: Body dysmorphic disorder (BDD) is a mental health disorder in which a person believes that their actual physical features appear worse than they truly are. The aim of this study is to examine the presence of BDD and its comorbidity with anxiety, depression, and obsessive-compulsive disorder (OCD) in patients with planned orthognathic surgery for dentofacial deformities.

Methods: In this study conducted on patients scheduled for orthognathic surgery, the presence of BDD, OCD, depression, anxiety, and stress was determined by administering the Florida Obsessive-Compulsive Inventory (FOCI), Depression, Anxiety, and Stress Scale-21 (DASS-21), and Body Image Disturbance Questionnaire (BIDQ) prior to the surgery. An independent samples t-test evaluated differences between group means, Pearson's correlation coefficient demonstrated the linear relationship between two variables, and the chi-square test assessed the relationship between two categorical variables. A p-value below 0.05 was deemed statistically significant. A total of 108 patients were included in the study, with 54 patients Class 2 and 54 patients Class 3.

Results: BIDQ, FOCI, depression, anxiety, and stress, males showed statistically significant higher results compared to females ($p < 0.05$). There were no statistically significant differences in BIDQ, FOCI, depression, and stress between Class 2 and Class 3 patients ($p > 0.05$). Of the 108 patients included in the study, a total of 20.4% (n=22) were found to be BDD positive. Among the female patients, 10% (n=6) were BDD positive, while among the male patients, 33.3% (n=16) were BDD positive. When evaluated in terms of skeletal classification, 14.8% (n=8) of Class 2 patients were BDD positive, while 25.9% (n=14) of Class 3 patients were BDD positive.

Conclusions: Surgeons performing orthognathic surgery should be familiar with common and often severe body image disturbances. When evaluating patients seeking orthognathic surgery, their psychological conditions should be carefully considered.

Keywords: Orthognathic surgery; Body dysmorphic disorder; Obsessive-compulsive disorder

INTRODUCTION

Body dysmorphic disorder (BDD) is a mental health disorder in which a person believes that their actual physical features appear worse than they truly are [1, 2] In this condition, the individual perceives themselves as excessively ugly, shapeless, or distorted, and this belief can negatively impact their social life, work life, and daily activities [3]. People with this disorder worry that these flaws will be noticed, mocked, or criticized by others. As a result, many individuals may limit or avoid social interactions, and they may lose their jobs or struggle academically [4, 5]. While BDD can be related to specific areas of the body such as the skin, hair, breasts, muscles, or genitalia, the face, which is a crucial aspect of individuals' social identity, tends to be the structure most affected by this disorder [1]. The prevalence of BDD in the general population ranges from 1% to 3%, but may be higher in some populations [1,4]. BDD can negatively impact social and occupational functioning and lead to other mental health problems such as depression and anxiety [5].

Individuals with BDD often seek general medical treatments, including surgery, to correct their perceived flaws [6]. However, in these cases, the person will still remain dissatisfied after treatment, and the disorder can progressively worsen and become more distressing [7]. Studies in the literature have reported a higher prevalence of BDD in patients with severe dentofacial deformities requiring orthognathic surgery compared to individuals without dentofacial deformities [8,9]. Furthermore, there is evidence of a reverse relationship between the severity of BDD symptoms and satisfaction with orthognathic surgery outcomes [10]. However, the relationship between the type of dentofacial deformity and BDD has not been examined in the literature.

Main Points

- Body Dysmorphic Disorder (BDD) prevalence is significantly higher in orthognathic surgery patients compared to the general population.
- Male patients exhibited higher scores in BDD, OCD, depression, anxiety, and stress compared to female patients.
- Class 3 skeletal deformity patients showed higher anxiety levels compared to Class 2, but no significant difference in BDD, OCD, depression, and stress.
- Surgeons should consider the psychological conditions, including BDD, when evaluating patients for orthognathic surgery.

The aim of this study is to examine the presence of BDD and its comorbidity with anxiety, depression, and obsessive-compulsive disorder (OCD) in patients with planned orthognathic surgery for dentofacial deformities. Additionally, the study aims to identify differences based on the skeletal relationship of the jaws that constitute the dentofacial deformity.

MATERIALS AND METHODS

This prospective cohort study has been approved by the Tokat Gaziosmanpaşa University Clinical Research Ethics Committee with the registration number 22-KAEK-043. Written and verbal consent has been obtained from the patients in accordance with the Helsinki Declaration. The study was conducted on patients referred to the Department of Oral and Maxillofacial Surgery at Tokat Gaziosmanpaşa University Faculty of Dentistry for the correction of dentofacial deformities and who underwent orthognathic surgery.

Inclusion criteria: (I) Patients aged between 18 and 40 years, (II) Patients with dentofacial deformities, (III) Patients who have completed skeletal growth and development, (IV) Patients who perceive their problematic area to be only their mouth and jaws without complaints about other parts of their body, (V) Patients without chronic or metabolic diseases (VI) Patients without a history of trauma in the jaw and facial region, (VII) Patients without any substance use habits.

Exclusion criteria: (I) Patients with cleft palate or lip, (II) Patients with planned genioplasty only, (III) Patients with a pre-existing psychiatric diagnosis, (IV) Patients using psychiatric medications, (V) Patients with dentofacial deformities but without planned orthognathic surgery.

Scales Used in the Study

The included patients were given three separate self-assessment questionnaires prior to surgery: the Florida Obsessive-Compulsive Inventory (FOCI) [11]1989 a, the Depression, Anxiety, and Stress Scale-21 (DASS-21)[12], and the Body Image Disturbance Questionnaire (BIDQ) [13].

The BIDQ contains 7 questions, with 2 sections: A and B, excluding the 3rd and 4th questions. Each question in section

A has Likert-type options ranging from 1 (none) to 5 (extreme), while section B asks individuals to describe the topic mentioned in the question in their own words in written form. By summing the scores of the 7 included questions, a value between 7 and 35 is obtained. Additionally, this total score can be divided by the number of questions to obtain a single average value. In this study, patients with an average score of 3 or higher were considered positive for BDD. Higher scores indicate a negative body perception and severe negative impact on psychosocial functioning, while lower scores indicate the absence of a distressing issue in body image. It was necessary to have an evaluation from a specialist psychiatrist to assess patients' self-statements. Therefore, in the questions where patients provided written explanations, their statements were not taken into account in the evaluation [13].

The FOCI consists of 5 items and requires individuals to rate the severity of repetitive thoughts and behaviors over the past 7 days. Patients with an average score of 2 or higher on this questionnaire were considered positive for OCD [11]1989 a.

The DASS-21 questionnaire assesses depression, anxiety, and stress parameters within a single test. Participants can respond to the questions on a scale of 0 (never) to 3 (always). Based on the total score, patients are classified as having normal, mild, moderate, severe, or extremely severe levels of depression, anxiety, and stress [12].

Statistical Analysis

The statistical analysis was performed using IBM SPSS Version 26.0 software (IBM Statistical Package for the Social Sciences (SPSS) Version 26, SPSS Inc., IBM Co., Somers, NY). Descriptive analyses were conducted to provide information about the general characteristics of the groups. Continuous variables were presented as mean ± standard deviation, while categorical variables were presented as n (%). The normal distribution of the data was examined through skewness and kurtosis analyses. Data with skewness and kurtosis values between -3 and +3 were considered to have a normal distribution. Independent samples t-test was used to assess the significance of the difference between the means obtained from two independent groups. The strength of the linear relationship between two variables was demonstrated using Pearson's correlation coefficient. The relationship between two categorical variables was evaluated using the chi-square test. If the p-value was less than 0.05, the result was considered statistically significant.

RESULTS

A total of 108 patients were included in the study, with 54 patients Class 2 and 54 patients Class 3, based on inclusion and exclusion criteria. Among the patients, there were 60 female individuals with an average age of 21.48±3.61, and 48 male individuals with an average age of 21.63±3.11. The age, gender, and skeletal relationship status of the patients are presented in Table 1.

The average total BIDQ scores for females were found to be 11.97 ± 5.624, while for males, it was 14.73 ± 6.93. When examining the average total depression scores, females had a score of 4.03 ± 3.769, whereas males had a score of 7.71 ± 5.21. Looking at the average total anxiety scores, females had a score of 4.95 ± 4.69, while males had a score of 7.71 ± 4.959. As for the average total stress scores, females had a score of 5.95 ± 4.83, while males had a score of 9.23 ± 5.665. When examining the average total OCD scores, females had a score of 5.30 ± 4.72, whereas males had a score of 8.19 ± 4.945. It was observed that males had higher scores than females in all scales (Table 2). When analyzing the effect of gender on BDD, OCD, depression, anxiety, and stress, males showed statistically significant higher results compared to females ($p < 0.05$).

The average total BIDQ scores for Class 2 patients were found to be 12.56 ± 6.297, while for Class 3 patients, it was 13.83 ± 6.416. When examining the average total depression scores, Class 2 patients had a score of 4.93 ± 5.061, whereas Class 3 patients had a score of 6.41 ± 4.466. Looking at the average total anxiety scores, Class 2 patients had a score of 5.04 ± 4.99, while Class 3 patients had a score of 7.31 ± 4.762. As for the average total stress scores, Class 2 patients had a score of 6.78 ± 5.69, while Class 3 patients had a score of 8.04 ± 5.313. When examining the average total OCD scores, Class 2 patients had a score of 5.8 ± 5.152, whereas Class 3 patients had a score of 7.37 ± 4.783 (Table 3). According to this table, patients with Class 3 skeletal deformities had higher scores in all scales compared to patients with Class 2 skeletal deformities. When evaluating the examined parameters, there were no statistically significant differences in BIDQ, FOCI, depression, and stress between Class 2 and Class 3 patients ($p > 0.05$) (Table 3). There was only a statistically significant difference in anxiety between Class 2 and Class 3 patients ($p < 0.05$).

Of the 108 patients included in the study, a total of 20.4% (n=22) were found to be BDD positive. Among the female patients, 10% (n=6) were BDD positive, while among the male patients,

33.3% (n=16) were BDD positive. When evaluated in terms of skeletal classification, 14.8% (n=8) of Class 2 patients were BDD positive, while 25.9% (n=14) of Class 3 patients were BDD positive. Among the BDD positive female patients (n=6), it was observed that 16.7% (n=1) had Class 2 skeletal deformity, while 83.3% (n=5) had Class 3 skeletal deformity. On the other hand, among the BDD positive male patients (n=16), 43.7% (n=7) had Class 2 skeletal deformity, while 56.3% (n=9) had Class 3 skeletal deformity. There was a statistically significant difference between females and males in terms of BDD occurrence in individuals (p=0.003), while there was no statistically significant difference between Class 2 and Class 3 deformities (p=0.152) (Table 4).

The Pearson correlation test was used to examine the correlation between the investigated parameters, and the results are shown in Table 5. As a result, there was a statistically significant correlation between depression, anxiety, stress, FOCI, and BIDQ scores (p <0.001). There was a very high correlation between depression and anxiety (r=0.827) and stress (r=0.852), while there was a moderate correlation between BDD and depression (r=0.575), and a high correlation between OCD (r=0.741). There was a very high correlation between anxiety and stress (r=0.859), a moderate correlation between anxiety and BDD (r=0.502), and a high correlation between OCD (r=0.752). There was a moderate correlation between stress and BDD (r=0.502).

Table 1. Age, gender and skeletal relationship status of the patients

		Skeletal Relationship of the Jaws		Age (Mean±SD)	Total
		Class 2	Class 3		
Gender	**Female**	35	25	21.48±3.61	60
	Male	19	29	21.63±3.11	48
Total		54	54		108

Table 2. BIDQ, FOCI, DASS-21 scores by gender groups

						95% Confidence Interval of Difference		
	Gender	**n**	**Mean**	**SD**	**t**	**Lower**	**Upper**	**p**
BIDQ	Female	60	11.97	5.624	2.951	-0.391	-0.076	0.004
	Male	48	14.73	6.930				
Depression	Female	60	4.03	3.769	-3.514	-1.429	-0.396	0.001
	Male	48	7.71	5.210				
Anxiety	Female	60	4.95	4.699	-2.944	-1.569	-0.306	0.004
	Male	48	7.71	4.959				
Stress	Female	60	5.95	4.983	-3.373	-1.424	-0.368	0.001
	Male	48	9.23	5.665				
FOCI	Female	60	5.30	4.720	-3.573	-0.493	-0.140	0.001
	Male	48	8.19	4.945				

Table 3. BIDQ, FOCI, DASS-21 scores by skeletal relationship groups

	Gender	n	Mean	SD	t	95% Confidence Interval of Difference		p
						Lower	Upper	
BIDQ	Class 2	54	12.56	6.297	-1.434	-0.265	0.043	0.155
	Class 3	54	13.83	6.416				
Depression	Class 2	54	4.93	5.061	-1.202	-0.834	0.204	0.232
	Class 3	54	6.41	4.466				
Anxiety	Class 2	54	5.04	4.990	-2.925	-1.554	-0.298	0.004
	Class 3	54	7.31	4.762				
Stress	Class 2	54	6.78	5.699	-0.895	-0.774	0.292	0.373
	Class 3	54	8.04	5.313				
FOCI	Class 2	54	5.80	5.152	-1.440	-0.308	0.049	0.153
	Class 3	54	7.37	4.783				

Table 4. Examination of the presence of BDD according to gender and skeletal relationship status

		BDD Negative	BDD Positive	p
Gender	**Female**	54	6	0.003
	Male	32	16	
Skeletal Relationship	**Class 2**	46	8	0.152
	Class 3	40	14	
Total		86	22	

Table 5. Examining the correlation between BIDQ, FOCI and DASS-21 scores.

		Depression	Anxiety	Stress	BIDQ	FOCI
Rho	**Depression**	1.000	0.827	0.852	0.575	0.741
	Anxiety	0.827	1.000	0.859	0.502	0.752
	Stress	0.852	0.859	1.000	0.482	0.714
	BIDQ	0.575	0.502	0.482	1.000	0.566
	FOCI	0.741	0.752	0.714	0.566	1.000
p	**Depression**	-	0.000	0.000	0.000	0.000
	Anxiety	0.000	-	0.000	0.000	0.000
	Stress	0.000	0.000	-	0.000	0.000
	BIDQ	0.000	0.000	0.000	-	0.000
	FOCI	0.000	0.000	0.000	0.000	-

DISCUSSION

BDD is a diagnosis that encompasses distress caused by perceived physical abnormalities, such as a scar or the shape or size of a body part, or dissatisfaction with another personal characteristic [14]. Individuals with BDD have unrealistic views about their appearance, believing they appear abnormal or deformed even though they are not. Many of these patients seek cosmetic procedures, including orthognathic surgery, for perceived flaws in their appearance [15]. This condition can have significant negative impacts on mental health, because available data indicates that cosmetic treatments often do not improve BDD symptoms, and patients with BDD are typically disappointed with the outcomes [16].

Our study investigated BDD and psychological issues such as obsession, depression, anxiety, and stress that accompany this disorder in patients scheduled for orthognathic surgery. Similar to the existing literature, self-report questionnaires were used to assess these parameters. The scores obtained from the questionnaires were evaluated, and the relationship between the obtained data was examined.

Studies indicate that the prevalence of BDD is significantly higher among individuals seeking aesthetic surgeries compared to the general population [17-19]. The rate of BDD in cosmetic surgery patients ranges from 2.21% to 56.67%, with a notable predominance in females (approximately 74-76%) [20]. Moreover, comorbidity with Obsessive-Compulsive Disorder (OCD) is also higher in this population, with rates around 15% compared to 0.2-7% in the general population [21, 22].

Ribeiro evaluated the prevalence of BDD in plastic surgery and dermatology patients through a systematic review and meta-analysis [23]. The rate of BDD occurrence in plastic surgery patients was found to be 15.04% (ranging from 2.21% to 56.67%) with a mean age of 34.54 ± 12.4. Furthermore, the majority of BDD-positive patients were females (74.38%). When examining dermatology patients, the rate of BDD occurrence was 12.65% (ranging from 4.52% to 36.16%) with a mean age of 27.79 ± 9.03. Once again the majority of patients were females (76.09%) [23].

In a study conducted by Buhlmann et al.[24] in Germany, a total of 2510 patients were included. They examined patients who had symptoms of BDD according to the Diagnostic and Statistical Manual of Mental Disorders Fourth Edition (DSM-IV) using self-report questionnaires to assess suicidal tendencies and the prevalence of BDD in plastic surgery patients. The overall prevalence of BDD in the general population was found to be 1.8% (n=45). Additionally, individuals with BDD reported significantly higher rates of cosmetic surgery history (15.6% vs. 3.0%) compared to those without BDD as well as higher rates of suicidal thoughts (31.0% vs. 3.5%) and suicide attempts due to appearance concerns (22.2% vs. 2.1%) [24]. In another study conducted by Mortada et al.[25] on patients visiting a cosmetic and plastic surgery clinic, a total of 344 patients were included with 86% of them being female. The patients were asked to complete BIDQ, which has high sensitivity in diagnosing BDD. Among the included patients, 19.4% were found to be positive for BDD. The most commonly requested surgeries by the patients were abdominoplasty (21.2%) and removal of skin lesions (21.2%) [25].

The prevalence of BDD is higher in patients seeking orthognathic surgery compared to the general population [15]. When examining studies investigating BDD prevalence in patients seeking orthodontic and orthognathic surgery in the literature, it is observed that the rate of BDD is higher in female patients [26, 27]. However, there are studies that show a higher rate of BDD in male patients, contrary to this [28]. According to the results of our study, 20.4% (n=22) of the 108 patients who underwent orthognathic surgery were found to be positive for BDD. The rate of BDD in female patients was 10% (n=6), while in male patients, this rate was 33.3% (n=16).

Patients with BDD often have other psychological problems such as obsession, depression, and anxiety [29]. The co-occurrence of Axis I disorders, especially major depressive disorder and anxiety disorders, with BDD is associated with increased impairment in functioning and a higher rate of suicide attempts [30, 31]. In our study, when investigating OCD in patients with BDD, 72.7% (n=16) of BDD-positive patients were found to have OCD. Among the OCD-positive patients, 45% were also positive for BDD.

Patients with BDD often have three times or more Axis I disorders compared to other psychiatric outpatients [32]. In a study conducted by Hollander et al., major depression was found to occur in 68% of BDD-positive patients [33]. Another study by Phillips and Diaz [34] reported a major depression rate of 82% in BDD-positive patients. In our study, it was found that 68% of BDD-positive patients exhibited moderate to severe symptoms

of depression.

Many studies in the literature show a high correlation between BDD and anxiety [35]. Studies have demonstrated anxiety rates ranging from 12% to 68.8% in patients with BDD symptoms [36]. In our study, 50% of BDD-positive patients exhibited severe anxiety, while 72.7% exhibited moderate to severe anxiety. Another study examining stress levels in BDD-positive patients reported statistically significant higher stress levels in individuals with BDD [37].

When examining patients seeking aesthetic surgical treatments in the literature, it is often observed that women have higher rates of psychological distress. However, there are also studies that indicate a higher prevalence of psychological distress in male patients. In a study conducted by Nicodemo et al.[38] on 29 patients with Class III skeletal deformities who were planning orthognathic surgery, the patients self-assessed their self-esteem using the Rosenberg Self-Esteem Scale and filled out the Self-Report Scale before surgery and at 6 months post-surgery. At the end of the study, increased self-esteem and decreased depressive symptoms were observed in female patients following the surgical intervention, while no changes in self-esteem or depression were observed in male patients [38]. In our study, when evaluating the parameters according to gender, it is observed that males have higher scores in all parameters compared to females. In the study by Mortada et al., they reported that women seeking aesthetic surgery had a higher prevalence of psychological disorders compared to men [25]. This could be attributed to the fact that the majority of patients seeking aesthetic and cosmetic surgery are women. However, more comprehensive studies with larger sample sizes and minimal confounding factors are needed to investigate the effect of gender on psychological disorders.

Contrary to the findings of our study, many studies in the literature have reported that levels of depression, anxiety, and stress are higher in female compared to male [39-41]. There are several potential reasons for the discrepancy between our results and those reported in the literature. Firstly, the impact of psychological conditions may vary across different age groups. Secondly, the cultural context of the society in which our study was conducted may influence how male express emotional and psychological difficulties. Male may be more reticent in expressing emotional problems and seeking help compared to female, leading to higher levels of depression and anxiety. Thirdly, the structure of the survey questions, the data collection process, and the analysis methods may have influenced our findings. Male might express themselves more openly and clearly in such surveys, which could result in differing outcomes.

Limitations

Our study had certain limitations. The evaluation of deformity severity was not included for Class 2 and Class 3 patients in our study. Additionally, a version of the Body Image Disturbance Questionnaire (BIDQ) adapted specifically for the maxillofacial region would allow for a more sensitive and accurate assessment. Furthermore, a comprehensive clinical and radiological evaluation by a specialist psychiatrist is necessary to diagnose psychological disorders such as BDD, OCD, depression, anxiety, and stress. Self-report questionnaires alone are not sufficient for making a diagnosis. In studies like these, it would be more ideal to consult a psychiatric specialist for patients exhibiting signs of psychological disorders such as BDD, OCD, depression, anxiety, and stress, and conduct further clinical evaluations.

CONCLUSION

In conclusion, this study examined the conditions of BDD, OCD, depression, anxiety, and stress in patients with Class 2 and Class 3 malocclusions undergoing orthognathic surgery. The prevalence rate of BDD was 20.4%, OCD rate was 32.4%. These results indicate a higher incidence of psychological problems in patients scheduled for orthognathic surgery compared to the general population.

Although Class 3 patients had higher scores in all parameters compared to Class 2 patients, no statistically significant difference was found between Class 2 and Class 3 patients in terms of BDD, OCD, depression, and stress conditions. Only anxiety was significantly higher in Class 3 patients. When examining the impact of gender on BDD, OCD, depression, anxiety, and stress, males exhibited statistically significantly higher results than females.

Therefore, surgeons performing orthognathic surgery should be familiar with common and often severe body image disturbances. Patients with BDD have unrealistic beliefs about their appearance, thinking they look abnormal or deformed when they are not. Many of these patients seek cosmetic procedures, including orthognathic surgery, for perceived aesthetic flaws. When evaluating patients seeking orthognathic surgery, their psychological conditions should be carefully considered. The patient's expectations should be thoroughly analyzed before

the operation, and the potential changes in their appearance and functional movements brought about by the surgery should be realistically explained. Future multidisciplinary studies involving maxillofacial surgeons, psychiatrists, and psychologists in larger patient populations will be useful in determining BDD and OCD comorbidities in the orthognathic surgery patient population.

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

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Patient consent: In this study conducted within the scope of the Decleration of Helsinki, written and verbal consent was obtained from the patients.

Ethics statement

This prospective cohort study has been approved by the Tokat Gaziosmanpaşa University Clinical Research Ethics Committee with the registration number 22-KAEK-043. Written and verbal consent has been obtained from the patients in accordance with the Helsinki Declaration.

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REFERENCES

[1] Bjornsson AS, Didie ER, Phillips KA (2010) Body dysmorphic disorder. Dialogues Clin Neurosci. 12(2):221–32. https://doi.org/10.31887/DCNS.2010.12.2/abjornsson

[2] Hong K, Nezgovorova V, Uzunova G, Schlussel D, Hollander E (2019) Pharmacological Treatment of Body Dysmorphic Disorder. Curr Neuropharmacol. 17(8):697–702. https://doi.org/10.2174/1570159X16666180426153940

[3] IsHak WW, Bolton MA, Bensoussan J-C, Dous G V, Nguyen TT, Powell-Hicks AL, et al. (2012) Quality of life in body dysmorphic disorder. CNS Spectr. 17(4):167–75. https://doi.org/10.1017/S1092852912000624

[4] Phillips KA (2009) Understanding body dysmorphic disorder. Oxford University Press, Oxford

[5] Ayub N, Kimong PJ, Ee GT (2018) A distorted body image: cognitive behavioral therapy for body dysmorphic disorder. In: Cognitive Behavioral Therapy-Theories and Applications, IntechOpen

[6] Greenberg JL, Weingarden H, Wilhelm S (2019) A practical guide to managing body dysmorphic disorder in the cosmetic surgery setting. JAMA Facial Plast Surg. 21(3):181–2. https://doi.org/10.1001/jamafacial.2018.1840

[7] Veale, D. (2014). Shame in body dysmorphic disorder. In Body Shame, Routledge, pp. 267-282

[8] Collins B, Gonzalez D, Gaudilliere DK, Shrestha P, Girod S (2014) Body dysmorphic disorder and psychological distress in orthognathic surgery patients. J Oral Maxillofac Surg. 72(8):1553–8. https://doi.org/10.1016/j.joms.2014.01.011

[9] Cunningham SJ, Feinmann C (1998) Psychological assessment of patients requesting orthognathic surgery and the relevance of body dysmorphic disorder. Br J Orthod. 25(4):293–8. https://doi.org/10.1093/ortho/25.4.293

[10] Kiyak HA, McNeill RW, West RA, Hohl T, Heaton PJ (1986) Personality characteristics as predictors and sequelae of surgical and conventional orthodontics. Am J Orthod. 89(5):383–92. https://doi.org/10.1016/0002-9416(86)90069-2

[11] Storch EA, Kaufman DAS, Bagner D, Merlo LJ, Shapira NA, Geffken GR, et al (2007) Florida Obsessive-Compulsive Inventory: development, reliability, and validity. J Clin Psychol. 63(9):851–9. https://doi.org/10.1002/jclp.20382

[12] Henry JD, Crawford JR (2005) The short-form version of the Depression Anxiety Stress Scales (DASS-21): construct validity and normative data in a large non-clinical sample. Br J Clin Psychol. 44(2):227–39. https://doi.org/10.1348/014466505X29657

[13] Cash TF, Phillips KA, Santos MT, Hrabosky JI (2004) Measuring "negative body image": validation of the Body Image Disturbance Questionnaire in a nonclinical population. Body Image. 1(4):363-372. https://doi.org/10.1016/j.bodyim.2004.10.001

[14] American Psychiatric Association D, Association AP (2013) Diagnostic and statistical manual of mental disorders: DSM-5. American psychiatric association Washington, DC

[15] Vulink NCC, Rosenberg A, Plooij JM, Koole R, Bergé SJ, Denys D (2008) Body dysmorphic disorder screening in maxillofacial outpatients presenting for orthognathic surgery. Int J Oral Maxillofac Surg. 37(11):985–91. https://doi.org/10.1016/j.ijom.2008.06.005

[16] Aouizerate B, Pujol H, Grabot D, Faytout M, Suire K, Braud C, et al (2003) Body dysmorphic disorder in a sample of cosmetic surgery applicants. Eur Psychiatry. 18(7):365–8. https://doi.org/10.1016/j.eurpsy.2003.02.001

[17] Dufresne Jr RG, Phillips KA, Vittorio CC, Wilkel CS (2001) A screening questionnaire for body dysmorphic disorder in a cosmetic dermatologic surgery practice. Dermatologic Surg. 27(5):457–62. https://doi.org/10.1046/j.1524-4725.2001.00190.x

[18] Crerand CE, Sarwer DB, Magee L, Gibbons LM, Lowe MR, Bartlett SP, et al (2004) Rate of body dysmorphic disorder among patients seeking facial plastic surgery. Psychiatric Annals. 34(12):958–65. https://doi.org/10.3928/0048-5713-20041201-19

[19] Picavet V, Gabriëls L, Jorissen M, Hellings PW (2011) Screening tools for body dysmorphic disorder in a cosmetic surgery setting. Laryngoscope. 121(12):2535–41. https://doi.org/10.1002/lary.21728

[20] Rosten A, Cunningham S, Newton JT (2018) Body dysmorphic disorder: a guide to identification and management for the orthodontic team. J Orthod. 45(3):163–8. https://doi.org/10.1080/14653125.2018.1490874

[21] Pavan C, Simonato P, Marini M, Mazzoleni F, Pavan L, Vindigni V (2008) Psychopathologic aspects of body dysmorphic disorder: a literature review. Aesthetic Plast Surg. 32(3):473–84. https://doi.org/10.1007/s00266-008-9113-2

[22] Hart AS, Phillips KA (2013) Symmetry concerns as a symptom of body dysmorphic disorder. J Obsessive Compuls Relat Disord. 2(3):292–8. https://doi.org/10.1016/j.jocrd.2013.04.004

[23] Ribeiro RVE (2017) Prevalence of body dysmorphic disorder in plastic surgery and dermatology patients: a systematic review with meta-analysis. Aesthetic Plast Surg. 41(4):964–70. https://doi.org/10.1007/s00266-017-0869-0

[24] Buhlmann U, Glaesmer H, Mewes R, Fama JM, Wilhelm S, Brähler E, et al (2010) Updates on the prevalence of body dysmorphic disorder: a population-based survey. Psychiatry Res. 178(1):171–5. https://doi.org/10.1016/j.psychres.2009.05.002

[25] Mortada H, Seraj H, Bokhari A (2020) Screening for body dysmorphic disorder among patients pursuing cosmetic surgeries in Saudi Arabia. Saudi Med J. 41(10):1111-20. https://doi.org/10.15537/smj.2020.10.25380

[26] Hepburn S, Cunningham S (2006) Body dysmorphic disorder in adult orthodontic patients. Am J Orthod Dentofac Orthop. 130(5):569–74. https://doi.org/10.1016/j.ajodo.2005.06.022

[27] Yassaei S, Moghadam MG, Aghili H, Tabatabaei SM (2014) Body dysmorphic disorder in Iranian orthodontic patients. Acta Med Iran. 52(6):454–7

[28] Sathyanarayana HP, Padmanabhan S, Balakrishnan R, Chitharanjan AB (2020) Prevalence of Body Dysmorphic Disorder among patients seeking orthodontic treatment. Prog Orthod. 21(1):1–5. https://doi.org/10.1186/s40510-020-00322-8.

[29] Otto MW, Wilhelm S, Cohen LS, Harlow BL (2001) Prevalence of body dysmorphic disorder in a community sample of women. Am J Psychiatry. 158(12):2061–3. https://doi.org/10.1176/appi.ajp.158.12.2061

[30] Phillips KA, Menard W, Fay C, Pagano ME (2005) Psychosocial functioning and quality of life in body dysmorphic disorder. Compr Psychiatry. 46(4):254–60. https://doi.org/10.1016/j.comppsych.2004.10.004

[31] Phillips KA, Menard W, Fay C, Weisberg R (2005) Demographic characteristics, phenomenology, comorbidity, and family history in 200 individuals with body dysmorphic disorder. Psychosomatics. 46(4):317–25. https://doi.org/10.1176/appi.psy.46.4.317

[32] Zimmerman M, Mattia JI (1998) Body dysmorphic disorder in psychiatric outpatients: recognition, prevalence, comorbidity, demographic, and clinical correlates. Compr Psychiatry. 39(5):265–70. https://doi.org/10.1016/s0010-440x(98)90034-7

[33] Hollander E, Cohen LJ, Simeon D (1993) Body dysmorphic disorder. Psychiatric Annals. 23(7):359–64. https://doi.org/10.3928/0048-5713-19930701-06

[34] Phillips KA, Diaz SF (1997) Gender differences in body dysmorphic disorder. J Nerv Ment Dis. 185(9):570–7. https://doi.org/10.1097/00005053-199709000-00006

[35] Fang A, Hofmann SG (2010) Relationship between social anxiety disorder and body dysmorphic disorder. Clin Psychol Rev. 30(8):1040–8. https://doi.org/10.1016/j.cpr.2010.08.001

[36] Coles ME, Phillips KA, Menard W, Pagano ME, Fay C, Weisberg RB, et al (2006) Body dysmorphic disorder and social phobia: cross-sectional and prospective data. Depress Anxiety. 23(1):26–33. https://doi.org/10.1002/da.20132

[37] DeMarco LM, Li LC, Phillips KA, McElroy SL (1998) Perceived stress in body dysmorphic disorder. J Nerv Ment Dis. 186(11):724–6. https://doi.org/10.1097/00005053-199811000-00011

[38] Nicodemo D, Pereira MD, Ferreira LM (2008) Self-esteem and depression in patients presenting angle class III malocclusion submitted for orthognathic surgery. Med Oral Patol Oral Cir Bucal. 13(1):48-51.

[39] Khesht-Masjedi MF, Shokrgozar S, Abdollahi E, et al (2019) The relationship between gender, age, anxiety, depression, and academic achievement among teenagers. J Family Med Prim Care. 8(3):799-804.

[40] Maslakçı A, Sürücü L. (2024) Gender effects on depression, anxiety, and stress regarding the fear of COVID-19. Trends in Psychology. 32(1):152-164.

[41] Gao W, Ping S, Liu X (2020) Gender differences in depression, anxiety, and stress among college students: a longitudinal study from China. Journal of affective disorders. 263(1):292-300.

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Original Research

Are Plaque Disclosing Agents Effective for Improving Self-Performed Dental Hygiene in Patients with Space Maintainers? A Randomized Controlled Clinical Trial

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ABSTRACT

Objective: In the event of premature loss of primary teeth, the placement of a space maintainer is the safest option for preserving dental arch length; however, the appliances and bands of space maintainers can change the contours of the teeth, causing plaque accumulation and making it difficult to maintain oral hygiene. The aim of this study was to evaluate changes in dental plaque accumulation and the effectiveness of a plaque disclosing agent (PDA) in providing oral hygiene with the use of space maintainers.

Methods: Patients were divided into two groups (Group A: intervention; Group B: control) and the study was carried out in four stages. At each stage, pocket depth (PD), gingival index (GI), and plaque index (PI) were measured.

Results: There was an increase in PD, GI, and PI after space maintainers were placed. Following oral hygiene education (with and without the use of a PDA), PD, GI, and PI significantly decreased in both groups.

Conclusion: PDAs are effective for improving oral hygiene, especially in young children; however, training should be repeated at frequent intervals to maintain good, continuous oral hygiene.

Keywords: dental plaque, gingival index, oral hygiene, plaque index, space maintainer

INTRODUCTION

Although preventive dental procedures are now widespread, epidemiological studies have shown that early childhood caries still occur frequently and lead to early tooth loss [1]. Decay or premature loss of primary teeth leads to the mesialization of adjacent teeth, resulting in a decrease in arch length, which can lead to malocclusions in permanent teeth. In the case of premature loss of primary teeth, placing a space maintainer is the safest option for preserving dental arch length [2].

Removable and fixed space maintainers are routinely used in children. The bands of fixed space maintainers, placed on the crowns of the teeth, or the clasps of removable space maintainers that are used for retention can sometimes extend slightly to the gingival sulcus and adversely affect gingival health. The appliances and bands of space maintainers can change the contours of the teeth, causing plaque accumulation and making it difficult to maintain oral hygiene, which can result in gingivitis and dental caries [3].

Space maintainers are in direct contact with oral microflora; thus, microbial biofilms can easily develop [4]. For this reason, patients with space maintainers may require more careful maintenance of oral hygiene. In particular, patients in younger age groups may need extra motivation to acquire and maintain good oral hygiene habits. Plaque disclosing agents (PDAs) can have a highly motivating effect as they can enable patients to better visually identify dental plaque [5]. Various studies have shown the effectiveness of PDAs in oral hygiene education programs [6] and professional dental prophylaxis practices [7]. However, there are no studies evaluating the effectiveness of PDAs in providing oral hygiene in areas of the mouth that may increase plaque accumulation, such as space maintainers. It is thought that visualizing the plaque accumulated around the space maintainers with PDA and showing to patients and their parents will increase oral hygiene motivation, by the way reduce plaque accumulation. Considering the motivational effect of PDAs on children's oral hygiene education, the aim of this study was to evaluate the effectiveness of PDA in improving oral hygiene in young children with space maintainers at different time intervals.

MATERIALS AND METHODS

This study was conducted in the Department of Pediatrics, Karabük Üniversity Oral and Dental Health Training and Research Hospital. The study was approved by the ethics committee of the Karabük University (approval no 2023/1519) and was conducted in full accordance with the World Medical Association Declaration of Helsinki. Written consent was obtained from participants' legal guardians.

Main Points;

- Because of the colour similarity of the tooth surface and dental plaque, it may be challenging to distinguish dental plaque, even on smooth surfaces. For this reason, making plaque visible, by using plaque-disclosing tablets or liquids, may be effective to ensure oral hygiene.
- In the case of premature loss of primary teeth, placing a space maintainer is the safest option for preserving dental arch length.
- Space maintainers are in direct contact with oral microflora, so they are ideal places for biofilm formation. For this reason, patients with space maintainers may require more careful maintenance of oral hygiene.
- It is thought that visualizing the plaque accumulated around the space maintainers with PDA and showing to patients and their parents will increase oral hygiene motivation, by the way reduce plaque accumulation.

Study Design

This interventional prospective study was conducted with 74 patients aged 4–9 years. The participants were selected by simple random drawing method from a group of patients who were recruited from the pedodontics clinic and needed space maintainers due to early tooth extraction.

Sample Size

According to the G Power analysis, the power of the study was found to be 90% when there were 34 people in each group. Due to possible issues, 80 pediatric patients were included in our study if the sample size decreased. As 6 patients could not continue the study, 74 participants were included in the study, of which 34 were girls and 40 were boys. A total of 40 participants were selected for the intervention group and received oral hygiene training using a PDA. The remaining 34 participants were included in the control group and received oral hygiene training without a PDA.

The sample size of the study was determined as a result of the power analysis performed in the G-power program. As there are no previous studies evaluating the effectiveness of PDAs in removing plaque accumulated around space maintainers, the sample size was calculated based on a study evaluating the role of PDAs in professional biofilm removal [8]. With an effect size of 1.2 and a standard deviation of 0.05 for pocket depth (PD), gingival index (GI), and plaque index (PI), 68 participants were required to provide 90% power. The required sample size was 80 to account for participants who did not attend their appointments. Six children were excluded from the study because they missed their follow-up appointments, reducing the sample size to 74.

Inclusion and Exclusion Criteria

Patients were included in the study if they were aged 4–9 years, if their families agreed to their participation, if they were cooperative in clinical examination, if they didn't have systemic disorders or disabilities and periodontal disease or gingival hyperplasia. Patients were excluded from the study if their families did not consent to their participation, if they were not cooperative in clinical examination, or if they had mental or

physical disabilities that may complicate their adaptation to the space maintainer and oral care.

Measurement Methods

Dental examination was performed using sterile examination kits containing a mirror (LS456 480/5, Carl Martin GmbH, Solingen, Germany), dental explorer (LS1091/33, Carl Martin GmbH, Solingen, Germany), and perio-probe (LS973/80 WHO, Carl Martin GmbH, Solingen,

Germany). PD, GI, and PI were measured from mesial, distal, buccal and lingual/palatal for each tooth. Pocket depth was measured in millimeters. The mean value for a tooth is the average of the values on the four surfaces. The mean index value for a patient was obtained by dividing the total value by the number of teeth.

According to Silness and Löe's plaque index [9]; 0 indicates no plaque; 1 indicates a thin layer of plaque present along the gingival margin that can be detected with an examination probe; 2 indicates a moderate layer of plaque along the gingival margin that can be detected visually; and 3 indicates an excessive layer of plaque along the gingival margin, and a layer of plaque can be observed in the interdental areas.

According to Löe and Silness's gingival index [10]; 0 indicates no visible signs of inflammation; 1 indicates a change in the colour and consistency of the gingiva, with no bleeding upon probing (by perio-probe / LS973/80 WHO, Carl Martin GmbH, Solingen, Germany); 2 indicates visible inflammation and bleeding upon probing; and 3 indicates pronounced redness, edema, and a tendency for spontaneous bleeding.

Data Collection

The included patients were randomly divided into two separate groups. For randomization, an opaque envelope included cards with text, control group or PDA group was used to determine which group the child would be allocated at the beginning of the study. For Group A (N 40 / intervention group), a PDA (TePe Plaq-Search™ Tablets -TePe Oral Hygiene Products AB, Malmö, Sweden) was used as a guide for patients and families to show dental plaque (Figure 1). The PDA was placed in the patient's mouth and the patient is asked to rub it against the teeth with the tongue until the tablet dissolves. All stained areas were shown to the patient and his/her parents. After the procedure, the areas stained with PDA were cleaned with water and a brush. For Group B (N=34 / control group), patients and their parents were shown the plaque accumulated around the space maintainers in front of the mirror (Figure 2). Then for both groups, they informed how to clean dental plaque. The study was carried out in four stages. Patients had a total of four appointments: the first for the installation of the space maintainer and the remaining three follow-up appointments for PDA application and other measurements.

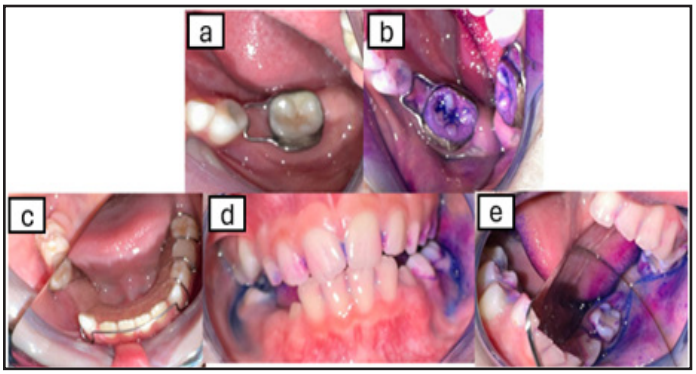

Figure 1. Intraoral images of Group A, **a.** Space maintainer was fixed, **b.** using PDA, **c**. removable space maintaner was placed, d and e) using PDA

Figure 2. Intraoral images of Group B **a.** Space maintainer was fixed, **b and c.** control session, **d.** removable space maintaner was placed, **e.** control session

The entire study was performed by a single pedodontics (the pedodontics installed all the space maintainers and was present at all the appointments for PDA application and other measurements). To ensure investigator standardization; PD, GI, and PI were assessed twice, 30 minutes apart, in 15 pediatric patients who were not part of the investigated sample. PD, GI, and PI measurements were repeated one week later at 30-minute

intervals in the same way. The kappa values for PD, GI, and PI were 0.87, 0.92, and 0.89, respectively.

Stage 1 (appointment for space maintainer installation and first index measurements)

The space maintainers of the patients were fixed, and in the same session, the PD, GI, and PI of the supporting teeth (in which the bands or clasps of the space maintainers were placed) were noted.

Stage 2 (1 month after first index measurements)

The patients were called upon for a follow-up appointment one month after the placement of their space maintainers. In this appointment, the PD, GI, and PI of the supporting teeth were measured. Then, a PDA was applied to the patients in Group A so that they could observe the dental plaque accumulated around their space maintainers and supporting teeth. In Group B, without the use of a PDA, dental plaque accumulated around the space maintainers and supporting teeth was shown to patients and their parents/caregivers with a mirror. In both groups, the patients and their parents/caregivers were informed about how to clean these dental areas with a visual demonstration.

Stage 3 (1 month after second index measurements)

The patients were called one month after their second index measurements. In this appointment, the PD, GI, and PI of the supporting teeth were measured. At this stage, any short-term differences in plaque accumulation between the groups were measured and recorded.

Stage 4 (3 months after third index measurements)

Patients were called three months after their third index measurement (five months after the placement of space maintainers). The PD, GI, and PI of the supporting teeth were measured. At this stage, any long-term differences in plaque accumulation between the groups were examined. The flow chart of the study is given in Figure 3.

Figure 3. Flow chart of the study

Statistical Analysis

The data were analysed using the SPSS (SPSS Inc., Chicago, Version 20) program. Comparisons of PD, GI, and PI between groups were made using dependent t-tests and the McNemar test. The statistical significance level was taken as $p < 0.05$.

RESULTS

The mean age was 6.8 ± 3.4 years. Demographic information about the patients and their space maintainers is given in Table 1.

Increases in PD, GI, and PI were observed in Stage 2 after the space maintainers were installed in the patients. These increases were greater in the mandible than in the maxilla, in boys than in girls, in the 7–9 age group than in the 4–6 age group, and for fixed space maintainers than for removable space maintainers. The mean PD, GI, and PI values of the patients are given in Figure 4, and their statistical evaluations are given in Table 2. In Stage 2, PD, GI, and PI increased in both groups. While PD and GI increased significantly (p=0.01 both PD and GI) in Group A, the increase in Group B was not significant (p values for PD, GI and PI were 0.14, 0.12, 0.06 respectively) In Stage 3, it was observed that PD, GI, and PI decreased in both groups. While this decrease was not significant in Group A (p values for PD, GI and PI were 0.11, 0.09, 0.2 respectively), there was a significant decrease in GI and PI in Group B (p values for GI and PI were 0.03 and, 0.01 respectively). In Stage 4, it was observed that all values increased significantly for both groups (p values for PD, GI and PI were 0.0001, 0.01, 0.01 respectively for Group A and 0.008, 0.03 and, 0.03 respectively for Group B).

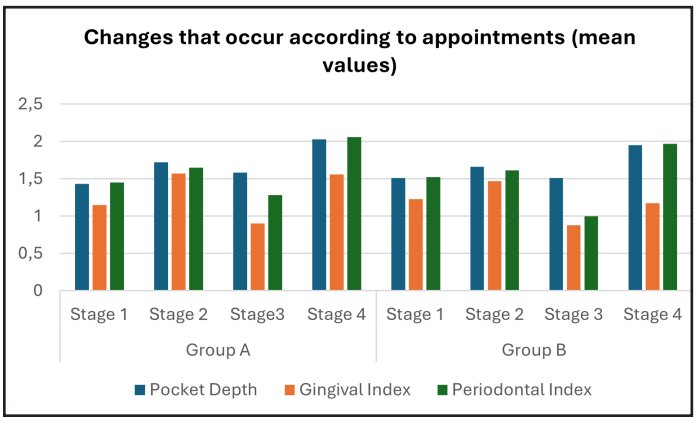

Figure 4. Changes that occur according to appointments (mean values)

The changes seen in Stage 2 according to the type of space maintainer used are given in Table 3. Fixed space maintainers caused a significant increase in PD, GI, and PI (p values for PD, GI and PI were 0.04, 0.005, 0.01 respectively).

After oral hygiene training was delivered to Groups A and B, decreases in PD, GI, and PI were observed. There was a significant decrease in GI and PI in patients with fixed space maintainers (p values for GI and PI were 0.001 and, 0.04 respectively) (Table 4).

At the last follow-up, it was observed that PD, GI, and PI increased significantly for those who used space maintainers in Group B (p values for PD, GI and PI were 0.03, 0.04, 0.000 respectively for fixed space maintainers and 0.01, 0.04, 0.02 respectively for removable space maintainers) (Table 5).

Table 1. Study Groups

		Group A (N)	Group B (N)
Gender	Girl	19	15
	Boy	21	19
Age group	4-6	17	17
	7-9	23	17
Type of space maintainers	Fixed space maintainers	25	25
	Removable space maintainers	9	6
	Fixed and removable space mainteiners	6	3
Jaws	Mandibula	23	20
	Maxilla	10	9
	Both of the jaws	7	5

Table 2. Changes that occur according to appointments (p values)

	Group A			Group B		
	1-2. Stage	**1-3. Stage**	**1-4. Stage**	**1-2. Stage**	**1-3. Stage**	**1-4. Stage**
Pocket Depth	**0.01**	0.11	**0.0001**	0.14	0.5	**0.008**
Gingival Index	**0.01**	0.09	**0.01**	0.12	**0.03**	**0.03**
Plaque Index	0.18	0.2	**0.001**	0.06	**0.01**	**0.03**

Statistical significance level was accepted as $p < 0.05$

Bold numbers indicate statistically significant values

Table 3. Changes in the 2nd session according to the type of space maintainers (mean values)

	Type of spacemaintainers	**Stage 1**	**Stage 2**	**p value**
Pocket Depth	Fixed	**1.46**	**1.78**	**0.04**
	Removale	1.39	1.47	0.07
	Both	1.61	1.86	0.22
Gingival Index	Fixed	**1.16**	**1.58**	**0.005**
	Removale	1.13	1.4	0.21
	Both	1.44	1.44	0.5
Plaque Index	Fixed	**1.4**	**1.96**	**0.01**
	Removale	1.73	1.66	0.43
	Both	1.55	1.44	0.40

Statistical significance level was accepted as $p < 0.05$

Bold numbers indicate statistically significant values

Table 4. Changes in the 3rd session according to the type of space maintainers (mean values)

	Type of spacemaintainers	**Grup A**			**Grup B**		
		Stage 2	**Stage 3**	**p value**	**Stagee 2**	**Stage 3**	**p value**
Pocket Depth	Fixed	1.79	1.62	0.19	1.57	1.48	0.30
	Removale	1.71	1.58	0.26	1.62	1.33	0.22
	Both	1.5	1.41	0.40	2.58	2.16	0.32
Gingival Index	Fixed	1.68	0.88	**0.001**	1.48	0.84	**0.001**
	Removale	1.44	1.22	0.25	1.33	1	0.29
	Both	1.33	0.66	**0.02**	1.66	1	0.18
Plaque Index	Fixed	1.76	1.22	**0.05**	1.56	1	**0.04**
	Removale	1.66	1.44	0.34	1.66	1	0.21
	Both	1.16	1.33	0.39	2	1	0.14

Statistical significance level was accepted as $p < 0.05$

Bold numbers indicate statistically significant values

Table 5. Changes in the 4th session according to the type of space maintainers

	Type of spacemaintainers	Grup A			Grup B		
		Stage 3	Stage 4	p value	Stage 3	Stage 4	p value
Pocket Depth	Fixed	1.62	1.92	0.05	1.48	1.86	**0.03**
	Removale	1.58	1.71	0.4	1.33	2	**0.01**
	Both	1.41	2	0.10	2.16	2.66	0.33
Gingival Index	Fixed	0.88	1.02	0.07	0.84	1.58	**0.04**
	Removale	1.22	1.55	0.3	1	1.56	**0.04**
	Both	0.66	1.33	0.09	1	2	0.14
Plaque Index	Fixed	1.22	2.22	**0.002**	1	2	**0.000**
	Removale	1.44	2.07	0.09	1	2.1	**0.02**
	Both	1.33	2.1	0.18	1	2.66	**0.03**

Statistical significance level was accepted as $p < 0.05$

Bold numbers indicate statistically significant values

DISCUSSION

Plaque accumulation is frequently observed and difficult to clean in areas where space maintainers are installed. Since space maintainers are in direct contact with oral microflora, they are ideal places for biofilm formation. The material from which space maintainers are made is also suitable for microorganism adhesion and biofilm formation [11]. In addition, the bands placed on the supporting teeth of fixed space maintainers and the retention clasps of removable space maintainers can promote plaque accumulation and gingival disease [12]. However, due to the colour similarity of the tooth surface and dental plaque, it may be challenging to distinguish dental plaque, even on smooth surfaces [13]. For this reason, making plaque visible, by using plaque-disclosing tablets or liquids, is considered the most effective way to ensure oral hygiene [14].

In this study, the effectiveness of plaque-disclosing tablets in preventing increased plaque accumulation and ensuring oral hygiene in patients aged 4-9, using space maintainers was evaluated.

The bands placed on supporting teeth for fixed space maintainers have sharp edges and penetrate deeper in the gingival sulcus than the Adams clasps of removable space maintainers. Additionally, food that becomes stuck under the loops can cause periodontal problems. It is known that the Adams clasps of removable space maintainers are positioned higher than the bands of fixed space maintainers and are placed in the free gum, which does not lead to an increase in PD [12]. In this study, a significant increase in PD was observed in patients with fixed space maintainers. Similar to this study, Arikan et al. [15] reported a significant increase in PD after the placement of a fixed space maintainer. Similarly, in this study, a significant increase in GI was observed in patients using fixed space maintainers. In a study by Hosseinipour et al. [12], a significant increase in the GI of the support teeth of both removable and fixed space maintainers at the end of the sixth month was observed. Fixed space maintainers change the contours of the teeth on which they are placed, which can promote food retention and make oral hygiene practice difficult. In this study, plaque accumulation increased in patients with fixed space maintainers while it decreased in those with removable space maintainers. This might be because removable space maintainers were not in the mouth when the user is eating or brushing their teeth.

In the literature, there are some limitations in eliminating dental plaque via routine tooth brushing [16-18]. A study involving 127 participants, including dentists, dental assistants, and dental students, reported that dental plaque remained on at least 10% of the tooth surfaces in all groups [19]. This shows that despite having high amounts of knowledge about dental and oral hygiene, it is still difficult to ensure that plaque is sufficient. In a study conducted by Van der Weijden and Hioe [18], mechanical plaque removal was more effective in individuals who were given oral hygiene training than in those who were not. Oral hygiene education is particularly important in controlling plaque

accumulation and oral hygiene, especially in patients using space maintainers or orthodontic appliances. In the present study, the effect of oral hygiene training on plaque accumulation around space maintainers was evaluated. Consistent with other studies, a decrease in PD, GI, and PI was detected in both the intervention and control groups in the present study. Soder et al. [20] stated that maxillary teeth were better cleaned than mandibular teeth in terms of the removal of dental plaque. Similarly, in our study, it was observed that the increase in plaque accumulation was greater after a space maintainer was placed in the mandible.

The studies report that PDAs increase children's oral hygiene motivation to maintain their oral hygiene [14,21]. In this study, significant decreases in GI and PI were observed in both groups one month after the patients were given oral hygiene education. The decrease in both groups showed that oral hygiene training without the use of PDA produces successful results. In the fourth session, in which the long-term effects of oral hygiene training were evaluated, a significant increase was observed only in PI for Group A, while a significant increase was observed in PD, GI, and PI for Group B. Although not all of the results were significant, the increase in PD, GI, and PI in both groups revealed the need to repeat oral hygiene training frequently. The fact that the increase in PD, GI, and PI in Group A was not significant shows that the use of the PDA produces effective results in oral hygiene maintenance. Similar to this study, Alencar et al. [7] reported that using a PDA effectively shows the accumulation areas of dental plaque; therefore, it ensures effective oral hygiene. The difficulty of reaching the palatal/lingual surfaces of the teeth while brushing and the developmental grooves on the occlusal surfaces can make cleaning plaque challenging [22]. Alencar et al. [7] reported that the use of a PDA was not very effective in cleaning the plaque on the palatal/lingual surfaces. In this study, fixed space maintainers increased the contours of the teeth and created recessed areas, making plaque accumulation difficult. This explains the increase in PD, GI, and PI in the second session.

Limitations

The major limitation of this study was that the study sample was selected from patients in a young age group. In young children, effective tooth brushing is more difficult due to poor mind–muscle coordination, and plaque accumulation may be higher in these patients. Thus, parental support is needed to ensure oral hygiene. Therefore, the cooperation of the parents was effective in the results of the study. It was also observed that the effectiveness of oral hygiene education decreased as time passed, resulting in increases in PD, GI, and PI. Thus, studies evaluating the effectiveness of oral hygiene training repeated at regular intervals are warranted.

Recommendations

It was observed that oral hygiene education with the help of a PDA increased the patient's ability to effectively control plaque accumulation. The use of PDAs may provide useful results in ensuring oral hygiene in patients who do not use any appliances.

CONCLUSION

The application of a PDA improves patients' ability to see the plaque and subsequently remove it, especially in areas that are difficult to access, such as areas around space maintainers. Oral hygiene education repeated at frequent intervals will be more effective for controlling dental plaque.

Conflict of interest: None

Funding: None

Ethical Approval: Karabuk University Non Invasive Ethical Committee / Protocol No: 2023/1519 Informed Consent: Verbal consent for intraoral examination was obtained from the children participating in the study, and written consent was obtained from their legal guardians on behalf of both themselves and their children.

REFERENCES

[1] Fields H (2019) Space Maintenance in the Primary Dentition. In: Nowak J, et al (eds), Amsterdam, pp 379-385. https://doi.org/10.1016/B978-0-323-60826-8.00026-2.

[2] Setia V, Pandit IK, Srivastava N, Gugnani N, Sekhon HK. Space maintainers in dentistry: past to present. J Clin Diagn Res. 2013 Oct;7(10):2402-5. https://doi.org/10.7860/JCDR/2013/6604.3539

[3] Goenka P, Sarawgi A, Marwah N, Gumber P, Dutta S. Simple fixed functional space maintainer. Int J Clin Pediatr Dent. 2014 Sep-Dec;7(3):225-8. https://doi.org/10.5005/jp-journals-10005-1272

[4] Kilian M, Chapple IL, Hannig M, Marsh PD, Meuric V, Pedersen AM, Tonetti MS, Wade WG, Zaura E. The oral

microbiome - an update for oral healthcare professionals. Br Dent J. 2016 Nov 18;221(10):657-666. https://doi.org/10.1038/sj.bdj.2016.865

[5] Oliveira LM, Pazinatto J, Zanatta FB. Are oral hygiene instructions with aid of plaque-disclosing methods effective in improving self-performed dental plaque control? A systematic review of randomized controlled trials. Int J Dent Hyg. 2021 Aug;19(3):239-254. https://doi.org/10.1111/idh.12491

[6] Fasoulas A, Pavlidou E, Petridis D, Mantzorou M, Seroglou K, Giaginis C. Detection of dental plaque with disclosing agents in the context of preventive oral hygiene training programs. Heliyon. 2019 Jul 10;5(7):e02064. https://doi.org/10.1016/j.heliyon.2019.e02064J

[7] de Alencar CR, de Oliveira GC, Tripodi CD, Gonçalves PS, Ionta FQ, Honorio HM, Oliveira TM, Rios D. Dental Plaque Disclosing as an Auxiliary Method for Professional Dental Prophylaxis in Early Childhood. Int J Clin Pediatr Dent. 2019 May-Jun;12(3):189-193. https://doi.org/10.5005/jp-journals-10005-1617

[8] Mensi M, Scotti E, Sordillo A, Agosti R, Calza S. Plaque disclosing agent as a guide for professional biofilm removal: A randomized controlled clinical trial. Int J Dent Hyg. 2020 Aug;18(3):285-294. https://doi.org/10.1111/idh.12442

[9] Silness J, Loe H (1964) Periodontal disease in pregnancy II. Correlation between oral hygiene and periodontal condition. Acta Odontol Scand 22:121–135. https://doi.org/10.3109/00016356408993968

[10] Loe H, Theilade E, Jensen SB. (1965) Experimental gingivitis in man, J. Periodontol. 36:177–187. https://doi.org/10.1902/jop.1965.36.3.177

[11] Gok B, Kirzioglu Z, Kivanc M. Green tea extract solutions can control bacterial biofilms formed on space maintainers. Niger J Clin Pract. 2020 Jun;23(6):783-791. https://doi.org/10.4103/njcp.njcp_246_19

[12] Hosseinipour ZS, Poorzandpoush K, Heidari A, Ahmadi M. Assessment of Periodontal Parameters Following the Use of Fixed and Removable Space Maintainers in 6-12-year Olds. Int J Clin Pediatr Dent. 2019 Sep-Oct;12(5):405-409. https://doi.org/10.5005/jp-journals-10005-1606

[13] You W, Hao A, Li S, Wang Y, Xia B. Deep learning-based dental plaque detection on primary teeth: a comparison with clinical assessments. BMC Oral Health. 2020 May 13;20(1):141. https://doi.org/10.1186/s12903-020-01114-6

[14] Peng Y, Wu R, Qu W, Wu W, Chen J, Fang J, Chen Y, Farella M, Mei L. Effect of visual method vs plaque disclosure in enhancing oral hygiene in adolescents and young adults: a single-blind randomized controlled trial. Am J Orthod Dentofacial Orthop. 2014 Mar;145(3):280-6. https://doi.org/10.1016/j.ajodo.2013.10.021

[15] Arikan F, Eronat N, Candan U, Boyacioğlu H. Periodontal conditions associated with space maintainers following two different dental health education techniques. J Clin Pediatr Dent. 2007 Summer;31(4):229-34. https://doi.org/10.17796/jcpd.31.4.9588m43n027t560n

[16] Ganss C, Schlueter N, Preiss S, Klimek J. Tooth brushing habits in uninstructed adults--frequency, technique, duration and force. Clin Oral Investig. 2009 Jun;13(2):203-8. https://doi.org/10.1007/s00784-008-0230-8

[17] Harnacke D, Stein K, Stein P, Margraf-Stiksrud J, Deinzer R. Training in different brushing techniques in relation to efficacy of oral hygiene in young adults: a randomized controlled trial. J Clin Periodontol. 2016 Jan;43(1):46-52. doi: 10.1111/jcpe.12489. Epub 2016 Feb 5. PMID: 26660396. https://doi.org/10.1111/jcpe.12489

[18] van der Weijden GA, Hioe KP. A systematic review of the effectiveness of self-performed mechanical plaque removal in adults with gingivitis using a manual toothbrush. J Clin Periodontol. 2005;32 Suppl 6:214-28. https://doi.org/10.1111/j.1600-051X.2005.00795.x

[19] Deinzer R, Schmidt R, Harnacke D, Meyle J, Ziebolz D, Hoffmann T, Wöstmann B. Finding an upper limit of what might be achievable by patients: oral cleanliness in dental professionals after self-performed manual oral hygiene. Clin Oral Investig. 2018 Mar;22(2):839-846. https://doi.org/10.1007/s00784-017-2160-9

[20] Söder B, Johannsen A, Lagerlöf F. Percent of plaque on individual tooth surfaces and differences in plaque area between adjacent teeth in healthy adults. Int J Dent Hyg. 2003 Feb;1(1):23-8. https://doi.org/10.1034/j.1601-5037.2003.00003.x

[21] Mamaklı E, Cengiz A, Akdağ H, Cesur G, Yaşar S, Altuner

E, Budak B, Uçak ME, İslamoğlu T, Akın H, Istıl B, Karayılmaz H. Diş plağının gösterilmesinin üniversite öğrencilerinin ağız ve diş sağlığına yönelik tutum ve davranışlarına olan etkisinin değerlendirilmesi. Akd Dent J. 2022;1(2):63-75.

[22] Yang BI, Park JA, Lee JY, Jin BH. Effects of Lingual and Palatal Site Toothbrushing on Periodontal Disease in the Elderly: A Cross-Sectional Study. Int J Environ Res Public Health. 2021 May 11;18(10):5067. https://doi.org/10.3390/ijerph18105067

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Original Research

Analysis of YouTube Videos on Circumcision: Evaluating Reliability and Quality for Patients and Parents

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ABSTRACT

Objective: Circumcision is a significant issue for child health and parents, and the reliability and quality of information published on platforms like YouTube can affect patients' access to accurate information. This study aimed to evaluate the quality and reliability of Turkish YouTube videos as a source of information about circumcision.

Methods: In this cross-sectional study, a search was conducted on YouTube using the keyword "circumcision" on June 1, 2024. After applying exclusion criteria, the 45 most viewed Turkish videos (more than 10,000 views) were included in the study. Videos that were irrelevant, contained advertisements, personal experiences, or were in languages other than Turkish were excluded. The duration, view count, likes, dislikes, number of comments, and upload date of the videos were recorded. The Video Power Index (VPI) was calculated to measure video popularity. Two pediatric surgeons independently assessed the quality and reliability of the videos using the Modified DISCERN (mDISCERN), Journal of the American Medical Association (JAMA), and Global Quality Scale (GQS) scores. Scale scores were compared between groups based on the purpose and publisher of the video using the Mann-Whitney U test. Correlations between video characteristics and scale scores were evaluated with the Spearman correlation coefficient, and inter-observer agreement was assessed with the intraclass correlation coefficient (ICC).

Results: According to the inclusion and exclusion criteria, 39 (86.7%) of the 45 videos were informational, and 6 (13.3%) were surgical. The average length of the videos was 213.5 ± 206 (35 - 1164) seconds. The average duration since the videos were published until June 1, 2024, was 1653 ± 980 (350 - 3985) days. The average view count of the videos was 73,862 ± 114,210 (11,736 – 679,985). The average Video Power Index of the videos was 39.9 ± 40.85 (3.69 - 247.1). The average mDISCERN score was 2.87 ± 1.24, the JAMA score was 2.71 ± 0.7, and the GQS score was 3.38 ± 1.19. According to the GQS scale, 22.3% (n=10) of the 45 videos were of low quality, 33.3% (n=15) were of medium quality, and 44.4% (n=20) were of high quality. All scale scores of informational videos were statistically significantly greater compared to the surgical videos (P=0.008, P=0.041, P=0.024, respectively).

Conclusion: YouTube is a significant source of information with the potential to influence the knowledge and behavior of a wide audience regarding circumcision. Patients and their relatives should consider videos uploaded by expert physicians. There is a need for more high-quality educational surgical circumcision videos and short, clear, unbiased, high-quality informational videos addressing controversial issues and containing necessary resources created by expert physicians. This will help increase the accurate knowledge of families about circumcision and consequently reduce unnecessary anxiety and expectations.

Keywords: Circumcision, YouTube™, Video analysis, Quality, Reliability

INTRODUCTION

The history of circumcision dates back thousands of years, making it one of the most commonly performed surgical procedures worldwide [1]. Circumcision involves the surgical removal of the foreskin (prepuce) from the tip of the penis. This procedure is widely performed globally for religious, cultural, and medical reasons [2]. Although circumcision is perceived as a simple and short procedure, it can lead to mild complications such as pain, edema, minor bleeding, and excessive foreskin removal [3], as well as more serious complications such as glans injury, urethral injury, and massive bleeding [4-6]. For parents, concerns arise from the preoperative fasting period, the time spent in the operating room, surgical and anesthesia complications, and the recovery period both in the hospital and at home [2].

Main Points

- Quality and Reliability of YouTube Videos on Circumcision: The study found that while Turkish circumcision videos on YouTube are generally more reliable and of higher quality compared to those in other studies, they are still insufficient. Videos uploaded by physicians were more reliable and of higher quality than those published by private hospitals, and informational videos were superior in quality and reliability compared to surgical videos.
- Influence of YouTube Videos on Patient Decision-Making: The study highlighted that unreliable videos can also achieve high view and like counts, potentially misleading patients and their families. It emphasizes the importance of considering videos uploaded by specialists in the field rather than relying solely on view and like counts when making health-related decisions.
- Need for High-Quality Educational Content: The study underscores the necessity for more high-quality educational surgical circumcision videos created by expert physicians and more high-quality informational videos that are short, clear, unbiased, address controversial issues, and include necessary sources. This would enhance the accurate knowledge of families about circumcision, reducing unnecessary anxiety and expectations.

In the past, most people obtained medical information by consulting healthcare professionals [7]. However, in modern medicine, patients' access to information has significantly changed with the widespread use of the internet and social media platforms [8]. Today, social media has become an accessible source of information for everyone to reach medical knowledge [9]. In addition to information provided by doctors on surgical procedures, potential risks, and treatment options, patients now also acquire information about their conditions from the internet and social media [10]. However, since YouTube is an open source where anyone can upload content, the information is often misleading or incorrect [11]. With the rapid proliferation of medical content on the internet today, the reliability and adequacy of this information remain uncertain [11].

Circumcision is a significant issue for child health and parents, and the reliability and quality of information disseminated on platforms like YouTube can affect the access of patients and their families to accurate information. There is no study in the literature that evaluates the quality of YouTube videos about circumcision. This study aimed to evaluate the quality and reliability of Turkish YouTube videos as a source of information about circumcision.

MATERIAL AND METHODS

Data Collection

The data used in our study were collected from YouTube on 1 June 2024. A search was made on YouTube using the keyword "sünnet" (circumcision)" and videos were ranked according to the number of views. Turkish language videos related to child circumcision with more than 10,000 views were determined as inclusion criteria. Irrelevant content, videos containing personal experiences, videos targeting adult patients, non-Turkish content, advertising videos, shorts videos and videos shorter than 15 seconds were determined as exclusion criteria and were not included in the analyses. As a result of YouTube search, 45 videos with Turkish content with more than 10,000 views were found. Data obtained from these videos and upload sources were recorded. Two independent paediatric surgical experts scored all videos using the Modified DISCERN (mDISCERN), Journal of the American Medical Association (JAMA), and Global Quality Scale (GQS) scoring systems. Video View ratio, video Like ratio and Video Power Index (VPI) were also calculated using the data obtained from the videos.

Evaluation Criteria

In this cross-sectional study, mDISCERN, JAMA, and GQS scales were used to assess the quality and reliability of Turkish-language YouTube videos. The mDISCERN Score was used to assess whether the videos contained accurate information about the level of information, treatment options and risks. The JAMA Score was used to assess the medical accuracy and reliability of the videos. The Global Quality Scale (GQS) Score was used to evaluate the overall quality and informative level of the videos.

The mDISCERN scale, which was first developed by Charnock et al. (1999) to assess the quality of health information sources, was later adapted as five items by Singh et al. (2012) [12,13]. In particular, it aims to increase the ability of patients or individuals seeking information on health-related issues to evaluate health information sources and access accurate information. The scale focuses on factors such as reliability, clarity, effective presentation and ability to provide accurate information on health-related issues. Each criterion is scored 1-0 (yes/no) and the total score ranges from 0 to 5. A higher scale score represents higher video quality [12,13].

JAMA Video Quality Analysis was developed by Silberg et al. (1997) to evaluate the quality of videos with medical content [14]. This scale evaluates factors such as accuracy, scientific validity, effective communication and the ability to provide useful information to the audience, especially in the field of medicine. It consists of four criteria (authorship, attribution, disclosure, and currency), each criterion is 1 point and the total score varies between 0 and 4. A higher score represents higher video reliability [14].

The GQS is a scale developed by Bernard et al. (2007) to evaluate the quality of videos with medical content in terms of patient use [15]. This scale supports the aims of providing accurate and reliable information in the field of health by evaluating the scientific accuracy, effective communication and the capacity of medical videos to benefit the viewer. The quality of the video content is scored from 1 to 5. A score of 1 indicates that the video is of poor quality and useless for patients, while a score of 5 indicates that the video is of high quality and useful for patients [15].

The main purpose of the Video Power Index (VPI) developed by Erdem et al. (2018) is to measure the popularity, interaction and audience attraction power of a video or content [16]. This scale aims to determine how effective a video content is on social media platforms and how much attention it receives from viewers. The VPI calculation uses the number of likes, dislikes, views, and the time elapsed since the video was uploaded to YouTube. The view ratio is calculated as ([number of views] / [days since the first upload]), the video-like ratio as ([100 × number of likes] / [number of likes + dislikes]), and the Video Power Index as (Video-like ratio × view ratio /100) [16].

Statistical Analyses

Statistical analyses were performed using SPSS software (Version 22.0, SPSS Inc., Chicago, IL, USA, License: Hitit University). Descriptive statistics for categorical variables were reported using frequency (n) and percentage (%). Descriptive statistics for numerical data with normal distribution were reported using mean ± SD, while those for numerical data not normally distributed were reported using median (min-max). The normality of the distribution of numerical data was examined using the Shapiro-Wilks test and some graphical approaches. The Mann-Whitney U test was used to compare numerical data between two independent groups because the normal distribution assumption was not met. To examine the correlations between numerical data, the Spearman correlation coefficient was used according to the data normal distribution. The intraclass correlation coefficient (ICC) was used to determine

the level of agreement between two independent observers. The ICC estimate value was considered indicative of poor reliability for values less than 0.5, moderate reliability for values between 0.5 and 0.75, good reliability for values between 0.75 and 0.9, and excellent reliability for values greater than 0.90. A value of $P< 0.05$ was considered statistically significant.

RESULTS

Of the 45 videos obtained according to the inclusion and exclusion criteria, 39 (86.7%) were informative, and 6 (13.3%) were surgical. The videos were uploaded by Physicians (36; 80%), private hospitals (8; 17.8%), and an independent user (1; 2.2%). The average length of the videos was 213.5 ± 206 (35 – 1,164) seconds. The average time elapsed since the videos were published until June 1, 2024, was 1,653 ± 980 (350 – 3,985) days. The average view count of the videos was 73,862 ± 114,210 (11,736 – 679,985). The average VPI of the videos was 39.9 ± 40.85 (3.69 - 247.1). Other statistical findings related to the videos are presented in Table 1.

The average mDISCERN score obtained for the videos was 2.87 ± 1.24, the average JAMA score was 2.71 ± 0.7, and the average GQS score was 3.38 ± 1.19. According to the GQS scale, 22.3% (n=10) of the 45 videos were of low quality, 33.3% (n=15) were of medium quality, and 44.4% (n=20) were of high quality. One of the 45 videos had a perfect mDISCERN score of 5, three had a perfect JAMA score of 4, and ten had a perfect GQS score of 5. Only one video received a perfect score on all quality scales. This video, posted by a pediatric surgery and urology specialist, explained the appropriate age range for circumcision and the methods of circumcision.

The most viewed video among all videos was a surgical circumcision video. Of the total six surgical videos, four contained only visual content. When categorized by titles, it was observed that most videos were about post-circumcision care. Post-circumcision care videos constituted 12 out of 45 videos. The most viewed informative video was also a post-circumcision care video. Only two videos mentioned complications, and two videos provided general information about circumcision.

The ICC values showing the level of agreement between the two independent pediatric surgeons evaluating the videos are presented in Table 2 with 95% confidence intervals. There was excellent statistical agreement between the evaluators for the mDISCERN, JAMA, and GQS scores (respectively, ICC: 0.925, ICC: 0.951, ICC: 0.946, $P<0.001$).

Although numerically the surgical videos had higher view counts, like counts, and VPI values compared to the informative videos, these differences were not statistically significant (respectively; $P=0.961$, $P=0.217$, $P=0.660$). Numerically, the length of the informative videos (seconds) was higher than the surgical videos, but this difference was not statistically significant ($P=0.271$). All scale scores of the informative videos were statistically significantly greater compared to the surgical videos (respectively; $P=0.008$, $P=0.041$, $P=0.024$; Table 3).

Numerically, the videos posted by physicians had higher view counts, like counts, mDISCERN, and GQS scores compared to the videos posted by private hospitals, but these differences were not statistically significant (respectively; $P=0.393$, $P=0.060$, $P=0.111$, $P=0.189$). Numerically, the JAMA scores of the videos posted by physicians were lower than those of the videos posted by private hospitals, but this difference was not statistically significant ($P=0.622$). The video length (seconds) and VPI values of the videos posted by physicians were statistically significantly greater than those of the videos posted by private hospitals (respectively; $P=0.007$, $P=0.001$; Table 4).

No statistically significant relationship was found between the mDISCERN, JAMA, and GQS scores and the time elapsed since the videos were published, video length, view count, like count, dislike count, comment count, view ratio, like ratio, and VPI values ($P>0.05$; Table 5).

A moderately positive significant correlation was determined between the time elapsed since the videos were published and the view count and dislike count (r=0.514, $P<0.001$; r=0.622, $P<0.001$). A highly negative significant correlation was determined between the time elapsed since the videos were published and the like ratio (r=-0.733, $P<0.001$). A moderately positive significant correlation was determined between the video length and like count (r=0.602, $P<0.001$). A low positive significant correlation was determined between the video length and comment count (r=0.392, $P=0.024$). No significant correlation was determined between the time elapsed since the videos were published and video length with other variables ($P>0.05$; Table 6).

Table 1. Characteristics of YouTube videos included in this study (n=45)

	n	%
Video content		
Informational Video	39	86.7
Surgery Video	6	13.3
Video sources		
Physician	36	80
Private Hospital	8	17.8
Independent users	1	2.2
	Mean ± SD	**Median (min-max)**
Video features		
Video length (seconds)	213.5 ± 206	144 (35 – 1,164)
Time since upload (days)	1,653 ± 980	1,423 (350 – 3,985)
Number of views	73,862 ± 114,210	39,010 (11,736 – 679,985)
Number of likes	309 ± 467.2	140 (16 – 2,189)
Number of dislikes	32.98 ± 78.97	14 (0 - 485)
Comments	72.03 ± 108.9	21 (0 - 459)
View ratio	44.48 ± 49.08	28.59 (4.24 - 307.4)
Like ratio	90.57 ± 9.79	95.53 (71.05 - 100)
VPI	39.9 ± 40.85	26.43 (3.69 - 247.1)
Scales		
Modified DISCERN	2.87 ± 1.24	3 (0 - 5)
JAMA	2.71 ± 0.7	3 (1 - 4)
GQS	3.38 ± 1.19	3 (1 - 5)

VPI: Video Power Index, JAMA: Journal of the American Medical Association, GQS: global quality scale

Table 2. Intraclass correlation coefficient (ICC) values showing the level of agreement between the observers

	Modified DISCERN		JAMA		GQS	
	ICC (%95 CI)	**P**	**ICC (%95 CI)**	**P**	**ICC (%95 CI)**	**P**
Observer I & Observer II	0.925 (0.915 – 0.932)	<0.001	0.951 (0.941 – 0.959)	<0.001	0.946 (0.937 – 0.953)	<0.001

CI: Confidence interval

Table 3. Statistical findings for the comparison of video length, number of views, number of likes, VPI, Modified DISCERN, JAMA and GQS between groups according to video purpose

	Informative videos (n=39)	Surgery related videos (n=6)	P values
Video length (seconds)	144 (35 - 1,164) (218.7 ± 216.8)	162 (60 - 386) (179.1 ± 120.4)	0.271
Number of views	34,883 (11,736 - 402,305) (60,634.8 ± 71,158.7)	61,097.5 (17,536 - 679,985) (159836.8 ± 257039.3)	0.961
Number of likes	129 (16 - 2,189) (289.5 ± 416.5)	150 (52 - 1,987) (435.8 ± 761.5)	0.217
VPI	24.55 (3.69 - 107.8) (35.23 ± 27.37)	35.79 (17.22 - 247.1) (70.27 ± 87.82)	0.660
Modified DISCERN	3 (0 - 5) (3.05 ± 1.191)	1.50 (1 - 3) (1.67 ± 0.81)	**0.008**
JAMA	3 (1 - 4) (2.82 ± 0.601)	2 (1 - 3) (2 ± 0.89)	**0.041**
GQS	3 (1 - 5) (3.54 ± 1.14)	2 (1 - 4) (2.33 ± 1.03)	**0.024**

Mann Whitney U test, VPI: Video Power Index, JAMA: Journal of the American Medical Association, GQS: global quality scale

Table 4. Statistical findings for the comparison of video length, number of views, number of likes, VPI, Modified DISCERN, JAMA and GQS between groups according to video publisher

	Physicians (n=36)	Private Hospitals (n=8)	P values
Video length (seconds)	149.5 (35 – 1,164) (232.4 ± 224)	150 (42 - 285) (137.2 ± 81.9)	**0.007**
Number of views	42,512.5 (11,736 – 679,985) (81,837.9 ± 124,184.4)	16,903 (14,891 – 175,531) (45,089.8 ± 56,079.2)	0.393
Number of likes	165.5 (26 – 2,189) (371.8 ± 503.8)	49 (16 - 125) (52.38 ± 34.15)	0.060
VPI	31.75 (8 - 247.1) (45.20 ± 43.19)	13.6 (3.69 - 67.97) (20.3 ± 20.2)	**0.001**
Modified DISCERN	3 (1 - 5) (3.08 ± 1.07)	3 (0 - 4) (2.25 ± 1.38)	0.111
JAMA	3 (1 - 4) (2.72 ± 0.7)	3 (2 - 3) (2.87 ± 0.35)	0.622
GQS	3 (1 - 5) (3.56 ± 1.13)	3 (1 - 4) (2.88 ± 1.12)	0.189

Mann Whitney U test, VPI: Video Power Index, JAMA: Journal of the American Medical Association, GQS: global quality scale

Table 5. Statistical findings for correlation analysis between video characteristics and Modified DISCERN, JAMA and GQS scale scores (n=45)

		Modified DISCERN	JAMA	GQS
Time since upload (days)	r	0.049	0.008	0.114
	P	0.749	0.956	0.457
Video length (seconds)	r	0.029	-0.028	0.072
	P	0.850	0.855	0.639
Number of views	r	-0.021	-0.202	0.100
	P	0.892	0.183	0.515
Number of likes	r	0.046	-0.144	0.136
	P	0.764	0.344	0.375
Number of dislikes	r	0.180	0.028	0.232
	P	0.237	0.854	0.125
Comments	r	0.203	-0.184	0.068
	P	0.258	0.307	0.709
View ratio	r	-0.094	-0.273	-0.014
	P	0.538	0.069	0.929
Like ratio	r	-0.109	-0.069	-0.222
	P	0.475	0.651	0.143
VPI	r	-0.120	-0.276	-0.049
	P	0.431	0.066	0.750

VPI: Video Power Index, JAMA: Journal of the American Medical Association, GQS: global quality scale

Table 6. Statistical findings for the correlation analysis between the features of the videos (n=45)

		Time since upload (days)	Video length (seconds)
Number of views	r	**0.514**	0.128
	P	**<0.001**	0.404
Number of likes	r	0.181	**0.602**
	P	0.233	**<0.001**
Number of dislikes	r	**0.622**	0.282
	P	**<0.001**	0.061
Comments	r	-0.020	**0.392**
	P	0.912	**0.024**
View ratio	r	-0.191	0.259
	P	0.209	0.086
Like ratio	r	**-0.733**	-0.017
	P	**<0.001**	0.910
VPI	r	-0.271	0.274
	P	0.071	0.069

VPI: Video Power Index, JAMA: Journal of the American Medical Association, GQS: global quality scale

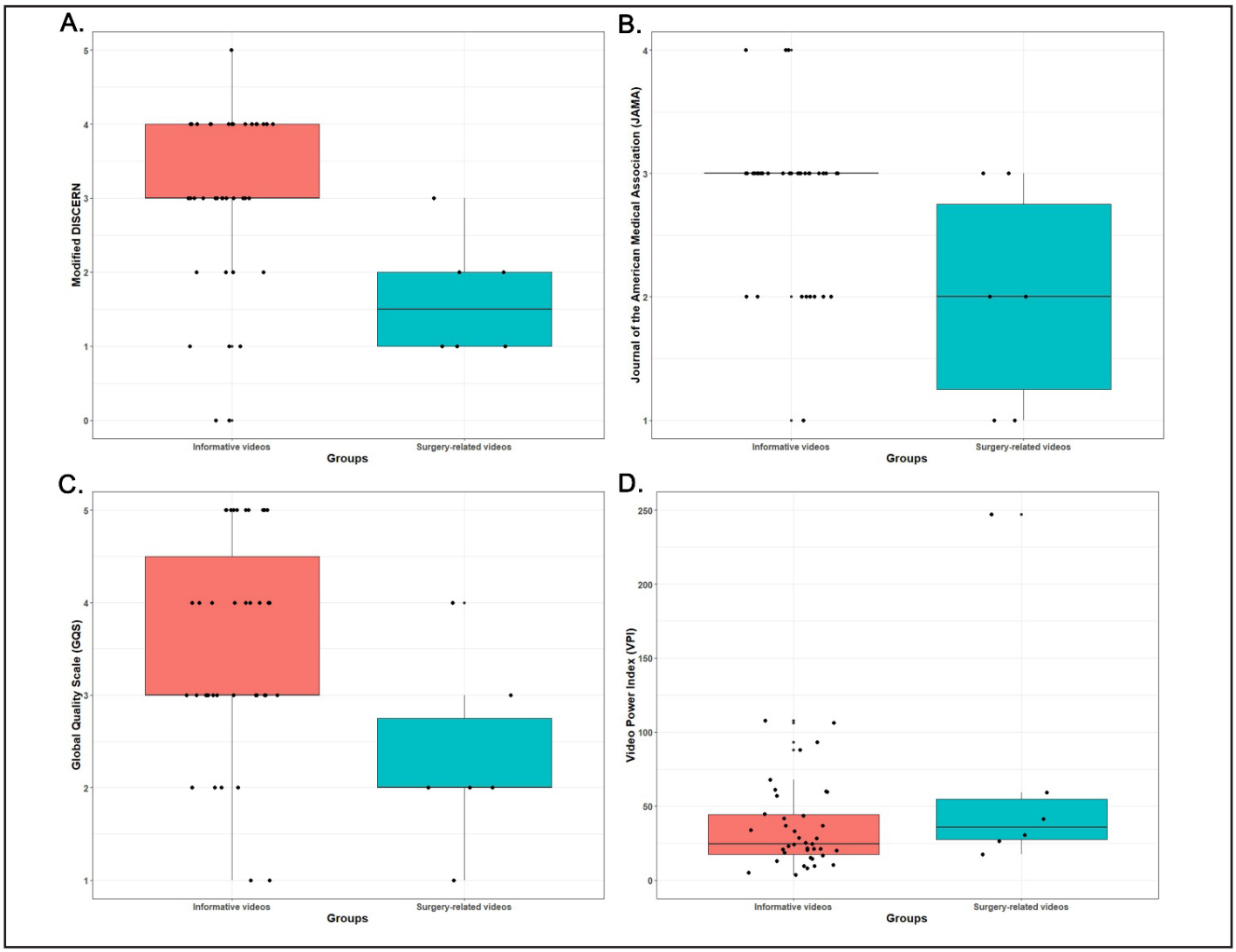

Figure 1. Boxplot with jitter showing the distribution of mDISCERN, Journal of the American Medical Association (JAMA), and Global Quality Scale (GQS) scores, and Video Power Index (VPI) valuesamong informative and surgery-related videos

DISCUSSION

Circumcision is one of the most widely applied surgical procedures worldwide due to religious, cultural, and medical reasons. Despite its short duration and frequency for physicians, parents face important decisions regarding the timing, type of anesthesia, and technique of circumcision, aside from medical necessities. During this anxious decision-making phase, families are most concerned about the appropriate ages for circumcision, pre-circumcision preparation, type of anesthesia, fasting duration, circumcision technique, hospital stay duration, and post-discharge process. Nowadays, parents can research their questions about circumcision before visiting a healthcare facility, and the information they obtain from social media influences their decision-making process and expectations.

YouTube has become a widely used source for gathering health information [9]. The quality and reliability of YouTube videos have been researched for various diseases [17-24]. In surveys, 86% of individuals using the internet to access health-related information believe the information is reliable, and 64% say it influences their treatment decisions [25]. In our study, we analyzed YouTube videos about circumcision. We found that the overall quality and reliability scores were moderate.

Zaliznyak et al. (2022) reported poor quality in their study analyzing 100 videos on neonatal circumcision on YouTube [26]. In contrast, our study found that circumcision videos were of higher quality and reliability. Ekenci et al. (2023) analyzed

50 videos on hydrocele on YouTube and found no videos containing useful, complete, and clear information for patients, with GQS and mDISCERN scale scores of 5 [27]. In our study, we identified 10 videos with a GQS score of 5. One of these videos had perfect scores across all assessment scales. This video, uploaded by a pediatric surgery and urology specialist, explained the appropriate age and methods for circumcision. The other videos did not mention additional sources of information for viewers, preventing them from achieving full mDISCERN scale scores. Only 3 out of 45 videos had a perfect JAMA scale score of 4. The other videos did not cite sources for the shared information about circumcision. To make the videos more reliable for viewers, more videos should cite sources and provide additional information, addressing controversial and uncertain topics.

When categorizing the content of informative videos by topic, "post-circumcision care" was the most frequently covered topic. The most viewed informative video was also about post-circumcision care. Only 2 videos mentioned complications, and 2 videos covered all aspects of circumcision generally. It can be said that more high-quality and reliable videos are needed on social media to provide comprehensive information about circumcision in a single video.

Of the 45 videos selected based on inclusion and exclusion criteria, 39 (86.7%) were informative, and only 6 (13.3%) were surgical videos. Among the 45 videos, the most viewed video was about the surgical technique of circumcision. Numerically, surgical videos had higher view counts, likes, and VPI values compared to informative videos, but this difference was not statistically significant. Despite being more viewed, the number of surgical videos was very low, and they were of lower quality compared to informative videos. Ekenci et al. (2023) found a similar low number of surgical videos in their study, but they did not compare the scale scores of surgical videos with informative videos [27]. In our study, all scale scores of informative videos were statistically significantly greater compared to the surgical videos. Four of the surgical videos only had visuals without any verbal or written information about the circumcision procedure, resulting in low mDISCERN, JAMA, and GQS scale scores. Based on these results and considering the view counts, it can be concluded that the number and quality of surgical videos should be increased. Additionally, there is a need for high-quality, reliable, and educational surgical circumcision videos produced by healthcare professionals that show all techniques and provide information, beneficial not only for families but also for trainee doctors.

Except for one, all informative videos were uploaded by physicians. The majority of these were shared by specialists in pediatric surgery, pediatric urology, and urology. Additionally, two pediatricians and one obstetrician-gynecologist had published informative videos on circumcision. Most of the physicians uploading circumcision informative videos were independent practitioners, with others working in private hospitals. Numerically, the view counts, likes, mDISCERN, and GQS scores of videos posted by physicians were higher compared to those posted by private hospitals, while JAMA scale scores were lower; however, this variation was not statistically significantly different. Thus, the quality of videos related to circumcision uploaded by independent practitioners and private hospitals was similar. This similarity is thought to be due to the involvement of physicians in the private hospital videos providing information on circumcision. One video uploaded by an independent user had very low mDISCERN, GQS, and JAMA scale scores (0, 1, and 1, respectively). Consistent with our findings, Barry et al. (2023) found that videos by healthcare professionals were of higher quality in their study investigating the quality and reliability of circumcision videos on YouTube [28]. Zaliznyak et al. (2022) also found that impartial videos produced by health channels or featuring physicians had the highest quality ratings in their study on neonatal circumcision videos, consistent with our study [26]. Nason et al. (2012) found that videos uploaded by academicians and physicians were of higher quality and reliability in their study on hydrocele videos [29]. Based on our findings, we can suggest that families should pay attention to whether the videos they watch are uploaded by a specialist physician in the field, as this would provide them with more reliable information.

In our study, consistent with Barry et al.'s (2023) findings on circumcision [28], no statistically significant relationship was found between mDISCERN, JAMA, and GQS scale scores and the duration since video publication, video duration, view count, like count, dislike count, comment count, view ratio, like ratio, and VPI values. Based on these data, we can say that popular videos do not always provide the most accurate information and that misleading content can also achieve high view and like counts. We suggest that viewers should not only consider the number of likes, dislikes, and comments when evaluating the information in videos.

Older circumcision videos had more views, while recently published videos received more likes. However, recent videos also did not sufficiently address additional sources of information, controversial and uncertain topics for viewers.

Limitations

This study has some limitations. Firstly, only the YouTube platform was used for video selection, excluding other social media and video-sharing platforms. This may limit the generalizability of the findings, but YouTube is the most important international video-sharing platform for patients and their families, addressing this limitation. Additionally, due to the current nature of the YouTube platform, video view counts change over time. Therefore, it should be known that the findings of video analyses based on such platforms may change over time. Secondly, subjective scales were used to evaluate the quality and reliability of the videos, which may reflect personal differences among evaluators. The high level of agreement among experts in our study addresses this limitation. However, more comprehensive new video evaluation scales could be used in future studies. Since the purpose of this study was to analyze Turkish videos on circumcision, the exclusion of videos in other languages can be considered another limitation. Furthermore, due to the small sample size of surgical videos in the groups created regarding the content of the videos, we recommend that future analyses be conducted with a larger number of surgical videos.

CONCLUSIONS

Based on our findings, we can say that the information patients obtain about circumcision from YouTube videos is not sufficiently reliable, high-quality, or adequate. Compared to other studies in the literature, Turkish circumcision videos were found to be more reliable and of higher quality, but still not sufficient. Videos uploaded by physicians were more reliable and of higher quality than those published by private hospitals, and informational videos were more reliable and of higher quality than surgical videos. It was observed that unreliable videos could also achieve high view and like counts. Patients and their families should prioritize videos uploaded by specialists in the field over view and like counts when evaluating videos. There is a need for more high-quality educational surgical circumcision videos created by expert physicians and more high-quality informational videos that are short, clear, unbiased, address controversial issues, and include necessary sources. This would increase families' accurate knowledge about circumcision, thereby reducing unnecessary anxiety and expectations. YouTube is a widely viewed information source with the potential to influence patients' knowledge and behavior about circumcision. We believe that quality standards for the verification and monitoring of health information videos should be implemented to ensure their accuracy and reliability.

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REFERENCES

[1] Morris BJ, Wamai RG, Henebeng EB, Tobian AA, Klausner JD, Banerjee J, et al. (2016) Estimation of country-specific and global prevalence of male circumcision. Population health metrics 14:4. https://doi.org/10.1186/s12963-016-0073-5

[2] Bar-Yaakov N, Mano R, Ekstein M, et al. (2022) Parental Regret Following Decision to Revise Circumcision. Frontiers in pediatrics 10:855893. https://doi.org/10.3389/fped.2022.855893

[3] Weiss HA, Larke N, Halperin D, Schenker I (2010) Complications of circumcision in male neonates, infants and children: a systematic review. BMC urology 10:2.

https://doi.org/10.1186/1471-2490-10-2

[4] Pippi Salle JL, Jesus LE, Lorenzo AJ, Romão RL, Figueroa VH, et al (2013) Glans amputation during routine neonatal circumcision: mechanism of injury and strategy for prevention. Journal of pediatric urology 9:763–768. https://doi.org/10.1016/j.jpurol.2012.09.012

[5] Ceylan K, Burhan K, Yilmaz Y, Can S, Kuş A, Mustafa G (2007) Severe complications of circumcision: an analysis of 48 cases. Journal of pediatric urology 3:32–35. https://doi.org/10.1016/j.jpurol.2006.02.009

[6] Prabhakaran S, Ljuhar D, Coleman R, Nataraja RM (2018) Circumcision in the paediatric patient: A review of indications, technique and complications. Journal of paediatrics and child health, 54: 1299–1307. https://doi.org/10.1111/jpc.14206

[7] Sturiale A, Dowais R, Porzio FC, et al. (2020) Youtube as a Source of Patients' and Specialists' Information on Hemorrhoids and Hemorrhoid Surgery. Reviews on recent clinical trials 15:219–226. https://doi.org/10.2174/1574887115666200525001619

[8] Farsi D (2021) Social media and health care, part I: literature review of social media use by health care providers. Journal of medical internet research 23(4), e23205.

[9] Jildeh TR, Okoroha KR, Guthrie ST, Parsons TW (2019) Social Media Use for Orthopaedic Surgeons. JBJS reviews 7: e7. https://doi.org/10.2106/JBJS.RVW.18.00085

[10] Patrick M, Venkatesh RD, Stukus DR (2022) Social media and its impact on health care. Annals of Allergy, Asthma & Immunology 128:139-145. https://doi.org/10.1016/j.anai.2021.09.014

[11] Madathil KC, Rivera-Rodriguez AJ, Greenstein JS, Gramopadhye AK (2015) Healthcare information on YouTube: A systematic review. Health informatics journal 21:173-194. https://doi.org/10.1177/1460458213512220

[12] Charnock D, Shepperd S, Needham G, Gann R (1999) DISCERN: an instrument for judging the quality of written consumer health information on treatment choices. Journal of epidemiology and community health 53:105-111. https://doi.org/10.1136/jech.53.2.105

[13] Singh AG, Singh S, Singh PP (2012) YouTube for information on rheumatoid arthritis--a wakeup call?. The Journal of rheumatology 39: 899-903. https://doi.org/10.3899/jrheum.111114

[14] Silberg WM, Lundberg GD, Musacchio RA (1997) Assessing, controlling, and assuring the quality of medical information on the Internet: Caveant lector et viewor--Let the reader and viewer beware. JAMA 277: 244–1245.

[15] Bernard A, Langille M, Hughes S, Rose C, Leddin D, Van Zanten SV (2007) A systematic review of patient inflammatory bowel disease information resources on the World Wide Web. The American journal of gastroenterology 102:2070–2077. https://doi.org/10.1111/j.1572-0241.2007.01325.x

[16] Erdem MN, Karaca S (2018) Evaluating the Accuracy and Quality of the Information in Kyphosis Videos Shared on YouTube. Spine 43:1334-1339. https://doi.org/10.1097/BRS.0000000000002691

[17] Anadolulu Aİ, Gerçel G, Durakbaşa ÇU (2023) Quality Assessment of YouTube Videos as an Information Source for Bowel Management in Children. Journal of pediatric surgery 58:2343-2346. https://doi.org/10.1016/j.jpedsurg.2023.08.014

[18] Ranade AS, Belthur MV, Oka GA, Malone JD (2020) YouTube as an information source for clubfoot: a quality analysis of video content. Journal of pediatric orthopedics. Part B 29:375-378. https://doi.org/10.1097/BPB.0000000000000694

[19] Semerci R, Şimşek E, Savaş E, Orhan E, Erbey F (2024) The quality and content analysis of YouTube videos about chemotherapy for children. Pediatric blood & cancer 71:30865. https://doi.org/10.1002/pbc.30865

[20] Toprak T, Tokat E. A quality analysis of nocturnal enuresis videos on YouTube. J Pediatr Urol. 2021 Aug;17:449.e1-449.e6. https://doi.org/10.1016/j.jpurol.2021.03.014

[21] Nishizaki N, Hirano D, Oishi K, Shimizu T (2022) YouTube videos in Japanese as a source of information on nocturnal enuresis: A content-quality and reliability analysis. Pediatrics international : official journal of the Japan Pediatric Society 64:15049. https://doi.org/10.1111/ped.15049

[22] Duymaz YK, Şahin Ş, Erkmen B, Uzar T, Önder S (2023)

Evaluating YouTube as a source of patient information for pediatric tracheostomy care. International journal of pediatric otorhinolaryngology 171:111580. https://doi.org/10.1016/j.ijporl.2023.111580

[23] Bai G, Pan X, Zhao T, Chen X, Liu G, Fu W (2022) Quality Assessment of YouTube Videos as an Information Source for Testicular Torsion. Frontiers in public health 10:905609. https://doi.org/10.3389/fpubh.2022.905609

[24] Hakami Z, Maganur PC, Khanagar SB, Naik S, Alhakami K, Bawazeer OA, Alassiry AM, Vishwanathaiah S (2022) Thumb-Sucking Habits and Oral Health: An Analysis of YouTube Content. Children (Basel, Switzerland) 9:225. https://doi.org/10.3390/children9020225

[25] Bağcı N, Taka K, Peker İ (2021) Ağız Kanseri Konusundaki YouTube Videolarının değerlendirilmesi. Yeditepe J Dent 17:102-107 https://doi.org/10.5505/yeditepe.2021.38278

[26] Zaliznyak M, Masterson JM, Duel B (2022) YouTube as a source for information on newborn male circumcision: Is YouTube a reliable patient resource?. Journal of pediatric urology 18:678.e1-678.e7. https://doi.org/10.1016/j.jpurol.2022.07.011

[27] Ekenci BY, Sarı H (2023) How quality and reliable are youtube videos about hydrocele for patients? Androl Bul 25:27-31 https://doi.org/10.24898/tandro.2023.75010

[28] Barry R, Murray G, Kravvas G, Bunker CB, Watchorn RE (2023) Circumcision and social media. A cross-sectional analysis of circumcision videos on Youtube. International journal of STD & AIDS 34: 505–507. https://doi.org/10.1177/09564624231163628

[29] Nason GJ, Tareen F, Quinn F (2013) Hydrocele on the web: an evaluation of Internet-based information. Scandinavian journal of urology 47:152–157. https://doi.org/10.3109/00365599.2012.719540

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Original Research

Effects of Pinealectomy and Melatonin Supplementation on Elements Metabolism in Rat Testicular Tissue

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ABSTRACT

Objective: The aim of this study was to investigate how pinealectomy and melatonin application affect elements metabolism in rat testicular tissue.

Methods: The study was carried out on 32 adult male Spraque-Dawley rats. Animals were divided into 4 equal groups. Group 1: Control, Group 2: Melatonin, Group 3: Pinealectomy, Group 4: Pinealectomy+Melatonin. Group 2 and 4 animals received daily 3mg/kg intraperitoneal (ip) melatonin supplementation for 4 weeks. The pineal glands of Group 3 and 4 animals were removed under general anesthesia. At the end of the applications, testicular tissue samples were taken from the animals sacrificed under general anesthesia. Elemental determinations (µg/gram/tissue) were performed in testicular tissue samples using the atomic emission method.

Results: The highest cobalt, molybdenum, nickel, manganese, phosphorus, and sodium levels ($p<0.001$) and the lowest potassium levels in the testicular tissue were obtained in the pinealectomy group (group 3) ($p<0.001$). Magnesium and selenium values in testicular tissue were highest in the pinealectomy group (group 3) ($p<0.001$), and were higher in the pinealectomy+melatonin group (group 4) than ingroup 1 (control) and group 2 (melatonin) ($p<0.001$). Testicular zinc levels were highest in group 2, where melatonin was administered, and lowest in group 3, which was the pinealectomy group ($p<0.001$).

Conclusion: The findings obtained as a result of the study show that pinealectomy significantly disrupts element metabolism in the testicular tissue of rats, and melatonin supplementation may have a regulatory effect on testicular elemental metabolism.

Keywords: Pinealectomy; melatonin administration; testicular elemental metabolism; rat.

INTRODUCTION

Melatonin (MEL) is a hormone produced mainly by the pineal gland in the brain, which is defined as a neuroendocrine organ [1]. MEL, which plays a role in the sleep-wake cycle, is also a powerful antioxidant that clears free radicals directly and indirectly [2]. MEL, which also serves as a control mechanism that prevents the early onset of puberty, is closely related to the reproductive system [3]. High concentrations of the MEL lead

to a delay in biochemical reactions that regulate puberty in both girls and boys [4]. The results of some reports show that MEL can also be found in testicular tissue [5]. This situation occurs when MEL crosses the blood-testis barrier [5, 6]. The strong antioxidant effect of melatonin has also been shown to prevent testicular tissue damage caused by chemotherapeutic agents [7, 8]. MEL also has a protective effect on the testicles against local inflammatory processes and lipid peroxidation [9]. Therefore, it is recommended that melatonin can be used in the treatment of infertility [10]. As a result, MEL, as an endogenous antioxidant, emerges as a strong candidate for the treatment of functional disorders caused by testicular tissue damage in the male reproductive system [11]. In addition to its known effects, there are limited publications on how MEL affects element metabolism in testicular tissue [11]. In these limited publications, it is mostly related to zinc metabolism, which is an important trace element [12]. The relationship between melatonin and elements is either the prevention of oxidative stress caused by some toxic elements in the testicular tissue with MEL support [13] or the combined application of some antioxidant elements with MEL in the prevention of oxidative stres [11, 14]. Although the effects of the pineal gland, an important neuroendocrine gland, on the male reproductive system are known, there is almost no research on how it affects the element metabolism in the testicular tissue. This study focused on the relationship between the pineal gland and testicular tissue.

MATERIALS AND METHODS

Animal Material and Groups

This study was carried out at Yeditepe University Experimental animals were obtained from the Experimental Medicine Research Institute of the same university. The study was conducted on Spraque-Dawley adult male rats. The study protocol was approved by the local ethics committee of the same center (21122009). A total of 32 male rats were used in the study and the animals were divided into 4 equal groups.

Group 1, Control: The group in which no application is made.

Main Points;

- Taken together, the results of our study highlight the important relationship between pineal gland and testicular element metabolism.
- This study is the first to investigate pineal gland and testicular element metabolism as a whole.

Group 2, Melatonin: Animals in this group were provided with melatonin support. MEL was given intraperitoneally (ip) (3 mg/kg/day for 4 weeks)

Group 3, Pinealectomy: The pineal glands of the animals in this group were removed under general anesthesia in order to create melatonin deficiency.

Group 4, Pinealectomy+Melatonin: Animals whose pineal glands were removed under general anesthesia were provided with MEL support via ip (3 mg/kg/day for 4 weeks).

Experimental Animals and Their Nutrition

Experimental animals were fed standard rat chow and tap water without dietary restrictions. At the end of the experimental phases, the animals were sacrificed and testicular tissue samples were taken for elemental analysis.

Sacrifice of Animals and Collection of Tissue Samples

After the completion of the experimental stages of the study, all animals were sacrificed under general anesthesia and serum samples were taken. General anesthesia was administered to all animals (with intramuscular administration of a combination of Ketalar (60 mg/kg), Parke-Davis and xylazine (5 mg/kg) "Rompun, Bayer") to avoid animal suffering. Testicular tissue samples taken from animals were stored at -80^{0}C until analysis.

Experimental Applications

MEL Application: Melatonin was commercially available (Sigma M-5250). Stock solution was prepared by dissolving melatonin in pure ethanol. A dose of 3 mg/kg MEL was prepared daily from the stock solution. MEL was injected into the peritoneal cavity of the animals in Groups 2 and 4 daily for 4 weeks [15].

Pinealectomy

Two methods attract attention in preventing the functions of the pineal gland in rats. The first of these is to suppress the function of the pineal gland by creating strong artificial light at night. The second method is to surgically remove the pineal gland. Surgical method was preferred in the current study. Pinealectomy was performed under general anesthesia in accordance with the method determined by Kuszack and Rodin [16]. A combination of ketamine hydrochloride (Ketalar, Parke-Davis) at a dose of 60 mg/kg and xylazine (Rompun, Bayer) at a dose of 5 mg/kg was used to perform pinealectomy operations.

The head of the experimental animal was placed in the stereotactic device. After the top of the skull skin was shaved, a 1.75 cm medial incision was made longitudinally, reaching the occipital protrusion. The periosteum of the bones where the sagittal and lambdoid sutures were located was scraped down to the temporal muscles. Then, with a dental drill, the skull bone was cut rostrocaudally, approximately 1.25 cm rectangularly and 0.75 cm mediolaterally. The bone fragment on the superior sagittal vein, transfer sinuses, and confluent sinum was held from the rostral angle and lifted. The dura was cut from the mediorostral edge of the transfer sinus along the lateral edges of the sagittal vein. Two ligatures were placed on the superior sagittal vein with 6-0 atraumatic silk, 1 mm apart. The sagittal sinus was cut between the two ligatures, and its posterior part was cut following the dissection of the dura until the pineal gland was exposed. The pineal gland was removed from the anterior side by grasping the stem with a thin-tipped forceps. Then, both ends of the sagittal vein were tied together and the skull skin was sutured with 5-0 silk.

Biochemical Analysis

Elements Analyzes in Testicular Tissue

Testicular tissue samples taken from experimental animals were placed in polyethylene capped tubes washed with NHO3 and deionized water to prevent contamination. Samples were stored at -80 C until the day of analysis. Then, the tissue samples were crushed into powder in a mortar and the wet weight of the tissue was recorded. Concentrated H2SO4 and concentrated HNO3 were added (gram tissue /ml H2SO4 / ml HNO3 = 1 / 1 / 10). It was kept in a closed system microwave oven (CEM – Marsx5) at 170 psa pressure and 200C for 20 minutes. Then, the final volume was filled to 25 ml with deionized water and the samples were read by waiting for a maximum of half an hour. Analysis process, S. Ü. It was carried out on the Atomic Emission (ICP – AES Varian Australia Pty LTD, Australia) device located in the Soil Department of the Faculty of Agriculture. Results were calculated as µg/gram tissue.

Statistical Analysis

Statistical evaluation of the results was made with SPSS 22.0 statistical software. Statistical analysis was performed with a computer package program. The following tests were applied respectively.

1. Arithmetic means and standard errors of all parameters were calculated.
2. Analysis of variance was applied to determine the differences between groups.
3. Least Significant Difference (LSD) Test was used to compare group averages in the variance analysis results that were found to be statistically significant. Differences at $P<0.05$ were considered significant.

RESULTS

No significant difference was detected between chromium, lead, calcium, sulfur, copper and iron parameters in the testicular tissue of the study groups.

The highest levels of cobalt, molybdenum, nickel, manganese, phosphorus and sodium in the testicular tissue were obtained in the pinealectomy group (group 3; $p<0.001$). Group 3 also had the lowest potassium levels ($p<0.001$).

Again, the highest magnesium and selenium values in testicular tissue were detected in group 3 ($p<0.001$). The same parameters were higher in group 4 than in groups 1 and 2 ($p<0.001$). Testicular zinc levels were highest in group 2, where MEL was applied, and lowest in group 3, where pinealectomy was applied ($p<0.001$; Table 1-4).

Table 1. Cobalt, Molybdenum, Chromium and Nickel Levels in Testicular Tissue of Study Groups (µg/g)

Groups	Cobalt	Molybdenum	Chrome	Nickel
Control (G1)	0.14±0.04^{B}	0.30±0.17^{B}	0.41±0.09	0.98±0.10^{B}
Melatonin (G2)	0.15±0.09^{B}	0.29±0.09^{B}	0.40±0.07	0.98±0.20^{B}
Px (G3)	0.24±0.10^{A}	0.80±0.47^{A}	0.38±0.04	2.09±1.30^{A}
Px+Melatonin(G4)	0.14±0.06^{B}	0.28±0.13^{B}	0.37±0.04	1.02±0.50^{B}

*Means with different letters in the same column are statistically significant ($P<0.001$).
(A> B)

Table 2. Manganese, Magnesium, Lead and Phosphorus Levels in Testicular Tissue of Study Groups (µg/g)

Groups	Manganese	Magnesium	Lead	Phosphorus
Control (G1)	0.54±0.07^{B}	87.28±12.29^{C}	0.11±0.04	275.2±99.8^{B}
Melatonin (G2)	0.53±0.05^{B}	84.60±14.57^{C}	0.12±0.04	278.4±86.4^{B}
Px (G3)	0.75±0.07^{A}	128.83±8.76^{A}	0.11±0.05	133.6±53.5^{A}
Px+Melatonin(G4)	0.52±0.05^{B}	110.00±8.30^{B}	0.11±0.05	273.9±57.0^{B}

*Means with different letters in the same column are statistically significant (P<0.001).
(A> B>C)

Table 3. Potassium, Sodium, Sulfur and Calcium Levels in the Testicular Tissue of the Study Groups (µg/g)

Groups	Potassium	Sodium	Sulfur	Calcium
Control (G1)	1360.7±87.0^{B}	1237.5±78.4^{A}	865.7±79.3	74.41±17.95
Melatonin (G2)	1372.3±94.9^{A}	1241.6±82.5^{B}	855.8±78.6	82.43±20.20
Px (G3)	980.9±57.4^{B}	1987.6±97.8^{A}	864.9±80.3	86.54±30.42
Px+Melatonin(G4)	1356.5±93.6^{A}	1258.9±92.2^{B}	849.3±69.6	83.10±25.59

*Means with different letters in the same column are statistically significant (P<0.001).
(A> B)

Table 4. Copper, Iron, Selenium and Zinc Levels in Testicular Tissue of the Study Groups (µg/g)

Groups	Copper	Iron	Selenium	Zinc
Control (G1)	2.97±0.54	22.50±2.30	1.71±0.53^{C}	23.17±4.70^{B}
Melatonin (G2)	2.77±0.18	23.20±3.15	0.70±0.35^{D}	29.63±2.85^{A}
Px (G3)	2.55±0.86	22.68±2.70	4.70±1.66^{A}	11.45±1.58^{C}
Px+Melatonin(G4)	2.74±0.11	23.40±2.55	1.80±0.62^{B}	24.62±2.54^{B}

*Means with different letters in the same column are statistically significant (P<0.001). (A> B>C).

DISCUSSION

The highest levels of cobalt, molybdenum, nickel, manganese, magnesium, selenium, and phosphorus in testicular tissue were obtained in the pinealectomy group (group 3). In Med-line searches, we could not find a publication with which we could directly compare our study in terms of the effect of the pineal gland on the element metabolism in the testicular tissue. Publications on the effects of the pineal gland and MEL on the metabolism of body elements other than testicular tissue are also very limited. It has been reported in a few publications that MEL has a regulatory effect on elemental metabolism in the body [17, 18]. Consistent with this, it has been shown that body element metabolism is impaired in rats after pinealectomy [19]. The high levels of cobalt, molybdenum, nickel, manganese, magnesium, selenium, and phosphorus in the testis that we obtained in our study show that MEL deprivation after pinealectomy significantly changes the element metabolism in the testicular tissue and strongly supports the findings of the researchers whose reports are presented above. The fact that MEL application after pinealectomy (group 4) returns the levels of the mentioned elements to control values shows the regulatory effect of MEL on the element metabolism in testicular tissue. Again, the highest sodium levels and lowest potassium levels in testicular tissue were obtained in the pinealectomy group (group 3). This finding we obtained in the testicular tissue is evidence that the sodium-potassium balance is disrupted in the testicular tissue after pinealectomy and is compatible with the reports of Mogulkoc and Baltaci [20] who reported that pinealectomy caused a deterioration in the fluid-

electrolyte balance in rats. In our study, we obtained the highest zinc level in testicular tissue in group 2, where MEL was applied, and the lowest zinc level in group 3, where pinealectomy was applied. Zinc, which is critical in the reproductive system [21], is the only metal found in almost every enzyme class [22]. The high concentration of zinc in both the testicles and glands of the male reproductive system is evidence of its critical importance in the reproductive system [23]. In rats, zinc deficiency causes an atrophic picture in the seminiferous tubules. Consistent with this, it also causes a deficiency in spermatogenesis [24]. Zinc is also critical in the physiological functions of sperm. Zinc is necessary for the integrity of the sperm membrane, regulation of sperm tail motility, and coordination of the spiral movements of the sperm tail [25]. There are also important relationships between zinc and MEL, which play an important role in testicular tissue and the male reproductive system [24]. While MEL increases the absorption of zinc, an important trace element, from the digestive system [26], on the contrary, pinealectomy results in zinc deficiency in the body [27, 28]. In our study, the low zinc levels we obtained after pinealectomy (group 3) or the high zinc levels we obtained after MEL application alone (group 2) are not only evidence of a significant and positive relationship between the pineal gland and zinc, but are also compatible with the studies whose findings are presented above. Defined as metal binding proteins, metallothioneins (MT) are low molecular weight proteins that play an important role in protecting against metal toxicity [29, 30]. The presence of specific binding sites for MEL in the intestines suggests that the pineal gland may be a fundamental mechanism in regulating the absorption of elements in the digestive system [31, 32]. In our study, the altered elemental metabolism in the testicular tissue obtained by pinealectomy or MEL application may be related to the altered elemental absorption in the gastrointestinal tract.

Taken together, the results of our study show that pinealectomy significantly disrupts elemental metabolism in the testicular tissue of rats, and MEL application may have a regulatory effect on testicular elemental metabolism. The current study is the first to consider elemental metabolism in the testicular tissue combined with the pineal gland and MEL. The results of the current study are at a level that will provide additional contributions to the known relationship between the pineal gland and the male reproductive system in terms of element metabolism.

Limitations

The limiting factor in the current study is that the relationship between the pineal gland and testicular tissue could not be demonstrated with various melatonin doses and application times. Addressing this gap in future studies may provide us with new and critical information.

CONCLUSION

Taken together, the results of our study highlight the important relationship between pineal gland and testicular element metabolism. This study is the first to investigate pineal gland and testicular element metabolism as a wholein rats.

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Informed Consent: The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethical Approval: This study was conducted in accordance with the Declaration of Helsinki. The study protocol was approved by the Experimental Animal Ethics Committee of Yeditepe University Experimental Medicine Research Institute (2009–21/12). This research was done on animals (rats).

Author Contributions: Conception**:** AU;ZK;BY – Design:RM; AKB - Supervision:RM; AKB- Fundings: -Materials:ZK - Data Collection and/or Processing:AU; ZK - Analysis and/or Interpretation: AU; ZK - Literature: AU; ZK - Review: AU; ZK - Writing: AU- Critical Review:RM;AKB

REFERENCES

[1] Ghorbani F, Karimi S, Boustan A, Ebrahimzadeh-Bideskan A, Saburi E (2023) Effect of melatonin on male offspring testis and sperm parameters in BALB/c mice after exposing their mother to METHamphetamine during pregnancy and lactation. Iran J Basic Med Sci 26(7):777-784. https://doi.org/10.22038/IJBMS.2023.69608.15158

[2] Joseph TT, Schuch V, Hossack DJ, Chakraborty R, Johnson EL (2024) Melatonin: the placental antioxidant and anti-inflammatory. Front Immunol 15:1339304. https://doi.org/10.3389/fimmu.2024.1339304

[3] Uğurlu AK, Bideci A, Demirel AM, Kaplanoğlu GT, Dayanır D, Gülbahar Ö, Bulut TSD, Döğer E, Çamurdan MO (2023) Is blue light exposure a cause of precocious puberty in male rats? Front Endocrinol (Lausanne) 14:1190445. https://doi.org/10.3389/fendo.2023.1190445

[4] Li C, Zhou X (2015) Melatonin and male reproduction. Clin Chim Acta 446:175-180. https://doi.org/10.1016/j.cca.2015.04.029.

[5] Zhang Y, Liu X, Bai X, Lin Y, Li Z, Fu J, Li M, Zhao T, Yang H, Xu R, Li J, Ju J, Cai B, Xu C, Yang B (2018) Melatonin prevents endothelial cell pyroptosis via regulation of long noncoding RNA MEG3/miR-223/NLRP3 axis. J Pineal Res 64(2). https://doi.org/10.1111/jpi.12449

[6] Zhang T, Zhou Y, Li L, Zhao Y, De Felici M, Reiter RJ, Shen W (2018) Melatonin protects prepuberal testis from deleterious effects of bisphenol A or diethylhexyl phthalate by preserving H3K9 methylation. J Pineal Res 65(2):e12497. https://doi.org/10.1111/jpi.12497

[7] Cao M, Wang Y, Yang F, Li J, Qin X (2021) Melatonin rescues the reproductive toxicity of low-dose glyphosate-based herbicide during mouse oocyte maturation via the GPER signaling pathway. J Pineal Res 70(3):e12718. https://doi.org/10.1111/jpi.12718

[8] Miao Z, Miao Z, Teng X, Xu S (2022) Melatonin alleviates lead-induced intestinal epithelial cell pyroptosis in the common carps (Cyprinus carpio) via miR-17-5p/TXNIP axis. Fish Shellfish Immunol 131:127-136. https://doi.org/10.1016/j.fsi.2022.09.071

[9] Zhang B, Zhong Y, Du J, Ye R, Fan B, Deng Y, Bai R, Feng Y, Yang X, Huang Y, Liang B, Zheng J, Rong W, Yang X, Huang Z [2024) 1,2-Dichloroethane induces testicular pyroptosis by activating piR-mmu-1019957/IRF7 pathway and the protective effects of melatonin. Environ Int 184:108480. https://doi.org/10.1016/j.envint.2024.108480

[10] [10] Heidarizadi S, Rashidi Z, Jalili C, Gholami M (2022) Overview of biological effects of melatonin on testis: A review. Andrologia 54(11):e14597. https://doi.org/10.1111/and.14597

[11] Semercioz A, Baltaci AK, Mogulkoc R, Avunduk MC (2017) Effect of zinc and melatonin on oxidative stress and serum Inhibin-B levels in a rat testicular torsion-detorsion model. Biochem Genet 55(5-6):395-409. https://doi.org/10.1007/s10528-017-9826-5

[12] Baltaci AK, Mogulkoc R, Baltaci SB (2019) Review: The role of zinc in the endocrine system. Pak J Pharm Sci 2019 ;32(1):231-239.

[13] Li T, Lv Y, Wu Z, Guo M, Liu R, Zeng W, Zheng Y (2023) Systematic assessment of hexavalent chromium-induced damage to male fertility and the preventive role of melatonin: a longitudinal study from the translational point of view. Mol Hum Reprod 2023;29(7):gaad020. https://doi.org/10.1093/molehr/gaad020

[14] Baş E, Nazıroğlu M (2019) Treatment with melatonin and selenium attenuates docetaxel-induced apoptosis and oxidative injury in kidney and testes of mice. Andrologia. 51(8):e13320. https://doi.org/10.1111/and.13320.

[15] Tuncer I, Sunar F, Toy H, Baltaci AK, Mogulkoc R (2011) Histological effects of zinc and melatonin on rat testes. Bratisl Lek Listy. 2011;112(8):425-427.

[16] Kuszack J, Rodin MA (1977) New technique of pinealectomy for adult rats. Pro Experimentis 32:283-284. https://doi.org/10.1007/BF02124114.

[17] Boguszewska A, Pasternak K (2024) [Melatonin and bio-elements]. Pol Merkur Lekarski 17(101):528-529.

[18] Cemek M, Emin Büyükokuroğlu M, Yürümez Y, Yavuz Y, Aslan A, Büyükben A, Aymelek F (2010) Tissue trace and major element levels in organophosphate insecticide fenthion (Lebaycid) toxicity in rats: prophylactic and therapeutic effect of exogenous melatonin. Ecotoxicol Environ Saf 73(2):206-212. https://doi.org/10.1016/j.ecoenv.2009.09.008

[19] Turgut M, Yenisey C, Bozkurt M, Ergin FA, Biçakçi T (2006) Analysis of zinc and magnesium levels in pinealectomized chicks: roles on development of spinal deformity? Biol Trace Elem Res 113(1):67-75. https://doi.org/10.1385/BTER:113:1:67

[20] Mogulkoc R, Baltaci AK (2008) The effect of pinealectomy on plasma vasopressin response to isotonic, hypertonic and hypovolemic treatments in rats supplemented with L-thyroxine. Acta Biol Hung 2008 59(2):163-72. https://doi.org/10.1556/ABiol.59.2008.2.3

[21] Te L, Liu J, Ma J, Wang S (2023) Correlation between serum zinc and testosterone: A systematic review. J Trace Elem Med Biol 76:127124. https://doi.org/10.1016/j.jtemb.2022.127124.

[22] Skalny AV, Aschner M, Tinkov AA (2021) Zinc. Adv Food Nutr Res 296:251-310. https://doi.org/10.1016/bs.afnr.2021.01.003.

[23] Osadchuk LV, Danilenko AD, Osadchuk AV (2021) An influence of zinc on male infertility. Urologiia (5):84-93.

[24] Ozturk A, Baltaci AK, Bediz CS, Mogulkoc R (2003) Güngör S. Effects of zinc and melatonin deficiency on testicular tissue of rats. Biol Trace Elem Res 96(1-3):255-62. https://doi.org/10.1385/BTER:96:1-3:255.

[25] Vickram S, Rohini K, Srinivasan S, Nancy Veenakumari D, Archana K, Anbarasu K, Jeyanthi P, Thanigaivel S, Gulothungan G, Rajendiran N, Srikumar PS (2021) Role of zinc (Zn) in human reproduction: A journey from initial spermatogenesis to childbirth. Int J Mol Sci 22(4):2188. https://doi.org/10.3390/ijms22042188.

[26] Unal O, Baltaci AK, Mogulkoc R, Avunduk MC (2021) Effect of pinealectomy and melatonin supplementation on metallothionein, ZnT2, ZIP2, ZIP4 and zinc levels in rat small intestine. Biotech Histochem 96(8):623-635. https://doi.org/10.1080/10520295.2021.1885738

[27] Baltaci AK, Mogulkoc R (2007) Pinealectomy and melatonin administration in rats: their effects on plasma leptin levels and relationship with zinc. Acta Biol Hung 58(4):335-343. https://doi.org/10.1556/ABiol.58.2007.4.1

[28] Baltaci AK, Mogulkoc R, Bediz CS, Pekel A (2005) Effects of zinc deficiency and pinealectomy on cellular immunity in rats infected with Toxoplasma gondii. Biol Trace Elem Res 104(1):47-56. https://doi.org/10.1385/BTER:104:1:047

[29] Fujie T, Segawa Y, Yoshida E, Kimura T, Fujiwara Y, Yamamoto C, Satoh M, Naka H, Kaji T (2016) Induction of metallothionein isoforms by copper diethyldithiocarbamate in cultured vascular endothelial cells. J Toxicol Sci 41(2):225-232. https://doi.org/10.2131/jts.41.225.

[30] Kimura T, Kambe T (2016) The Functions of Metallothionein and ZIP and ZnT Transporters: An Overview and Perspective. Int J Mol Sci 17(3):336. https://doi.org/10.3390/ijms17030336.

[31] Lee PP, Pang SF (1992) Identification and characterization of melatonin binding sites in the gastrointestinal tract of ducks. Life Sci 50(2):117-25. https://doi.org/10.1016/0024-3205(92)90293-x.

[32] Lee PP, Pang SF (1993) Melatonin and its receptors in the gastrointestinal tract. Biol Signals 2(4):181-93. https://doi.org/10.1159/000109491.

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Original Research

Evaluation of the Results of Intracytoplasmic Sperm Injection and Microdissection Testicular Sperm Extraction Treatments in Patients with Nonobstructive Azoospermia According to Etiological Factors: A Retrospective Analysis

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ABSTRACT

Objective: This study aimed to retrospectively compare the results of microdissection testicular sperm extraction (microTESE) and Intracytoplasmic sperm injection (ICSI) treatments in nonobstructive azoospermia (NOA) patients with different aetiologies. Determinants (clinical characteristics) for microTESE outcomes were compared between patients with successful sperm retrieval (SSR) and sperm retrieval failure (SRF).

Material and Method: A total of 510 NOA patients who underwent microTESE between January 2015 and January 2024 were included in this study. Patients were classified according to the cause of NOA and SSR, fertilisation rate, clinical pregnancy, and overall live birth rate were evaluated.

Results: The SSR rate was 44.1% in the whole population. The idiopathic patient group had the lowest SSR rate (X^2: 34.81; p<0.01). There was no difference between the groups in terms of fertilisation rate, clinical pregnancy and overall live birth rate. There was a negative correlation between age and SSR rates in patients with idiopathic NOA (t:-0.27; p<0.01). SSR rates were higher in patients with cryptorchidism (right: t:0.8; P:0.003; left: t:0.72; p:0.002) and mumps orchitis (right: t:0.76; P<0.01; left: t:0.76; p=0.003).

Conclusion: Etiology has a significant role in terms of SSR in patients with NOA. SSR was found to be significantly less in patients with idiopathic NOA compared to other causes. In addition, age and testicular volume were significant predictive factors for SSR in patients with idiopathic and acquired NOA.

Keywords: nonobstructive azoospermia, microtese, intracytoplasmic sperm injection, male infertility

INTRODUCTION

Infertility is observed in 8-12% of the population and azoospermia occurs as a cause of this condition in some patients [1]. Azoospermia may result from obstructive and non-obstructive causes [2]. Nonobstructive azoospermia (NOA) is the absence of spermatozoa in semen [3]. Although there are

many factors among the etiologies of NOA, Klinefelter syndrome (KS), Y chromosome microdeletions (YCMDs) cryptorchidism, idiopathic factors and mumps orchitis are the most common etiologies [4].

Intracytoplasmic sperm injection (ICSI) technique is widely used in patients with NOA. The main aim is to extract live spermatozoa for assisted reproductive techniques in these patients [5]. Conventional testicular sperm extraction (cTESE) and microdissection testicular sperm extraction (microTESE) are the methods used to obtain viable spermatozoa [5]. MicroTESE has a higher sperm retrieval rate (SSR) than cTESE. In the literature, the results of microTESE and ICSI treatments in NOA patients differ according to etiologic factors [6-8].

In our study, we aimed to compare the results of microTESE and ICSI treatments in NOA patients according to the etiologic factor.

MATERIALS AND METHODS

Ethics committee approval was obtained from the ethics committee of our hospital with decision number 2024/02-19 on 04.03.2024. Our study was conducted according to the ethical standards of the 1964 Declaration of Helsinki.

A total of 510 NOA patients who underwent microTESE between January 2015 and January 2024 for ICSI were included in this study. Inclusion criteria were absence of sperm in at least 2 spermiogram tests, obstructive azoospermia, lack of radiologic and genetic testing, and hormonal evaluation. Exclusion criteria were defined as the presence of any disease that may be associated with infertility in the woman and patients with incomplete data.

In the study, patients were divided into 5 groups according to the cause of NOA. The patient groups were idiopathic, KS, YCMDs, cryptorchidism and mumps orchitis. A detailed medical history and physical examination were performed in all patients. Semen analysis, hormonal evaluation and scrotal ultrasound were performed to exclude obstructive pathologies. All patients in the study underwent genetic testing after NOA was considered, and patients who could not be diagnosed despite these tests were defined as idiopathic NOA. The study collected microTESE results (SSR ratio) and ICSI results including fertilization, clinical pregnancy and live birth rates. All NOA patients with KS in the study group were non-mosaic Klinefelter males. All patients with YCMD had a partial mutation in the AZFc region on the Y chromosome. In all patients with cryptorchidism, testicular descent to the inguinal region was observed.

Main Points:

- Azoospermia may result from obstructive and nonobstructive etiological factors.
- Etiology has a significant role in terms of SSR in patients with NOA.
- MicroTESE has a higher sperm retrieval rate (SSR) than cTESE
- Idiopathic NOA patients have the lowest SSR rate.
- Age and testicular volume are important parameters for SSR in idiopathic and acquired NOA patients.

Surgical Procedure of MicroTESE

Following the midline incision, the tissues were crossed with blunt and sharp dissections and the tunica albugenia was reached. The tunica albuginea was opened with a sagittal incision of approximately 1,5 cm from the avascular line. The testicular tissue was examined under a microscope at 20 magnification and samples from the seminiferous tubules, which were fuller and more whitish, were taken and sent for examination by the embryologist. If no sperm was found in the examination of the tissues sent from the opened testicle, local anesthesia was applied to the contralateral vas deferens, the testicle was opened and samples were sent from the testicle on that side in the same way and sperm were searched. One sample was taken in pathology without scapating both testes and sent in a bovine solution. Tunica albuginea was closed with 4/0 monocryl. The scrotum skin was closed separately with 3/0 monocryl.

Sperm Processing

The tissues obtained from the microTESE procedure were dissected by the embryologist using two needles. The dissected tissues were analyzed in a micromanipulator under 200 or 400 times magnification. The tissues with spermatozoa were dissected thoroughly and taken into a tube with the help of a pipette. The tube was then vortexed for 35 minutes. The vortexed tube was removed from the incubator until the medium was warmed up. Two tubes were prepared in two layers by placing 90% and 45% gradient solutions (1ml each from bottom to top) in a 15ml conical falcon tube. TESE tissue in the previously

incubated tube was added to the heated conical tube with the help of a pipette without shaking the layers. The conical tubes were centrifuged at 2000 rpm for 20 minutes and the supernatant was removed with a pipette until 0.5 ml remained at the bottom of the tubes. The remaining 0.5ml at the bottom was mixed with 23ml of wash medium. Centrifuged at 1800 rpm for another 10 minutes. The supernatant portion was discarded with a pipette until 0.4ml remained at the bottom of the tube. The remaining portion was placed in the incubator until ICSI.

Intracytoplasmic Sperm Injection Prosedure

Following the hormone protocol, ovulation induction was performed with a single dose of hCG approximately 36 hours before ovum retrieval. Oocyte cumulus complexes were collected by puncture of follicles under ultrasound guidance and placed in a universal IVF medium. After 30 seconds of hyaluronidase (80IU/ml) application, the cumulus was separated from the oocyte by up and down movements with a Pasteur pipette. Oocytes were incubated at 37°C and 5% CO2 until injection. A pool was created with the help of mediums to put sperm in the sterile polystyrene IVF container. The prepared sperm was added to the pool with a pipette. The spermatozoa were placed in PVP (a liquid that slows down sperm movement and allows the micropipette to draw sperm under control) solution with the help of a micropipette. The sperm whose tail was broken and immobilized with the help of a micropipette was removed from the PVP solution and an ICSI procedure was performed.

Definitions of ICSI Outcomes

Clinical outcomes were determined as fertilization, clinical pregnancy and live birth rates. Fertilization was defined as the presence of two pronuclei (2pn) and two polar bodies after the ICSI procedure. Pregnancy was defined as a spontaneous increase in HCG levels after >10 days. Clinical pregnancy was defined as the appearance of a gestational sac on USG >5 weeks after ICSI.

Statistical Analysis

Statistical analysis in this study was performed with SPSS version 22.0 (SPSS Inc, Chicago, IL, United States of America). Continuous variables were analyzed as mean ± standard deviation (SD) and categorical variables were expressed as proportion (%). Data distribution was analyzed using the Kolmogorov-Smirnov test. Group differences in demographic and clinical data were compared with one-way analysis of variance (ANOVA), and post hoc contrasts were made with chi-square test for categorical variables. Furthermore, associations between clinical characteristics and SSR rates were analyzed by Kendall's correlation analysis.

RESULTS

Results of MicroTESE Results According to Aetiologies

Non-clinical characteristics according to different aetiologies are given in Table 1. The results of patients who underwent microTESE for ICSI are given in Table 2**.** The overall SSR rate was 44.1% (225/510). When SSR rates were analyzed, the idiopathic group had the lowest SSR rate compared to the other groups (36.4%, 125/343). SSR rates in other groups were as follows: KS: 49.3%, YCMDs: 60%, cryptorchidism: 77.1%, mumps orchitis: 70.5% (X^2 : 34.81; $p<0.01$). Predictors of successful sperm retrical by aetiology are given in Table 3. In SSR patients, age was significantly lower in the idiopathic group (t:-1.92; $p<0.05$). Mean testicular volume was higher in patients with cryptorchidism (left: t:5.3, $p<0.001$; right: t:4.23, $p<0.001$) and mumps orchitis (left: :2.8, p:0.003; right: t:2.72, p:0.002). No significant difference was found in hormone levels (FSH, LH, T) in patients with SSR and SRF.

Comparison of ICSI Results According to Aetiologies

Fertilisation rate was determined as the number of patients with successful sperm retrieval. Clinical pregnancy was calculated according to the fertilisation rate. Live birth was calculated according to the clinical pregnancy rate. In our study, the overall fertilisation rate was 73.3% (165/225). No difference was found in fertilisation rates according to etiology (idiopathic: 76%, KS: 72.9%, YCMDs: 62.5%, cryptorchidism: 70.3%, mumps orchitis: 75%, X^2 : 0.72; p:0.691) (Table 2).

The clinical pregnancy rate was 66.67% (110/165). No difference was found in clinical pregnancy according to etiology (idiopathic: 68.4%, KS: 66.6%, YCMDs: 46.4%, cryptorchidism: 68.4%, mumps orchitis: 77.7%, X^2 : 3,76; p:0.404) (Table 2).

The overall live birth rate was 68.2% (75/110). No difference was found in overall live birth according to etiology (idiopathic: 73.8%, KS: 50%, YCMDs: 42.8%, cryptorchidism: 76.9, mumps orchitis: 71.4%, X^2 : 2.04; p:0.428) (Table 2).

Table 1. Demographic, Laboratory and Radiological Data

	Whole Cohort (n=510)	Idiopathic (n=343)	Klinefelter (n=75)	Y chromosome micro deletion (n=40)	Cryptorchidism (n=35)	Mumps orchitis (n=17)
Age (years)	32.7 ± 4.61	33.1 ± 3.7	30.8 ± 4.2	33.7 ±6.1	27.5 ± 4.3	29.5 ± 2.8
FSH (IU/L)	19.2± 14.1	13.9 ± 11.8	39.2± 15.8	17.2 ±8.1	18.3 ± 6.2	21.7 ± 11.3
LH (IU/L	10.4 ± 7.9	8.1 ± 5.3	22.1 ± 10.4	8.8 ± 3.8	11.9 ± 4.1	13.2 ± 5.2
Testesteron (ng/mL)	9.2 ± 5.8	10.3 ± 6.6	5.8 ± 4.23	11.8 ±4.2	11.1 ± 1.3	10.9 ± 5.6
Left Testis Volume (mL)	6.4 ± 3.4	7.6 ± 3.3	2.1 ± 0.6	7.2 ± 2.3	4.9 ± 1.5	4.9 ± 1.8
Right Testis Volume (mL)	6.3 ± 3.1	7.4 ± 3.2	2 ± 0.6	7.4 ± 2.4	5.1 ± 1.3	4.8 ± 1.7

Table 2. Results of microTESE and ICSI according to Etiological Factors

(n/%)	Whole Cohort (n=510)	Idiopathic (n=343)	Klinefelter (n=75)	Y chromosome micro deletion (n=40)	Cryptorchidism (n=35)	Mumps orchitis (n=17)
Successful sperm retrieval	225 (44.1%)	125 (36.4%)*	37 (49.3%)	24 (60%)	27 (77.1%)	12 (70.5%)
Fertilization	165 (73.3%)	95 (76%)	27 (72.9%)	15 (62.5%)	19 (70.3%)	9 (75%)
Clinical pregnancy	110 (66.6%)	65 (68.4%)	18 (66.6%)	7 (46.6%)	13 (68.4%)	7 (77.7%)
Live birth	75 (68.2%)	48 (73.8%)	9 (50%)	3 (42.8%)	10 (76.9%)	5 (71.4%)

Table 3. Predictors of SSR success according to ethylogical factors

	Whole Cohort (44.1%) SSR (225) / SRF (285)	Idiopathic (36.4%) SSR (125) / SRF (218)	Klinefelter (49.3%) SSR (37) / SRF (38)	Y chromosome micro deletion (60%) SSR (24) / SRF (16)	Cryptorchidism (77.1%) SSR (27) / SRF (8)	Mumps orchitis (70.5%) SSR (12) / SRF (5)
Age (years)	30.96 ± 4.27*/ 32.92 ± 3.96*	30.8 ± 3.6*/ 34.43 ± 3.4*	30.9 ± 4.1/ 30.9 ± 5.2	31.9 ± 5.8/ 31.6 ± 5.2	30.8 ± 3.6/ 32.1 ± 5.2	31.3 ± 3.7/ 32.3 ± 2.1
FSH (IU/L)	20.9 ± 16.7/ 18.6 ± 14.8	15.5 ± 11.8/ 12.7 ± 10.8	37.6 ± 13.9/ 40.5 ± 14.8	17.1 ± 7.8/ 15.4 ± 8.5	20.6 ± 6.1/ 18.5 ± 3.2	21.7 ± 11.8/ 21.5 ± 13.3
LH (IU/L)	12.3 ±8.3/ 10.5 ± 8.2	7.7 ± 4.8/ 7.8 ± 5.3	24.8 ± 8.4/ 22.2 ± 9.6	9.1 ± 4.4/ 8.6 ± 4.1	11.9 ± 4.4/ 12.5 ± 1.7	11.7 ± 5.6/ 12.9 ± 4.7
Testesteron (ng/mL)	9.8 ± 5.2/ 9.4 ± 6.3	9.8 ± 6.1/ 9.4 ± 6.2	5.5 ± 4.2/ 5.5 ± 4.1	13.1 ± 4.3/ 12.7 ± 4.6	10.5 ± 1.6/ 11.3 ± 1.1	10.3 ± 4.1/ 9.8 ± 6.7
Left Testis Volume (mL)	6.1 ± 3.2/ 6.2 ± 3.5	7.9 ± 2.91/ 7.3 ± 3.4	2.1 ± 0.6/ 2.2 ± 0.7	8 ± 2.4/ 7.3 ± 2.2	5.3 ± 1.4*/ 2.6 ± 0.7*	5.4 ± 1.6*/ 3.2 ± 1.2*
Right Testis Volume (mL)	6.3 ± 3.7/ 6.1 ± 3.5	7.7 ± 3/ 7.2 ± 3.4	2.3 ± 0.6/ 2.1 ± 0.7	8.4 ± 2.3/ 7.1 ± 2.5	6.1 ± 1.3*/ 3.4 ± 0.7*	5.5 ± 1.7*/ 2.4 ± 1.2*

SSR, successful sperm retrieval; **SRF**, sperm retrieval failure

Results Between Age, Testicular Volume and SSR in Patients Undergoing Microtese

There was a negative correlation between age and SSR rates in the idiopathic patient group (t:-0.27; p<0.01). There was a positive correlation between testicular volume and SSR rates in patients with cryptorchidism (right: t:0.8; p=0.003; left: t:0.72; P:0.002) and mumps orchitis (right: t:0.76; p<0.01; left: t:0.76; p:0.003).

DISCUSSION

The factors affecting the treatment in NOA patients who underwent ICSI after TESE treatment have always been a subject investigated by researchers interested in infertility [9]. In the studies performed, microTESE is considered to be the most successful of the TESE methods performed for ICSI. While the SSR rate is between 16.7-45% in patients who underwent C-TESE, this rate is between 42.9-63% in patients who underwent microTESE and the rate of finding live spermatozoa is approximately 1.5 times higher compared to C-TESE [9]. In our study, SSR rate was found to be 44.1%. SSR was found to be lowest in the idiopathic group and highest in the cryptorchidism group.

In one study, it was found that age had no effect on SSR in microTESE, whereas in other studies, it was shown that TESE performed at an early age had better SSR rates than TESE performed at an older age [10,11]. In our study, early TESE in patients with idiopathic NOA resulted in better SSR rates.

In the literature, it has been shown that a testicular volume has no effect on sperm detection rates and that similar success can be achieved even in testicular volumes below 2 ml [12]. Nevertheless, many studies have reported that microTESE success is higher in patients with larger testicular volume. Corona et al. reported that the SSR rate was significantly higher in NOA with testicular volume >12 cc compared to patients with testicular volume <12 cc [13]. In our study, it was observed that SSR rate was affected by testicular volume. This was especially significant in patients with cryptorchidism and mumps orchitis. We think that age and testicular volume are important factors for SSR.

Studies on the factors affecting the results of microTESE have been conducted primarily on SSR. In this study, we evaluated the results of ICSI and microTESE treatments in NOA patients according to the etiological factors. The overall fertilization, clinical pregnancy and live birth rates for all NOA patients were 73.3%, 66.6%, and 68.2%, respectively, with no difference between groups. In another study in which these factors were analyzed, clinical pregnancy rates were 39% and live birth rates were 39%. No effect of clinical characteristics and hormone levels on ICSI was found [14]. In a study related to fertilization, clinical pregnancy, and live birth rates, these rates were found to be higher in patients with orchitis among the etiological factors, whereas these rates were found to be the lowest in patients with YCMDs [15]. In our study, SSR rates were higher in patients with YCMD and KS compared to other similar studies in the literature.

There is a relationship between deletion localisation and SSR rate in YCMD patients. No sperm is observed in AZFa and AZFb microdeletion patients. In patients with AZFc microdeletion, there is a possibility of spermatozoa [16]. SSR is 30-50% in patients with KS [17]. In our study, all patients with KS were non-mosaic KS and the SSR rate in patients with YCMD and KS was found to be similar to the literature.

Zang et al. [15] reported that clinical pregnancy and live birth rates of 46.9% and 40.6% in patients with idiopathic NOA, 54.4% and 50.4% in patients with KS, 20.3% and 18.8% in patients with YCMDs, 53.9% and 46.2% in patients with cryptorchidism, respectively. In our study, the clinical pregnancy rate in the general cohort was 66.6% and the live birth rate was 68.2%. When analyzed between groups, the highest clinical pregnancy rate was observed in mumps orchitis at 77.7%, and the lowest was observed in the YCMDs group at 46.6%. In terms of live birth rate, the highest pregnancy rate was observed in patients with cryptorchidism at 76.9% and the lowest was observed in the YCMDs patient group at 42.8%. The higher clinical pregnancy and live birth rate in YCMDs patients compared to the literature is due to the fact that, unlike other studies, both fresh and frozen microTESE were performed in NOA patients instead of only fresh MTESE. We think that this led to higher clinical pregnancy and live birth rates.

There are some limitations in our study. The data of the patients were collected retrospectively. In addition, etiological factors were classified under only 5 main groups. Other rare factors that cause NOA were not included in the study.

CONCLUSION

Etiological factors have an important role in terms of SSR in NOA patients. Among the etiological factors, idiopathic NOA patients have the lowest SSR rate. Age and testicular volume are important parameters for SSR in idiopathic and acquired NOA patients. Our study showed that the etiological factors affect the success of microTESE and ICSI in NOA patients.

Consent to Participate: For this type of retrospective study, formal consent is not required.

Inform of Publication: The results of the study were not published in full or in part in the form of an abstract.

Research Involving Human Participants and/or Animals: This article does not contain any studies with animals performed by any of the authors. All procedures performed in studies involving human participants were by 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.

Conflict of Interest: The authors declare no competing interests.

No artificial intelligence was used in the writing of our study.

Ethics Approval: The study was approved by The University of Health Sciences, IzmırTepecik Training and Research Hospital Ethical Committee, Izmır, Türkiye (Decision No: 2024/02-19, Date: 04.03.2024).

Authors Contributions; Conception: YA, MZK, AHİ; Design: YA, MZK, AHİ; Supervision: YA, MZK; Fundings: -; Materials: -; Data Collection: ED,YA; Analysis: MZK, ED; Literature Review: YA, ED; Writer: YA, ED; Critical Review: MZK, YA, AHİ.

REFERENCES

[1] Agarwal A, Baskaran S, Parekh N, Cho CL, Henkel R, Vij S, Arafa M, Panner Selvam MK, Shah R (2021) Male infertility. Lancet. 397(10271):319-333. https://doi.org/10.1016/S0140-6736(20)32667-2

[2] Akhavizadegan H (2024) Male Genetic Evaluation in Infertility, Recurrent Abortion and Recurrent in Vitro Fertilization Failure; A Clinical Approach. Urol J.9;21(4):221-225. https://doi.org/ 10.22037/uj.v20i.8044

[3] Jiang H, Zhang Y, Ma H, Fan S, Zhang H, Shi Q (2022) Identification of pathogenic mutations from nonobstructive azoospermia patients. Biol Reprod. 107:85-94. https://doi.org/10.1093/biolre/ioac089

[4] Chen X, Ma Y, Zou S, Wang S, Qiu J, Xiao Q, Zhou L, Ping P (2019) Comparison and outcomes of nonobstructive azoospermia patients with different etiology undergoing MicroTESE and ICSI treatments. Transl Androl Urol. 8: 366-73. https://doi.org/10.21037/tau.2019.04.08

[5] Tharakan T, Luo R, Jayasena CN, Minhas S (2021) Non-obstructive azoospermia: current and future perspectives. Fac Rev. 10:7. https://doi.org/10.12703/r/10-7

[6] Wang K, Tang D, Zhu Q, Yang D, Yang H, Peng J, Ding D, Wang J, Wang Q, Cao Y, Chen B, Zhang Z (2023) Micro-TESE surgery combined with ICSI regimen in the treatment of non-obstructive azoospermia patients and its effect analysis. Zygote. 31(1):55-61. https://doi.org/10.1017/S096719942200051X

[7] Mao J, Deng C, Zhao L, Liu D, Lin H, Zhang Z, Yang Y, Zhang H, Li R, Hong K, Jiang H (2023) Micro-TESE strategy in patients with NOA caused by AZFc deletion: synchronous or asynchronous? Zygote. 31(1):25-30. https://doi.org/10.1017/S0967199422000466

[8] Sharma A, Minhas S, Dhillo WS, Jayasena CN (2021) Male infertility due to testicular disorders. J Clin Endocrinol Metab. 106(2):e442-e459. https://doi.org/10.1210/clinem/dgaa781

[9] Esteves SC (2015) Clinical management of infertile men with nonobstructive azoospermia. Asian J Androl. 17(3):459-70. https://doi.org/10.4103/1008-682X.148719

[10] Topuz B, Ebiloğu T, Sarıkaya S, Kaya E, Fidan U, Korkmaz C, Ceyhan ST, Bedir S, Gürdal M, Karataş ÖF (2021) The timing of micro-TESE: what is the ideal age for male and female partner to bring a child to home? Rev Assoc Med Bras (1992). 67(7):958-965. https://doi.org/10.1590/1806-9282.20210290

[11] Pook CJ, Cocca A, Grandone A, Al-Hussini M, Lam W (2021) The Evidence for Fertility Preservation in Pediatric Klinefelter Syndrome. Front Reprod Health. 3:629179. https://doi.org/10.3389/frph.2021.629179

[12] Bryson CF, Ramasamy R, Sheehan M, Palermo GD, Rosenwaks Z, Schlegel PN (2014) Severe testicular atrophy does not affect the success of microdissection testicular sperm extraction. J Urol. 191(1):175-8. https://doi.org/10.1016/j.juro.2013.07.065

[13] Corona G, Minhas S, Giwercman A, Bettocchi C, Dinkelman-Smit M, Dohle G, Fusco F, Kadioglou A, Kliesch S, Kopa Z, Krausz C, Pelliccione F, Pizzocaro A, Rassweiler J, Verze P, Vignozzi L, Weidner W, Maggi M, Sofikitis N (2019) Sperm recovery and ICSI outcomes in men with non-obstructive azoospermia: a systematic review and meta-analysis. Hum Reprod Update. 25(6):733-757. https://doi.org/10.1093/humupd/dmz028

[14] Achermann APP, Pereira TA, Esteves SC (2021) Microdissection testicular sperm extraction (micro-TESE) in men with infertility due to nonobstructive azoospermia: summary of current literature. Int Urol Nephrol. 53(11):2193-2210. https://doi.org/10.1007/s11255-021-02979-4

[15] Zhang HL, Zhao LM, Mao JM, Liu DF, Tang WH, Lin HC, Zhang L, Lian Y, Hong K, Jiang H (2021) Sperm retrieval rates and clinical outcomes for patients with different causes of azoospermia who undergo microdissection testicular sperm extraction-intracytoplasmic sperm injection. Asian J Androl. 23(1):59-63. https://doi.org/10.4103/aja.aja_12_20

[16] The Trinh S, Nguyen NN, Thi Thu Le H, Thi My Pham H, Tien Trieu S, Tran NTM, Sy Ho H, Van Tran D, Van Trinh T, Trong Hoang Nguyen H, Pham Minh N, Duc Dang T, Huu Dinh V, Thi Doan H (2023) Screening Y Chromosome Microdeletion in 1121 Men with Low Sperm Concentration and the Outcomes of Microdissection Testicular Sperm Extraction (mTESE) for Sperm Retrieval from Azoospermic Patients. Appl Clin Genet. 16:155-164. https://doi.org/10.2147/TACG.S420030

[17] Küçükaslan AŞ, Çetintaş VB, Altıntaş R, Vardarlı AT, Mutlu Z, Ulukuş M, Semerci B, Eroğlu Z (2013) Identification of Y chromosome microdeletions in infertile Turkish men. Turk J Urol. 39(3):170-4. https://doi.org/10.5152/tud.2013.035

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Original Research

Efficacy of Triple Shoulder Injection with Steroids and Ozone in the Treatment of Chronic Shoulder Pain and Range of Motion Limitation

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ABSTRACT

Objective: This study aimed to evaluate and compare the therapeutic effects of fluoroscopy-guided triple shoulder injections with steroids and, a combination of steroids and ozone.

Methods: Data were retrospectively collected from the files of 70 patients diagnosed with non-specific chronic shoulder pain and received triple shoulder injections. The patients were divided into two groups. One group included triple shoulder injections with steroids under fluoroscopic guidance, while the other group included combination of steroids and ozone. Pain intensity was evaluated with the Visual Analog Scale (VAS), the patient's quality of life and functionality were assessed using the Shoulder Pain and Disability Index (SPADI), and the active range of motion (ROM) of shoulder abduction, external rotation, and flexion were measured by goniometry. Beck Depression Inventory (BDI) was used to measure the severity of depression before the procedure. All measurements were recorded at baseline and 1, 3, and 6 months after the procedure.

Results: Baseline characteristics were similar in both groups. There was no statistical difference in VAS scores between the two groups at baseline, 1st, and 3rd months. However, in the 6th months, the VAS scores of patients treated with steroids plus ozone were significantly lower than those of patients treated with steroids ($P<0.001$). Both groups showed significant improvements in SPADI pain, disability, and total subscores compared to pre-treatment values ($P < 0.001$). There was a significant improvement in the ROM of the shoulder joint in both groups ($p<0.001$). However, patients who received ozone and steroid treatments showed a significantly greater increase in SPADI and ROM of shoulder joints in the 6th months ($p<0.001$). There was a positive correlation between BDI score and pain duration, and the severity of depression had no statistically significant effect on VAS scores.

Conclusion: Results of this study revealed that triple shoulder injection with steroids or a combination of steroids and ozone proved to be an effective therapeutic approach for patients suffering from shoulder pain and limited mobility. The combination of ozone with steroids may lead to better results than using steroids alone. Furthermore, a long duration of pain increases the risk of chronic depression in patients.

Keywords: Shoulder pain, Shoulder joint, Ozone treatment, Lumbar epidural steroid

INTRODUCTION

Shoulder pain, often caused by repetitive or excessive activities, is a common musculoskeletal disorder with a lifetime prevalence of 61% [1]. It's often associated with a decreased range of motion. [2]. Common causes of shoulder pain are osteoarthritis of the glenohumeral or acromioclavicular joint, adhesive capsulitis, tendinitis, bursitis, synovial impingement, and rotator cuff tears [3]. Treatments aim to restore shoulder movements, reduce the patient's pain, and usually require a multimodal approach. Initial treatment includes analgesics, anti-inflammatory drugs, and physical therapy. If pain relief is inadequate, other minimally invasive treatments are used. Fluoroscopy-guided triple joint injection is a shoulder intervention technique that includes injection of steroid and local analgesic mixture into the glenohumeral joint, subacromial-subdeltoid bursa, and acromioclavicular joint in a single session by entering from the AC joint [4]. Steroid injection could be effective even in advanced patients. The disadvantage is that the steroid has a relatively short duration of therapeutic action and is often not reproducible due to the potential risk of osteoporosis, osteonecrosis, and infection [5,6].

In recent years, ozone therapy has started to be used in the treatment of musculoskeletal disorders, low back pain, lumbar disc herniation, and osteoarthritis especially in the knee. Ozone provides controlled activation of antioxidant systems, increases blood flow, and thus increases oxygenation and the release of growth factors and cytokines. Intra-articular ozone injection has emerged as an alternative to steroids since it is effective, well-tolerated, and without serious side effects [7-9].

The primary objective of this study was to compare the efficacy of triple shoulder injection with steroid alone and the combination of steroid and ozone in patients with shoulder pain and limitation of movement. The secondary aim was also to observe how it affects pain and disability after treatment by analysing the severity of depression.

Main Points

- It has been found that triple shoulder steroid injections with or without ozone showed significant efficacy on shoulder pain and disability, however, treatment outcomes were better in combination therapy.
- This study demonstrates the potential benefits of ozone therapy as an adjunct treatment for improved outcomes.

MATERIALS AND METHODS

The study was performed in accordance with the Helsinki Declaration criteria, after the approval of the ethics committee (decision dated 13.01.2022, No: İ01-01-22) in the Ankara University Faculty of Medicine. Patients' records collected between January and June 2021 were analyzed retrospectively. After reviewing 108 patient files, 38 were excluded due to missing information. Seventy patients, with shoulder pain and range of motion limitation secondary to subacromial-subdeltoid bursitis, glenohumeral, and acromioclavicular joint degeneration, biceps/rotator cuff tears, tendinopathy, glenoid labrum injuries demonstrated on shoulder magnetic resonance image (MRI), were included in the study. In addition, patients whose psychological status was evaluated with the Beck Depression Inventory (BDI) were considered.

Inclusion criteria were patients aged between 30-80 years, body mass index (BMI)<40 kg/m^2, positive shoulder abnormal passive range of motion (ROM), Visual Analog Scale (VAS)>4 shoulder pain for at least 3 months, no causes such as dislocation, subluxation, fracture on the direct radiograph (acute shoulder pain causes) and exclusion criteria were patients younger 30 and older 80 years, coagulation disorder, oral anticoagulant use, pain of cervical origin (excluded by routine cervical MRI and physical examination findings), glucose 6-phosphate dehydrogenase deficiency, and pregnancy.

The same experienced pain medicine specialist performed all injections. Age, gender, BMI, affected side, symptom duration, and smoking of all patients were recorded.

Patients were divided into two treatment groups; steroid (40 mg triamcinolone, 1 ml) in 4 ml saline and steroid plus 15 cc ozone injection at a concentration of 15 µg/ml. In our clinic, the combination of steroids and ozone has been used as a routine treatment for patients experiencing long-term pain and presenting with multiple coexisting MRI findings in patients with chronic nonspecific shoulder pain. Given the recommendation and utilization of intra-articular 5-20 ml ozone injections at a concentration of 5-20 µg/ml in studies, our clinic has preferred 15 cc ozone injection at a concentration of 15 µg/ml.

The procedures were performed in a supine position on the fluoroscopy table. The glenohumeral joint and acromioclavicular joint were visualized in the Anterior-Posterior (A-P) position, and then the 10° oblique position. A 21 G needle spinal needle reached the glenohumeral joint through the acromioclavicular joint and subacromial-subdeltoid bursa, after confirming the needle position with contrast medium (iohexol), injections were performed respectively. Ozone gas was obtained from the ozone generator (Dr J. Hänsler Ozonosan, Iffezheim, Germany) in our clinic. Figure 1 demonstrated the triple shoulder injection during procedure. One group received 3 mL of a mixture of Triamcinolone acetonide and saline into the glenohumeral joint. In addition to the first group, 7 cc ozone was injected into the glenohumeral joint in the other group. Then the needle was withdrawn into the subacromial bursa. It was confirmed by administering 2 mL of contrast medium that the needle was in the subacromial bursa and 1 ml of a mixture of steroid and saline was administered. The other patients received 5cc ozone additionally. The needle tip was withdrawn into the acromioclavicular joint and 1 mL of steroid and saline mixture was injected into the joint. The other patients received 3 cc of ozone into the acromioclavicular joint. For all patients, daily physical therapy was suggested after intervention.

Shoulder pain intensity was assessed using the VAS score. Quality of life and the functional assessment of the patients were evaluated using the Shoulder Pain and Disability Index [SPADI] [10]. These measures were taken from all patients before injection and at 1st, 3rd and 6th months after procedure. VAS scores were also recorded at the 24th hours. Furthermore, BDI [11] was used to assess the severity of depression before the procedure. The study also examined the impact of depression levels on the VAS score and the disability experienced by the participants after six months.

Patients' recorded goniometric measurements of shoulder active ROM of flexion, abduction, and external rotation were also analyzed.

The VAS score for the assessment of pain ranges between 0-10 (min-max pain degree). The SPADI includes a total of 13 questions, related pain severity (5 items) and disability (8 items). The items in the BDI are mainly based on behaviors and symptoms specific to depression and are described in a series of sentences, and each sentence is numbered from 0 to 3.

The primary and secondary outcomes were compared with the baseline and between the two groups.

Statistical Analysis

Data were presented as mean ± standard deviation (SD) and minimum-maximum or number and percentage (n, %) as appropriate. Normality analysis was performed using the Shapiro-Wilk test, skewness-curvature, and histograms. Normally distributed variables are presented as mean and SD Numerical dependent variables were compared between groups using independent samples T-test and Mann-Whitney U test. The Mann–Whitney U test was used to assess nonparametric continuous variables. Categorical variables were compared using the Chi-squared test. Repeated measures were analyzed

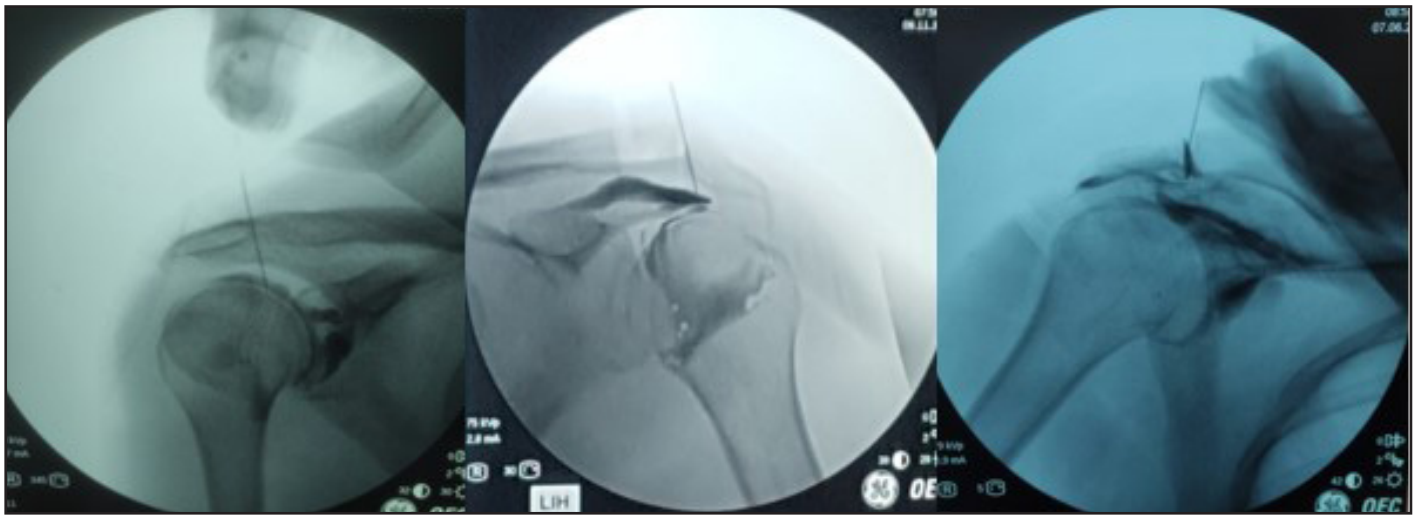

Figure 1. Fluoroscopy-guided Triple Shoulder Injection

using Friedman and Wilcoxon tests. Changes over time were compared using the Bonferroni correction. The Jamovi project (2022, Jamovi version 2.3, computer software) was used for statistical analysis, and $p<0.05$ was considered statistically significant.

RESULTS

The study involved 70 patients who received triple shoulder injections with triamcinolone or combination of triamcinolone and ozone. There was no difference between the patients in age, gender, BMI, smoking status, pain duration, baseline VAS, SPADI total, and BDI scores. Detailed demographic and clinical characteristics of the patients in each group were presented in Table 1.

There was no correlation between VAS values at all times and age, gender, BMI, smoking status, pain duration, and BDI scores.

There was a positive, moderate correlation between pain duration and BDI score (Pearson correlation $r=0.602$, $p<0.001$).

Table 2 showed the distribution of patients according to MRI findings and Table 3 showed changes in baseline VAS and SPADI subscores at 1, 3, and 6 months. Although multiple MRI findings coexisted in most patients, not all findings in one patient were symptomatic.

No difference was found in the patients' VAS scores between the two groups at 1 and 3 months. After 6 months of treatment, it was observed that the patients who received ozone in addition to steroids showed significantly lower VAS scores than the group receiving only steroids ($p<0.001$). However, both groups showed significant improvement in their VAS scores in six months (both $p<0.001$).

Although the decrease in SPADI pain, disability, and total subscores in both groups over time was statistically significant ($p<0.001$), there were statistically significant changes in favour of the patients who received ozone and steroid injection in the 3rd and 6th months as shown in Table 3.

The active ROM values of both groups showed a statistically significant increase compared with the baseline for all time points, however, this improvement was especially greater in the group receiving combined treatment at the 6th months. The detailed changes in the shoulder range of motion after treatment are shown in Table 4.

No significant complications reported in the patients, while 5 patients receiving combination therapy had a short-term feeling of pressure in the shoulder.

Table 1. Clinical Characteristics of the Patients

		Ozone+Steroid Injection	Only Steroid Injection	P value
		n=35	n=35	
Age (year±STD)		58.71±11.91	59.8±12.25	0.708[a]
Gender (n)	Female	21	20	1.000[b]
	Male	14	15	
BMI		26.08(18.3-35.1)	25(16.9-36.6)	0.332[a]
Smoking Status	(+)	10	13	0.611[b]
	(-)	25	22	
Pain duration (Month)		13(3-72)	11(3-36)	0.729[c]
VAS basal		8(5-10)	8(6-10)	0.966[c]
VAS 24h.		5(1-9)	4(3-7)	0.214[c]
SPADI Total score basal		79.33±8.66	82.19±6.23	0.119[a]
BDI Score		14.34±7.15	15.86±7.22	0.382[a]

STD: Standart Deviation, BMI: Body Mass Index, VAS: Visual Analog Scale, SPADI: Shoulder pain and disability index, BDI: Beck's Depression Inventory, a: Independent Samples T-test, b: Chi-Square test, c: Mann-Whitney U test, d: Wilcoxon signed ranks test

Table 2. Baseline MRI findings

	Steroid and Ozone Injection		Only steroid Injection	
	(n=35)	%	(n=35)	%
Side				
Right	23	65,71	21	60
Left	12	34,28	14	40
AC-joint osteoarthritis	10	28,6	10	28,6
Mild	3	8,6	2	5,7
Moderate	7	20	7	20
Severe	0	0	1	2,85
SA/SD bursitis	13	37,14	15	42,85
Mild	3	8,6	4	11,42
Moderate	6	17,14	7	20
Severe	4	11,42	4	11,42
Partial thickness tear in RC	4	11,42	5	14,3
Full-thickness tear in RC	5	14,3	8	22,85
Tendinosis in RC	2	5,7	2	5,7
Partial thickness tear with/without tendinosis in the long head of the biceps	12	34,3	8	22,85
Full-thickness tear in the long head of the biceps	1	2,85	6	17,14
GH-joint osteoarthritis	18	51,42	15	42,85
Mild	3	8,6	1	2,85
Moderate	12	34,3	10	28,6
Severe	3	8,6	4	11,42
Tear in glenoid labrum	7	20	6	17,14
Small tear	6	17,14	6	17,14
Large tear	1	2,85	0	0

AC acromioclavicular, SA subacromial, SD subdeltoid, RC rotator cuf, GH glenohumeral

Table 3. The changes in baseline VAS and SPADI subscores at 1, 3, and 6 months

	Steroid and Ozone Injection	Steroid Injection	p*
	median(min-max)/mean rank	median(min-max)/mean rank	
VAS basal	8(5-10)/4	8(6-10)/4	0.966
VAS 1m	4(2-8)/1.8	4(3-6)/1.46	0.499
VAS 3m	4(2-7)/1.84	4(3-6)/1.67	0.067
VAS 6m	4(3-7)/2.36	6(4-7)/2.87	**<0.001**
p**	**<0.001**	**<0.001**	
S.Pain score basal	80(60-90)/4	78(66-92)/3.81	0.773
S.Pain score 1m	44(28-74)/1.6	46(32-58)/1.4	0.110
S.Pain score 3m	48(22-70)/1.87	54(32-78)/1.9	**0.041**
S.Pain score 6m	54(28-70)/2.53	70(40-82)/2.89	**0.004**

p**	**<0.001**	**<0.001**	
S.Disab. score basal	82.5(55-97)/4	85(67.5-93-7)/4	0.091
S.Disab. score 1m	42.5(18.7-80)/1.8	43.7(26.2-56.2)/1.34	0.529
S.Disab. score 3m	43.7(28.7-72.5)/1.8	48.7(25-5-68.7)/2.06	**0.004**
S.Disab. score 6m	47.5(37.5-72.5)/2.36	55(41.2-81.2)/2.6	**<0.001**
p**	**<0.001**	**<0.001**	
S.Total score basal	81.5(59.9-93)/4	81.5(68.4-93)/4	0.264
S.Total score 1m	42.2(23-77.6)/1.67	43.8/33-56.1)/1.37	0.210
S.Total score 3m	44.6(26.1-71.5)/1.8	49.2(37.6-71.5)/1.9	**0.011**
S.Total score 6m	49.2(38.4-71.5)/2.53	59.9(40.7-80.7)/2.73	**<0.001**
p**	**<0.001**	**<0.001**	

p*: Mann-Whitney U Test, p**: Friedmann Test

Table 4. The Changes in the Range of Motion of the Joint over Time and Between the Groups

	Ozone+Steroid Injection	**Only Steroid Injection**	**p***
	median(min-max)/mean rank	**median(min-max)/mean rank**	
Flexion at basal	110(78-176)/1	115(78-168)/1.03	0.557
Flexion at 1. month	155(95-180)/2.14	145(111-175)/2.43	0.284
Flexion at 3. months	165(118-180)/3.16	157(120-173)/3.54	**0.035**
Flexion at 6. Months	168(124-180)/3.70	152(120-172)/3	**<0.001**
p**	<0.001	<0.001	
External rotation at basal	65(36-90)/1.06	58(35-81)/1.10	0.659
External rotation at 1. Month	75(43-90)/2.29	66(45-88)/2.73	**0.010**
External rotation at 3. Months	78(45-90)/3.04	68(49-88)/3.3	**0.001**
External rotation at 6. Months	80(45-90)/3.61	70(42-88)/2.87	**<0.001**
p**	<0.001	<0.001	
Abduction at basal	120(75-170)/1.14	105(162-77)/1.17	0.100
Abduction at 1. Month	137(102-172)/2.04	137(107-164)/2.61	0.545
Abduction at 3. Months	150(110-175)/3.01	148(110-170)/3.56	0.359
Abduction at 6. Months	158(110-180)/3.8	143(102-168)/2.65	**<0.001**
p**	<0.001	<0.001	

p*: Mann-Whitney U Test, p**: Friedmann Test

DISCUSSION

The results of the present study showed that patients who were treated with fluoroscopy-guided triple shoulder injection either with triamcinolone alone or triamcinolone plus ozone demonstrated significant improvement in their VAS scores, SPADI subscores, and active ROM in all three intervals. However, patients who received additionally the ozone injection showed greater improvement. In addition, patients' level of depression did not affect their pain severity and disability.

The healing process of chronic shoulder pain can take a long time to heal especially in untreated patients. Steroid injections are commonly used to reduce pain and facilitate exercise in patients with shoulder pain to increase range of motion. It is

important to know that steroid injections may have local side effects such as infection, cartilage injury, aseptic bone necrosis, tendon rupture, tissue atrophy, fat necrosis, calcification, and depigmentation. Additionally, there are systemic side effects that can occur with the local application of corticosteroids, including impaired glucose tolerance, immunosuppression, osteoporosis, and exacerbation of psychosis [12]. It's important to remember that every medication comes with potential risks but no major side effects related to steroid use were demonstrated during this study.

Ozone, which has no major side effects, is effective in treating inflammatory and degenerative disorders of the musculoskeletal system. It has anti-inflammatory and analgesic effects in the short and long term. Studies have reported the effects of ozone on various conditions including lumbar facet joint syndrome, subacromial bursitis, carpal tunnel syndrome, hip bursitis, shoulder adhesive capsulitis, herniated disc, and temporomandibular joint disorder [13]. Only five patients in the present study reported a temporary sensation of pressure in their shoulders. This indicated that the procedure was generally well-tolerated and safe.

Ozone has multiple benefits for joint health. It reduces oxidative stress, promotes joint repair, increases oxygenation, releases growth factors and cytokines, and inhibits pro-inflammatory cytokines like interferon alpha, TNF-alpha, and ILs [3]. After reviewing the available literature on the use of ozone in treating shoulder disorders, it is evident that most studies have shown the effectiveness of ozone therapy either alone or in combination with steroids. Although there have been limited studies on the shoulder joint, many studies have been conducted on the effects of ozone on knee osteoarthritis [14]. The literature on shoulder pain indicates that ozone therapy has proven to be beneficial for subacromial bursitis, calcific tendinitis, capsulitis, and partial rotator cuff tears [15]. Moreover, some studies have demonstrated the positive effects of ozone therapy on musculoskeletal disorders, both in the short and long term with a single intervention [16]. In our study, we performed the triple shoulder injection technique in a single session and observed long-term treatment efficacy in both treatment groups. While our study did not include patients receiving ozone therapy alone, it might be a promising alternative for patients who cannot receive steroid treatment due to contraindications or intolerable side effects. Furthermore, unlike steroids, ozone therapy is a reproducible treatment with additional advantages.

In a recent study on adhesive capsulitis treatment, Foula et al [17] included three groups of patients who received intra-articular ozone, steroid, and pulse radiofrequency (PRF) injections. The study found that steroid injection showed greater pain relief at rest compared to ozone and PRF, starting from week one after the intervention. However, the ozone and PRF groups had a delayed effect but better VAS scores at week 8 post-intervention. All three intervention modalities showed significant improvement in SPADI, with no significant difference in pain and disability components. In a study conducted by Benvenuti [18], it was reported that a patient suffering from limited joint range of motion and pain was given 10 ml of intra-articular ozone at a concentration of 15 μg/ml. Additionally, 0.5 to 1 ml was injected into the subacromial bursa and the long head of the biceps muscle. This led to a reduction in overall pain and recovery of shoulder function. As in our study, the severity of patients' pain decreased with ozone treatment. However, we did not intend to inject into the bicipital sulcus as was done in the previous study, this indicated that ozone was capable of diffusing throughout the tissue, providing relief beyond the site of injection.

Ghazani et al [6] suggested that although corticosteroids had short-term benefits, they lost their effect in the long term, while ozone had a longer therapeutic effect and improved pain and disability in the longer term. They reported a study of 30 patients with impingement syndrome and found that after injecting a combination of ozone and steroids into the bursa (with or without intra-articular injection), both VAS and SPADI scores improved after 2 weeks and 2 months. There was no significant difference between the range of motion and ultrasound measurements. The corticosteroid was more effective in improving pain and disability scores than a single injection of ozone. After following the patients for 6 months, we observed that both treatment methods showed improvement in the patients' VAS, SPADI, and ROM values. However, patients who received a combination of ozone and steroid injections showed better improvements compared to patients who received steroid alone injections.

To maintain treatment efficacy and ensure long-term clinical improvement, Kara and Gürçay [19] suggested that injections should be repeated one to three times per week. In some studies, patients were given a higher number of injections than in the current study. For instance, Moretti et al [20] evaluated ozone's effectiveness in treating shoulder tendinopathies, and their patients received 10 ml ozone (6-10 μg) at the site of maximum pain and 10 to 15 ml ozone (15-20 μg) for joints. These injections

were given twice a week for a total of 6 sessions for shoulder osteoarthritis. The study also had a control group consisting of 40 patients who were treated with anti-inflammatory mesotherapy. The patients who received ozone injections experienced a greater reduction in the VAS (from 9.4 to 2.9), while in the mesotherapy group, the reduction was from 9 to 6. The study found that ozone treatment was significantly better than anti-inflammatory mesotherapy in improving painful shoulders. In a study conducted by Scarchilli [21], 14 individuals who were suffering from shoulder pain received an injection of ozone into their shoulder joint and subacromial bursa. Patient's pain decreased from 8.5 to 3, and further improved to 1.5 after two months. Furthermore, five weekly injections into the posterior intra-articular or lateral access improved their 2-month mean constant score by approximately 85%.

Corticosteroid injections were found to be effective in treating tendinopathies in the short term, but not in the long term according to Coombs et al [22]. In addition, it has been found that ozone injection was an effective treatment for acute or chronic tendinitis, even in cases where calcium deposits are present [23]. A study by Gjonovich et al [24] used a larger dose of ozone (35 ml) at a concentration of 12-15 μg/ml to treat a painful shoulder complicated by rotator cuff lesions. The study reported 67% positive results with good pain control and excellent recovery of joint function.

In the management of chronic musculoskeletal pain, it is important to consider a psychological approach in addition to pain control. Recent studies have shown that depression, anxiety, and sleep problems are common among individuals with chronic musculoskeletal pain [25]. In contrast, the association between depressive symptoms and functional and symptomatic disability in patients with chronic shoulder pain was poorly understood [26]. In a study of 130 patients (96 with rotator cuff disease, 24 with adhesive capsulitis, and 10 with calcific tendinitis), Cho et al [25] reported a high prevalence of depression, anxiety, and sleep disturbance in patients with shoulder pain for 3 months or longer compared with healthy subjects. The authors suggested that chronic shoulder pain puts patients at risk of developing depression and anxiety. Furthermore, in their study of 76 patients with frozen shoulders, Toprak et al [27]. found a high prevalence and close association between pain, anxiety, and sleep disturbance. The VAS and Beck Anxiety Inventory were significantly higher in the patients with frozen shoulders than in the controls ($p<0.001$). However, there was no significant difference between patients and controls Beck Depression Inventory scores ($p=0.067$). The current study revealed that the BDI scores of patients increased as the duration of pain increased and the level of depression have no impact on the pain and disability. In order to achieve complete treatment, it is crucial to consider the patient's psychological well-being and plan a multimodal treatment approach

It is important to note that structural changes such as degenerative joint changes, subacromial-subdeltoid bursitis, biceps and rotator cuff tendon disorders, and tears, which are demonstrated by shoulder MRI, are prevalent in both symptomatic and asymptomatic patients [3]. It is crucial to keep in mind that a considerable number of patients might experience multiple co-existing disorders concurrently. This means a complex challenge to their diagnosis and treatment.

Limitations

The main limitation of this study was retrospective nature and the small sample size. Additionally, the lack of long-term follow-up (longer than 6 months) of the patients was another limitation. In this study, triple shoulder injections were administered in a single session, were not repeated. There was also no control group that received sham or ozone alone injections in the study. However, the lack of research on the combination of steroid and ozone injections into the shoulder joint, along with the assessment of psychological factors, makes this study special.

CONCLUSION

The results of the study suggested that fluoroscopy-guided triple shoulder injection with both treatments improved patient's VAS, SPADI, and ROM of shoulder joint outcomes at the 6-months follow-up. However, results were in favour of patients treated with combination therapy. This highlights the potential benefits of ozone therapy as an adjunct treatment for improved outcomes and ozone could be an alternative treatment option for patients with shoulder pain. Additionally, the effect of a depressive state on the severity of pain could not be shown at any time. Shortening the duration of pain in patients might also reduce the tendency to depression. Further studies with larger sample sizes and randomized controlled trials with longer follow-ups would be needed to make a definitive decision about the efficacy of triple shoulder injection with steroids plus ozone for short- and long-term pain relief.

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REFERENCES

[1] Carroll MB, Motley SA, Smith B, Ramsey BC, Baggett AS (2018) Comparing corticosteroid preparation and dose in the improvement of shoulder function and pain: a randomized, single-blind pilot study. Am J Phys Med Rehabil. 97:450–455. http://dx.doi.org/10.1097/phm.0000000000000758

[2] Lee SW, Tiu T, Roberts J, Lee B, Bartels MN, Oh-Park M (2018) Point-of-care ultrasonography findings and care use among patients undergoing ultrasound-guided shoulder injections. Am J Phys Med Rehabil. 97:56–61. http://dx.doi.org/10.1097/phm.0000000000000807

[3] Cuce I, Sencan, S, Demir FU, Koc A, Çalış M (2019) Efficacy of fluoroscopy-guided triple shoulder injection for older patients with nonspecific shoulder pain. Eur Geriatr Med 10: 595–602. https://doi.org/10.1007/s41999-018-00159-9

[4] Erdine S (2011) Üçlü omuz bloğu. In: Erdine S (eds), Algolojide Girişimsel Yöntemler. İstanbul, Nobel kitabevi, pp 195-198.

[5] Gaujoux-Viala C, Dougados M, Gossec L (2009) Efficacy and safety of steroid injections for shoulder and elbow tendonitis: a meta-analysis of randomized controlled trials. Ann Rheum Dis. 68:1843–1849. http://dx.doi.org/10.1136/ard.2008.099572

[6] Babaei-Ghazani A, Fadavi HR, Eftekharsadat B, Ebadi S, Ahadi T, Ghazaei F, Khabbaz MS (2019). A Randomized Control Trial of Comparing Ultrasound-Guided Ozone (O2-O3) vs Corticosteroid Injection in Patients With Shoulder Impingement. Am J Phys Med Rehabil. November. 98(11):1018-1025. http://dx.doi.org/10.1097/phm.0000000000001240

[7] Bocci VA (2006) Scientific and medical aspects of ozone therapy. State of the art. Arch Med Res 37:425–435. https://doi.org/10.1016/j.arcmed.2005.08.006

[8] Shanta H, Amruta S (2017). Ozone Therapy: An Excellent Treatment for Various Diseases International. Journal of Pharmacy and pharmaceutical research 10(3), 303-311.

[9] Babaei-Ghazani A, Najarzadeh S, Mansoori K, Forogh B, Madani SP, Ebadi S, Fadavi HR, Eftekharsadat B (2018) The effects of ultrasound-guided corticosteroid injection compared to oxygen–ozone (O 2–O 3) injection in patients with knee osteoarthritis: a randomized controlled trial. Clin Rheumatol. 37:2517–2527. https://doi.org/10.1007/s10067-018-4147-6

[10] Bumin G, Tüzün EH, Tonga E (2008). The Shoulder Pain and Disability Index (SPADI): Cross-cultural adaptation, reliability, and validity of the Turkish version. J Back Musculoskelet Rehabil. 21(1): 57-62. http://dx.doi.org/10.3233/bmr-2008-21108

[11] Kapci EG, Uslu R, Turkcapar H, Karaoglan A (2008). Beck Depression Inventory-II: evaluation of the psychometric properties and cut-off points in a Turkish adult population. Depress Anxiety, 25(10), E104-E110. https://doi.org/10.1002/da.20371

[12] Kamel, SI, Rosas HG, Gorbachova T (2023) Local and Systemic Side Effects of Corticosteroid Injections for Musculoskeletal Indications. Am J Roentgenol. 1-12. https://doi.org/10.2214/AJR.23.30458

[13] Seyam O, Smith NL, Reid I, Gandhi J, Jiang W, Khan SA (2018) Clinical utility of ozone therapy for musculoskeletal disorders. Med Gas Res. Sep 25;8(3):103-110. http://dx.doi.org/10.4103/2045-9912.241075

[14] Atar MÖ, Korkmaz N, Aslan SG, Tezen Ö, Köylü Sinem Uyar, Demir Yasin, Kesikburun S (2023) Comparison of ultrasound-guided subacromial corticosteroid and ozone

(O2-O3) injections in the treatment of chronic rotator cuff tendinopathy: a randomized clinical trial. Korean J Pain 36(1):128-136 http://dx.doi.org/10.3344/kjp.22221

[15] Gurger M, Once G, Yilmaz E, Demir Ş, Calik I, Say Y, Kavakli A, Key S, Gurbuz MU, Bingollu O (2021) The effect of the platelet-rich plasma and ozone therapy on tendon-to-bone healing in the rabbit rotator cuff repair model. J Orthop Surg Res 16(1): 1-9 https://doi.org/10.1186/s13018-021-02320-w

[16] Giurazza F, Guarnieri G, Murphy KJ, Muto M (2017) Intradiscal O2O3: rationale, injection technique,short-and long-term outcomes for the treatment of low back pain due to disc herniation. Can Assoc Radiol J 68:171–177. https://doi.org/10.1016/j.carj.2016.12.007

[17] Foula AS, Sabry LS, Elmulla AF, Kamel MA, Hozien AI (2023) Ultrasound-guided Shoulder Intraarticular Ozone Injection Versus Pulsed Radiofrequency Application for Shoulder Adhesive Capsulitis: A Randomized Controlled Trial. Pain Physician. Jul;26(4):E329-E340. PMID: 37535775.

[18] Benvenuti P (2006) Oxygen-ozone treatment of the knee, shoulder, and hip: A personal experience. Rivista Italiana di Ossigeno-Ozonoterapia 5:135-144. https://sid.ir/paper/655510/en

[19] Kara M, Gürçay E (2020) A Single-Dose Injection of Ozone Is Not an Alternative of Corticosteroid Injection in Shoulder Impingement Syndrome. Am J Phys Med Rehabil. 99(7):e89. http://dx.doi.org/10.1097/phm.0000000000001303

[20] Moretti B, Lanzisera R, Sisti GL, Moretti L, Patella V, Simone C (2005) O2-O3 therapy in tendinopathies and entrapment syndromes. Rivista Italiana di Ossigenoozonoterapia. 4(1):20-29.

[21] Scarchilli A (2008) Indications and limits of intra-articular oxygen-ozone therapy for rotator cuff tendinopathy. Int J Ozone Ther. 7:49–52

[22] Coombes BK, Bisset L, Vicenzino B (2010) Efficacy and safety of corticosteroid injections and other injections for management of tendinopathy: a systematic review of randomized controlled trials. Lancet. 376:1751–1767. http://dx.doi.org/10.1016/s0140-6736(10)61160-9

[23] Ikonomidis S, Iliakis E, Eleftheriadou A, Bratanis D, Thomaidis R (2003) Conservative treatment of acute or chronic tendinitis with oxygen-ozone mixture. A double-blind clinical trial. Rivista Italiana di Ossigeno-Ozonoterapia. 2: 67–71.

[24] Gjonovich A, Girotto L, GF Sattin (2002) L'Ossigeno-Ozonoterapia nella spalla dolorosa. (Article in Italian) Esperienza clinica. Riv Ital Ossigeno- Ozonoterapia 1: 37-40.

[25] Cho CH, Jung SW, Park JY, Song KW, Yu KI (2013) Is shoulder pain for three months or longer correlated with depression, anxiety, and sleep disturbance? J Shoulder Elbow Surg. 22(2): 222-228. https://doi.org/10.1016/j.jse.2012.04.001

[26] Roh YH., Lee BK, Noh JH, Oh JH, Gong HS, Baek GH (2012) Effect of depressive symptoms on perceived disability in patients with chronic shoulder pain. Arch Orthop Trauma Surg 132: 1251–1257. https://doi.org/10.1007/s00402-012-1545-0

[27] Toprak M, Erden M (2019) 'Sleep Quality, Pain, Anxiety, Depression and Quality of Life in Patients with Frozen Shoulder'. J Back Musculoskelet Rehabil. 32(2);287 – 291. http://dx.doi.org/10.3233/bmr-171010

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Original Research

Prognostic and Predictive Significance of HER2-low Expression in Metastatic Hormone Receptor Positive Breast Cancer Patients Receiving CDK4-6 Inhibitor Therapy

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ABSTRACT

Objective: This study aimed to analyze the predictive and prognostic value of HER2-low expression in hormone receptor (HR) positive human epidermal growth factor receptor-2 (HER2) negative metastatic breast cancer patients receiving cyclin-dependent kinase-4/6 inhibitor (CDK4/6i) therapy.

Methods: This retrospective study included patients who received CDK4/6i plus endocrine therapy (ET). The pathological and clinical characteristics and survival times of the patients were compared and analyzed.

Results: Our study included 122 patients. There were HER2-zero 88(72%) and HER2-low 34 (28%) patients. The median progression free survival (mPFS) of all patients who received CDK4/6i+ET was 21 (95% confidence interval (CI),18.5–23.5) months, while mPFS was not reached in the HER2-zero group, and mPFS in the HER2-low group was 12 (95%CI, 6.8–17.1) months (p=0.001). The mPFS was shorter in patients with primary endocrine resistance (6 vs. 21 months, p=0.001). There was a change in the HER2-low status of 26(45%) patients with recurrence compared to the first biopsy. In the HER2-zero and HER2-low groups, 22(25%) and 24(71%) patients, respectively, progressed with CDK4/6i+ET (p=0.001). Estrogen receptor (ER) levels less than and greater than 50% resulted different mPFS (6 and 21 months, respectively) (p=0.025). Median PFS differed based on CDK4/6i+ET combination, treatment line, and best treatment response (all p=0.001). In multivariate analysis, HER2 status(p=0.018), chemotherapy status(p=0.006), best response status with CDK4/6i (p=0.001) for PFS, and best response status with CDK4/6i therapy (p=0.007) for OS were significant.

Conclusions: In patients with HR+HER- metastatic breast cancer receiving CDK4/6i therapy, the duration of mPFS was lower in the HER2-low group than that in the HER2-zero group. HER2-low expression is a predictive biomarker of response to CDK4/6 inhibitor therapy.

Keywords: Breast cancer, Endocrine resistance, Cyclin-Dependent Kinases, Her2-low.

INTRODUCTION

Breast cancer is the most common and important cancer in women [1]. Breast cancer is divided into four groups according to HER2, HR and ki-67 proliferation index [2]. Currently, it is difficult to routinely assess gene expression profiles, and the treatment and management of patients are based on the HR and HER2 status.

HER2 expression is reported according to College of American Pathologists (CAP) guidelines [3]. HER2 expression status was further discussed after trastuzumab deruxtecan showed superior efficacy in the DESTINY-Breast-04 trial [4]. In this study, HER2 immunohistochemistry (IHC) score1+ or 2+ but in situ hybridization (ISH) negative patients are defined as "HER2-low". HER2-low expression is present in 30% of patients with triple-negative breast cancer [5], 43% of patient with non-metastatic breast cancer [6]. The HER2-low ratio was 48% in the HR-negative group and 67% in the HR-positive group [7]. There are differences in the results of previous studies regarding the prognostic significance of HER2-low status in breast cancer. HER2-low expression in early-stage breast cancer were associated with better survival [7]. In metastatic triple negative patients, HER2 expression had no prognostic significance [5].

A complex bidirectional double crosstalk mechanism between the ER and HER2 pathways has been implicated in endocrine resistance in patients with luminal-B breast cancer [8]. Therefore, it is important to investigate the predictive and prognostic value of HER2-low expression in HR+HER2-patients receiving CDK4/6i therapy. Douganiotis G et al. found that there was no significant difference in PFS in patients receiving CDK4/6i treatment; However, PFS was shorter in HER2-low patients [9].

Main Points;

- HER-2 low status is present in approximately half of all breast cancers patients.
- HER2 expression is dynamic and can change with disease progression.
- In patients with HR+HER-metastatic breast cancer receiving CDK4/6i therapy, the duration of mPFS was lower in the HER2-low group than that in the HER2-zero group.
- HER2-low expression is a predictive biomarker of response to CDK4/6 inhibitor therapy.

Carlino et al. found that HER2-low or HER2-zero expression had no significant effect on patients receiving palbociclib [10]. Bao et al. showed that PFS was significantly shorter in HER2-low patients who received CDK4/6i [11].

Different results have been obtained by studies on the predictive and prognostic value of HER2-low expression in patients with HR+ and HER2- metastatic breast cancer receiving CDK4/6i therapy. This study aimed to comprehensively evaluate the prognostic and predictive value of HER2-low expression in patients with HR positive HER2 negative metastatic breast cancer receiving CDK4/6i therapy at a single center.

MATERIALS AND METHODS

Study Design

This study was conducted at the Oncology Clinic of the Gaziantep University Faculty of Medicine (Ethics Committee of the Gaziantep University Faculty of Medicine, no.2023/48). Inclusion criteria: Patients age older 18 years, who received CDK4/6i treatment for HR positive and HER2 negative metastatic breast cancer, and for whom follow-up and treatment-related information could be obtained. Patients diagnosed with male breast cancer, second malignancy, performance score (PS)>2, and those with no information regarding treatment or response were excluded from the study.

Patients who received CDK4/6i treatment between May 2020 and February 2023 were screened retrospectively at a single center. A total of 122 patients who met the inclusion criteria were included in this study. Parameters such as age at diagnosis, sex, PS, HER2 status at diagnosis, PR, ER levels and, ki-67 proliferation index in biopsy, stage at diagnosis, endocrine treatment received, endocrine resistance status, luminal type, treatments received before or after CDK4/6i treatment, date of progression, last control, and mortality date were obtained from the patient files or electronic systems.

Variables and Outcome Definition

In Turkey, ribociclib and palbociclib were included on the list of reimbursement agencies in May 2020. However, abemaciclib is not included in the list. All patients in the study were at stage-4; some had de novo metastasis while some had metastasis with recurrence during follow-up. Some patients received cytotoxic chemotherapy during the neoadjuvant phase, whereas others received cytotoxic chemotherapy before or after CDK4/6i treatment during the metastatic phase. Before CDK4/6i was

included in the scope of reimbursement, some patients received cytotoxic chemotherapy prior to CDK4/6i treatment during the metastatic period, regardless of tumor burden.

For standardization, ER, PR, HER2, and ki-67 proliferation index values in breast biopsies at the time of diagnosis were analyzed. In patients who were diagnosed at the local stage and developed recurrence during follow-up, a repeat biopsy was usually performed for metastatic lesions. Patients who were HER2 positive in the control biopsy were excluded from the study. The discordance in HER2 status between diagnostic and repeat biopsies of patients with recurrence was also analyzed.

HER2-zero was defined as an IHC score 0, and HER2-low was defined as IHC score 1 or 2++ but with ISH-negative results. For ER, <1% was defined as negative, 1–9% as weakly positive, 10–49% as moderately positive, and 50–100% as strongly positive. PR and ki-67 were analyzed by dividing into two groups: between 1% and 20%, and ≥20%.

During CDK4/6i treatment, response evaluation is usually performed using physical examination, radiological evaluation, hemogram, biochemistry, and tumor markers. The patients' best responses to treatment were screened retrospectively. Endocrine resistance was analyzed as a factor that may affect the PFS duration of patients treated with CDK4/6 inhibitors. Endocrine resistance, and endocrine sensitivity were analyzed according to the advanced breast cancer (ABC) 4 guidelines [12].

PFS was defined as the time from initiation of CDK4/6i treatment to the date of progression, last control, or mortality. OS was defined as the time from CDK4/6i initiation to the date of the last follow-up or mortality.

Statistical Analysis

The distribution pattern and descriptive characteristics of the variables were analyzed. Since the variables are generally non-parametric, the Mann-Whitney U test was often used for comparison. The association between HER2 status and clinicopathological features was evaluated using the chi-squared or Fisher's exact test. Survival of HER2 groups was analyzed using the Kaplan-Meier method. The multivariate Cox model included HER2 expression status, ER-positive levels, combination of CDK4/6i and ET, chemotherapy status, and best response to CDK4/6i treatment. Data were recorded in the SPSS program (SPSS Inc., Chicago, IL, USA) and statistical analysis were performed. $p<0.05$ was accepted for significance.

RESULTS

Patient Characteristics

A total of 122 eligible patients were included. Among these patients, 88 (72%) were HER2-zero and 34 (28%) were HER2-low. Among the HER2-low patients, 19 (16%) had an IHC score1+ and 15 (12%) had an IHC score2++/ISH-negative. The median age of the study cohort was 48 (27–87)years. Ribociclib and palbociclib were administered to 81 (66%) and 41 (34%) patients respectively. While 72 (59%) patients had de novo metastasis, 50 (41%) had recurrence. Among patients with recurrence, 47 patients received adjuvant ET before recurrence. The HER2 groups were compared according to the patients' baseline characteristics (**Table-1**). The baseline characteristics were similar between the groups. However, a higher proportion of patients in the HER2-zero group did not receive cytotoxic chemotherapy (HER2-zero group: 39.8%; HER2-low group: 8.8%) (p=0.004).

In patients with recurrence, the conversion from HER2-zero at diagnosis to HER2-low at recurrence was 16%, the conversion from HER2-low at diagnosis to HER2-zero at recurrence was 29%, while the HER2 status remained unchanged in 55% of patients. HER2 groups were compared in terms of ER level, PR level, ki-67 proliferation index, tumor grade, luminal type, histological type, and CDK4/6i+ET combination (**Table-2**). The frequency of lung, liver, brain, lymph node, and bone metastases was similar between HER2 groups (all $p>0.05$). A significant correlation was observed between the best response to CDK4/6i treatment and endocrine resistance. While three patients with complete response were endocrine sensitive, 9 of the 11 patients who progressed had secondary endocrine resistance (p=0.028). There were no correlations between the development of endocrine resistance and the PR level, ER level, ki-67 proliferation index, or HER2 expression status (all $p>0.05$).

In the HER2-zero group, 52 (59%) patients received first-line treatment and 19 (21%) patients received third-line treatment and subsequent CDK4/6 inhibitor therapy, while in the HER2-low group, 9 (26%) patients received first-line treatment and 18 (52%) patients received third-line treatment and subsequently underwent CDK4/6 treatment (p=0.001). In the HER2-zero and HER2-low groups, 22 (25%) and 24 (71%) patients, respectively, progressed with CDK4/6i+ET (p=0.001). The HER2 expression status did not affect the best response to CDK4/6i+ET treatment (p=0.497). Prior to CDK4/6i treatment, 40 (33%) patients had a median history of 17.5(3–65) months of ET during metastasis.

Table 1. Patient and treatment characteristics of HER2-zero and HER2-low patients

Demographics		**All (n:122) n (%)**	**Her2-zero (n:88) n(%)**	**Her2-low (n:34) n(%)**	**p value**
Age(years)	(median (min-max))	48(27-87)	49 (27-85)	47.5 (29-87)	0.444
	<65	100(82)	71 (80.7)	29 (85.3)	0.552
	≥65	22(18)	17 (19.3)	5 (14.7)	
Performance score(PS)	PS-0	64(52.5)	50 (56.8)	14 (41.2)	0.323
	PS-1	53(43.4)	35(39.8)	18 (52.9)	
	PS-2	5(4.1)	3(3.4)	2 (5.9)	
Stage at diagnosis	Stage-1	2(1.6)	2(2.3)	0 (0)	0.648
	Stage-2	11(9)	7 (8)	4 (11.8)	
	Stage-3	38(31.1)	26(29.5)	12 (35.3)	
	Stage-4	71(58.2)	53(60.2)	18 (52.9)	
Adjuvant endocrine therapy	Tamoxifen	11(23.4)	8(23.5)	3 (23.1)	0.766
	Tamoxifen+GnRH	18(38.3)	14(41.2)	4 (30.8)	
	Aromatase inhibitor(Aİ)	17(36.2)	11 (32.4)	6 (46.2)	
	Aİ+ GnRH	1(2.1)	1 (2.9)	0 (0)	
Menopause status	Premenopause	47(38.5)	33 (37.5)	14 (41.2)	0.887
	Perimenopause	13(10.7)	10 (11.4)	3 (8.8)	
	Postmenopause	62(50.8)	45 (51.1)	17 (50)	
Metastasis status	Recurrence patients	50(41)	35 (39.8)	15 (44.1)	0.662
	De novo metastasis	72(59)	53 (60.2)	19 (55.9)	
Chemotherapy status	Neo/adjuvant received	46(37.7)	30 (34.1)	16 (47.1)	***0.004***
	Received in the metastatic stage	38(31.1)	23 (26.1)	15 (44.1)	
	Did not receive	38(31.1)	35 (39.8)	3 (8.8)	
Radiotherapy status	Palliative	28(23)	17 (19.3)	11 (32.4)	0.202
	Adjuvant	37(30.3)	26 (29.5)	11 (32.4)	
	Did not receive	57(46.7)	45 (51.1)	12 (35.3)	
CDK4-6i	Ribociclib	81(66.4)	61 (69.3)	20 (58.8)	0.271
	Palbociclib	41(33.6)	27 (30.7)	14 (41.2)	
Endocrine resistance	Endocrine sensitive	58(47.5)	42 (47.7)	16 (47.1)	0.874
	Primary resistance.	15(12.3)	10 (11.4)	5 (14.7)	
	Secondary resistance.	49(40.2)	36 (40.9)	13 (38.2)	

GnRH: Gonadotropin-releasing hormone, CDK4/6i:Cyclin-dependent kinase 4 and 6 inhibitors.

Table 2. Distribution of tumor characteristics and combination of CDK4/6i and endocrine therapy in both groups

Demographics		**All (n:122) n(%)**	**Her2-0 (n:88) n(%)**	**Her2-low (n:34) n(%)**	**p value**
Histological Type	Invasive ductal carcinoma	84(68.9)	58 (65.9)	26 (76.5)	0.435
	Invasive lobular carcinoma	15(12.3)	11 (12.5)	4 (11.8)	
	NOS	23(18.9)	19 (21.6)	4 (11.8)	
Percentage of ER	1-9%	2(1.6)	0 (0)	2 (5.9)	0.071
	10-49%	7(5.7)	5 (5.7)	2 (5.9)	
	50-100%	113(92.6)	83 (94.3)	30 (88.2)	
Percentage of PR	Negative	10(8.2)	6 (6.8)	4 (11.8)	0.589
	1-20 %	19(15.6)	13 (14.8)	6 (17.6)	
	≥20%	93(76.2)	69 (78.4)	24 (70.6)	
ki-67 proliferation index	Unknown	22(18)	14 (15.9)	8 (23.5)	0.506
	0-20%	36(29.5)	28 (31.8)	8 (23.5)	
	≥20%	64(52.5)	46 (52.3)	18 (52.9)	
Grade	grade-1	8(6.6)	7 (8)	1 (2.9)	0.726
	grade-2	59(48.4)	42 (47.7)	17 (50)	
	grade-3	21(17.2)	14 (15.9)	7 (20.6)	
	Unknown	34(27.9)	25 (28.4)	9 (26.5)	
Luminal type	luminal-A	33(27)	26 (29.5)	7 (20.6)	0.318
	luminal-B	89873)	62 (70.5)	27 (79.4)	
Combination of CDK4/6i and endocrine therapy	Palbociclib+letrozole	24(19.7)	16 (18.2)	8 (23.5)	0.737
	Ribociclib+letrozole	47(38.5)	35 (39.8)	12 (35.3)	
	Palbociclib+fulvestrant	17(13.9)	11 (12.5)	6 (17.6)	
	Ribociclib+fulvestrant	34(27.9)	26 (29.5)	8 (23.5)	
Concomitant endocrine therapy	Aromatase inhibitor	71(58.2)	51 (58)	20 (58.8)	0.930
	Fulvestrant	51(41.8)	37 (42)	14 (41.2)	

NOS: No Specific Type, ER: Estrogen receptor, PR: Progesterone receptor,CDK4/6i: Cyclin-dependent kinase 4 and 6 inhibitors

Survival Analysis

There were differences in the duration of mPFS in subgroups based on HER2 status, best response to CDK4/6i treatment, concomitant ET, CDK4/6i treatment line, ER level, and chemotherapy status (Table-3). In the HER2-zero group, median PFS duration was not reached. Median PFS was 12 (95% CI, 6.8–17.1) months in the HER2-low group and 21 (95% CI, 18.5–23.5) months in all patients (p=0.001) (Figure-1A). Median PFS was 20 (95% CI, 9.2–30.8) months in the IHC score1+ group and 6 (95% CI, 3.4–8.6) months in the IHC score2++/ISH negative group (p=0.001). In the multivariate analysis, HER2 status at diagnosis, cytotoxic chemotherapy status, and best response to CDK4/6i+ET treatment were significant parameters for PFS (p = 0.018, p = 0.006, p = 0.001, respectively) (Table-4). At the time of analysis, 46 (38%) patients had progressed with CDK4/6i+ET therapy and 76 (62%) patients were on CDK4/6i+ET therapy.

Table 3. Comparison of groups with different PFS and overall survival by Kaplan–Meier method

		PFS		OS	
Variables		**Median(95%CI) month**	**p value**	**Mean(95%CI) month**	**p value**
HER2 status	HER2-zero	NR	**0.001**	25.7(23.3-28.2)	0.195
	HER2-low	12(6.8-17.2)		30.3(24.3-36.4)	
	All patients	21(18.5-23.5)		32.9(9.5-36.4)	
CDK4/6i	Ribociclib	20(17.4-22.6)	0.530	33.4(29.3-37.6)	0.631
	Palbociclib	21(10.9-31)		23.5(20-26.9)	
Change in HER2 status	Her2-zero then became Her2-low	NR	**0.001**	25.4(20.7-30.2)	**0.031**
	Her2-low then became Her2-zero	9(4-13.9)		20.2(15.2-25.3)	
	Her2 unchanged	17(10.8-23.2)		21.5(16.8-26.2)	
	There was no repeat biopsy	NR		36.7(32.5-41)	
Metastasis status	Recurrence	17(8.5-25.5)	0.052	22.6(19.1-26.2)	**0.018**
	De novo metastatic	27(18.8-35.2)		35.1(31-39.3)	
Best response to CDK4/6i	partial response	23(18.2-27.8)	**0.001**	37.5(33.8-40.9)	**0.001**
	stable disease	9(7.2-10.7)		21.8(17.2-26.5)	
	progressive disease	3(1.9-4.1)		8.3(4.5-12)	
Progression with CDK4/6i	No			29.6(28.1-31.1)	**0.001**
	Yes			25.3(20-30.5)	
Concurrent endocrine therapy	aromatase inhibitor	NR	**0.01**	36.3(32-40.8)	**0.003**
	Fulvestrant	18(8.3-27.7)		21(17.5-24.8)	
CDK4/6i therapy line	1.line	NR	**0.001**	26.3(23.5-29)	0.527
	2.line	NR		24.2(19.5-28.8)	
	3.line	12(7.4-16.6)		32.4(24.6-40.2)	
	4.line and later	9(3.9-14)		17.9(13.6-22.3)	
Primary endocrine resistance	No	21(14.5-27.5)	**0.001**	34(31.4-38)	**0.001**
	Yes	6(4.7-7.2)		16(10-22.9)	
Estrogen receptor status	1-9%	NR	**0.025**	10(0.3-19.7)	0.172
	10-49%	6(2.1-9.8)		18.7(9.8-27.5)	
	50-100%	21(18.7-23.2)		33.5(30-37)	
CDK4-6i plus endocrine therapy	Palbociclib+letrozole	NR	**0.001**	28.7(26.3-31)	**0.001**
	Ribociclib+letrozole	NR		34(28-39.9)	
	Palbociclib+fulvestrant	7(4.7-9.2)		14.4(8.6-20)	
	Ribociclib+fulvestrant	19(13.9-24)		24.1(20-28)	
chemotherapy status	Did not receive	NR	**0.001**	21.3(17.9-24.8)	**0.047**
	Received in the metastatic stage	12(0-25.2)		31(25-37)	
	Neoadjuvant received	17(8.8-25.2)		28.9(26-31.6)	

PFS: Progression-free survival, OS: Overall survival, NR: Not reached,CDK4/6i: Cyclin-dependent kinase 4 and 6 inhibitors

Table 4. Univariable and multivariable analysis of parameters affecting mPFS duration

		Univariable		Multivariable	
Variables		**HR(95% CI)**	**p value**	**HR(95% CI)**	**p value**
HER2	Score-0	Reference	**0.001**	Reference	**0.018**
	Score-(+1)	1.8(0.9-3.8)		2.08(0.90-4.79)	
	Score-(+2)/ISH(-)	4.3(2.1-8.8)		3.21(1.38-7.44)	
ER	≥50%	Reference	**0.05**	Reference	0.223
	10-49%	5.5(1.3-23)		4.59(0.76-27.8)	
	1-9%	1.4(0.5-3.9)		1.60(0.49-5.12)	
CDK4-6i+ET	Palbociclib+letrozole	Reference	**0.015**	Reference	0.753
	Ribociclib+letrozole	1.3(0.5-3.5)		1.32(0.46-3.77)	
	Palbociclib+fulvestran	4.1(1.5-11.2)		1.68(0.54-5.16)	
	Ribociclib+fulvestrant	2(0.8-5.2)		1.05(0.38-2.88)	
chemotherapy status	Did not receive	Reference	**0.003**	Reference	**0.006**
	Received in the metastatic stage	10.8(2.5-46)		3.56(0.69-18.3)	
	Neoadjuvant received	12.1(2.8-51.6)		8.99(1.77-45)	
CDK4-6i+ET best response	partial regression	Reference	**0.001**	Reference	**0.001**
	stable disease	3(1.5-6)		2.98(1.38-6.44)	
	progressive disease	54(19-149)		83.5(21.9-318)	

ER: Estrogen receptor, CDK4/6i: Cyclin-dependent kinase 4 and 6 inhibitors, ET: Endocrine therapy, ISH:in situ hybridization

There was no significant difference in mPFS between patients receiving ribociclib and those receiving palbociclib (p=0.530). The mPFS was 6 (95% CI, 4.8–72) months in patients with primary endocrine resistance and 21 (95% CI, 14.5–27.4) months in patients without primary endocrine resistance (p=0.001) (Figure-1B). The mPFS was not reached in patients receiving CDK4/6i concomitant letrozole, while mPFS was 18 (95%CI,8.3–27.7) months in patients receiving fulvestrant (p=0.01) (Figure-2A). The mPFS was 21 (95% CI, 18.8–23.2) months in patients with ER levels >50% and 6(95% CI, 2.1–9.8) months in patients with ER levels<50% (p=0.025). There was a difference in mPFS based on the best response to CDK4/6i+ET treatment and the treatment line in which cytotoxic chemotherapy was administered (all p<0.001) (Figure-2B). There was a difference in mPFS between CDK4/6i+ET combinations, for example, mPFS at 7 (95% CI, 4.7–9.3) months in the fulvestrant+palbociclib group and 19 (95% CI, 14–24) months in the fulvestrant+ribociclib group (p=0.001) (Figure-3A).

In patients with discordance between diagnosis and repeat biopsy, the duration of mPFS could not be reached in patients who were HER2-zero initially and then became HER2-low. In patients whose HER2 status did not change in the second biopsy, mPFS was 17 (955 CI, 10.8–23.2) months. The mPFS was 9 (95% CI,4.1–13.9) months in patients who were initially HER2-low and then became HER2-zero (p=0.001) (**Figure-3B**).

The median PFS was 20 months in tumor histologica grade 1 and 2 patients and 7 (95% CI,3.3–10.6) months in grade 3 patients (p=0.120). The ORR was 69% and the DCR was 90%. In 12 patients, treatment was newly initiated or the best-response status could not be reached. The mPFS decreased significantly with increasing CDK4/6i+ET treatment line (p=0.001).

In the HER2-zero group, the 24-month survival was 95% and the 36-month survival was 86%, while in the HER2-low group, the 24-month survival was 93% and the 36-month survival was 89% (p=0.578). There were differences in mOS between the subgroups, discordance in HER2 status, metastasis status, CDK4-6i+ET combinations, primary endocrine resistance, chemotherapy status, concurrent ET agent, best response to CDK4/6i, and progression with CDK4/6i treatment (**Table-3**).

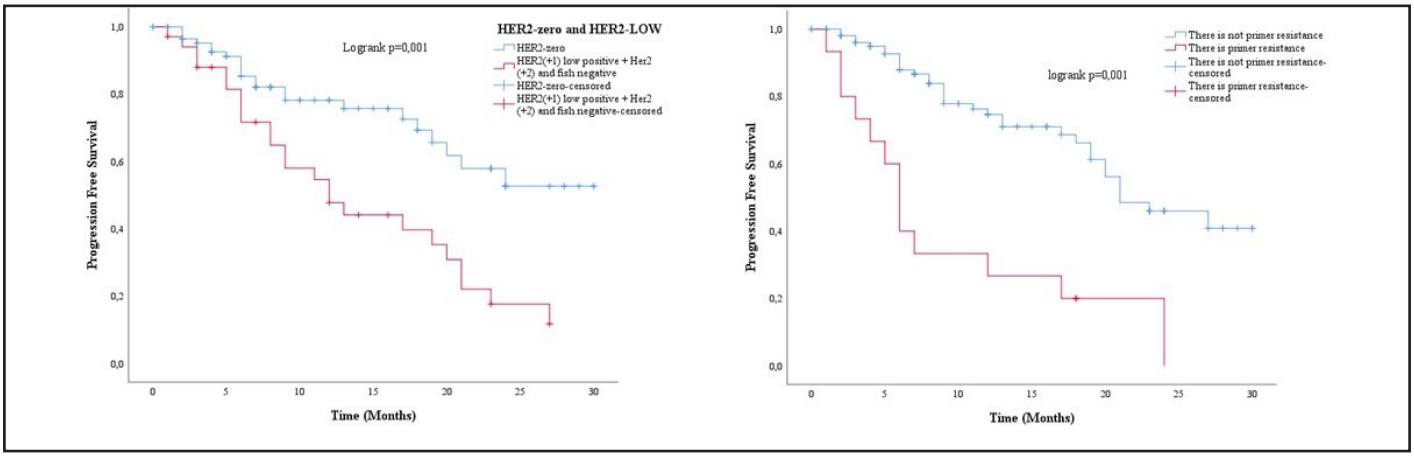

Figure 1.A. Median PFS is significantly longer in HER2-zero than that in HER2-low patients. **B)** Median PFS was significantly shorter in patients with primary endocrine resistance.

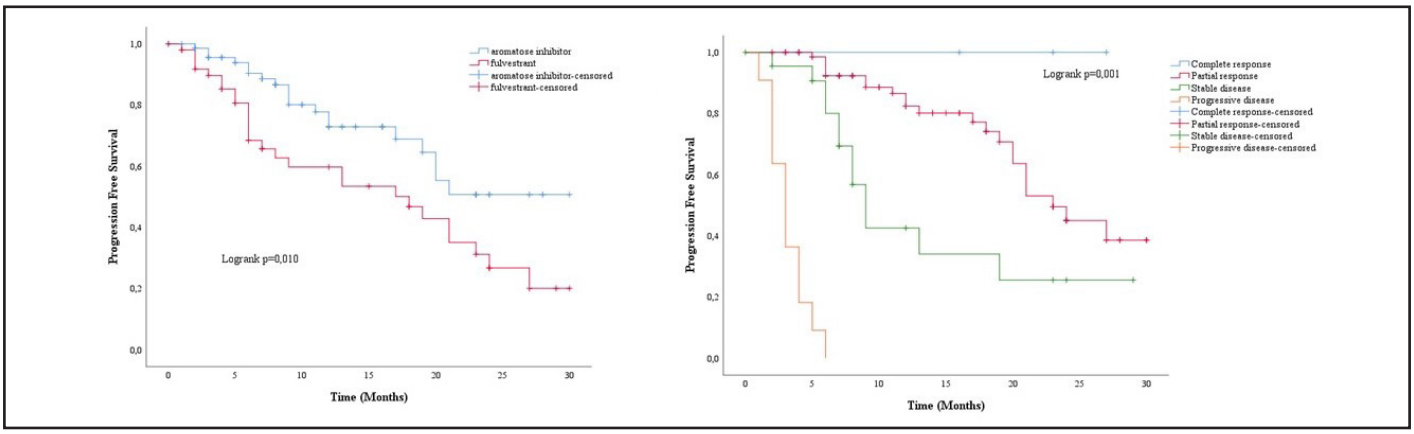

Figure 2.A. Regardless of the CDK4/6i agent, mPFS was different according to concomitant letrozole or fulvestrant treatment. **B)** The best response status obtained with CDK4/6i+ET combination predicted the duration of mPFS.

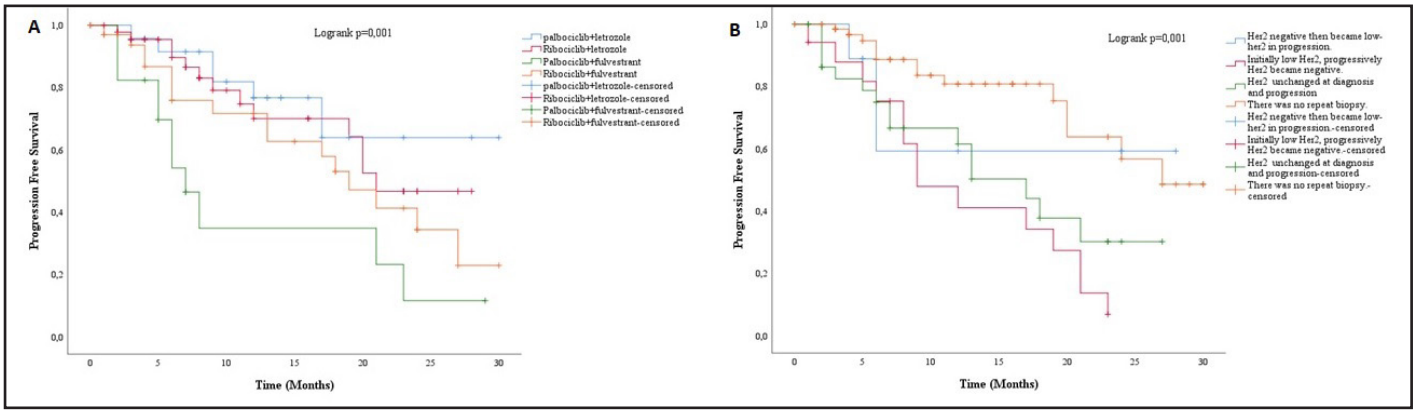

Figure 3.A. A difference was observed in the duration of mPFS across different treatment combinations. Similar mPFS was observed in patients treated with CDK4-6i plus letrozole combination, while its was significantly lower in those treated with fulvestrant+palbociclib combination. **B**) Moreover,differences were found in the mPFS times in patients whose HER2 status changed between biopsy specimen obtained at diagnosis and at recurrence. Patients with HER2-zero in the initial biopsy had significantly longer mPFS than those with HER2-low in the initial biopsy (p=0.001).

In the multivariate analysis, best response to CDK4/6i alone was an independent parameter affecting OS duration (p=0.007). Following progression with CDK4/6i+ET, patients received subsequent treatments: 4 (11%) received ET alone, 9 (25%) received chemotherapy and ET, and 23 (64%) received chemotherapy only. In these patients, the mOS was 9 (95%CI, 3.8–14.2) months and the best mOS was observed in the group that received chemotherapy and ET (p=0.001).

DISCUSSION

In this study, the duration of mPFS with CDK4/6i+ET was lower in the HER2-low group than in the HER2-zero group (p=0.001). Similarly, the mPFS duration was significantly lower in patients with an IHC score2++/ISH- than in patients with IHC score1+ (6 vs. 20 months). In a study by Bao et al., mPFS was 8.9 months in HER2-low patients receiving CDK4/6i+ET treatment, whereas mPFS was 18.8 months in HER2-zero patients (p=0.01) [11]. In another study, the duration of mPFS was lower in HER2-low patients receiving CDK4/6i therapy than that in HER2-zero patients (1.74 vs.3.35 years), but the difference was not significant [9]. In a study by Francesca et al., there was no difference in mPFS between HER2 groups who received palbociclib treatment alone [10]. The results of our study is consistent with that of two of three similar studies in the literature. HER2-low expression is a negative predictive biomarker in patients treated with CDK4/6i+ET.

The incidence of HER2-low expression varies among breast cancer studies. While the incidence of HER2-low was 28% in our study, it occurred at different rates (30–77%) in other studies [9-11]. In a database study analyzing 65,000 patients, the incidence of HER2-low status in the ER+ cohort was 65%, while that in the ER- cohort was 38% [13]. Differences in the incidence of HER2-low expression may be related analytical processes, ethnicity, and patient group. In the DESTINY-Breast04 study, trastuzumab deruxtecan was compared with physician-selected chemotherapy in previously treated HER2-low patients, and mPFS was found to be 10.1 months vs. 5.4 months in both patients with IHC score1+ and score2++/ISH [4]. HER2-low expression is present in nearly half of breast cancer patients and is a predictive biomarker for the response to antibody-drug conjugates (ADCs).

In our study, there was a change in the HER2-low status of 26 (45%) patients with recurrence compared to the first biopsy. While the mPFS duration could not be reached in those who changed from HER2-zero to HER-low, it was 9 months in those who changed from HER-low to HER-zero. In other words, the mPFS duration of patients with discordance between biopsies was determined using the HER2 expression status in the first biopsy. In a study conducted in China, changes in HER2-low status were analyzed in 247 patients with recurrent breast cancer. The twenty-five (49%) patients who were initially HER2-zero and 19 (27%) who were initially HER2-positive converted to HER2-low. Changes in HER2 status were observed in up to 20% of all study patients [14]. The HER2-low expression status is dynamic, similar to the hormone profile in breast cancer, and may change during progression. Tissue sampling during disease progression is essential to detect the potential benefits of ADC and dynamic HER2 expression.

In our study, patients with complete response to CDK4/6i+ET treatment were endocrine sensitive, whereas patients with progressive disease with CDK4/6i+ET treatment often had secondary endocrine resistance. If the patient had secondary endocrine resistance, the expected response and duration of response to CDK4/6i+ET treatment were reduced compared to those in endocrine-sensitive patients. There are common pathways involved in the mechanisms of endocrine resistance and resistance to CDK4/6i therapy [15]. Furthermore, in our study, 25% of the patients in the HER2-zero group and 71% of the patients in the HER2-low group progressed with CDK4/6i+ET (p=0.001). The presence of HER2-low expression, such as in endocrine resistance, decreased the efficacy of CDK4/6i+ET treatment and more patients progressed. The role of HER2 activation in endocrine resistance in patients with HR+HER- breast cancer has only been demonstrated in a limited number of preclinical studies [16].

The mPFS was lower in patients with tumor histological grade-3 tumors than that in patients with grades 1-2 tumors (6 vs. 20 months). This may be related to the low efficacy of CDK4/6 inhibitors, especially in high-grade and aggressive tumors [17]. There was no difference between the median PFS of our patients who received ribociclib or palbociclib treatment. In a study in which real-life data were analyzed, the PFS times were similar for palbociclib and ribociclib (28 and 29 months, respectively) [18]. In our study, mPFS was significantly different between patients who received cytotoxic chemotherapy before CDK4/6 in the metastatic period and those who received CDK4/6i treatment as first-line treatment [19]. In a similar study, PFS with CDK4/6i was shorter in patients with recurrence and visceral metastases. Although the reason for this is not clear, because patients with

recurrence usually receive ET as an adjuvant treatment, endocrine resistance may develop in these patients; therefore, the response to CDK4/6i+ET treatment is reduced.

In our study, we found that the duration of mPFS increased significantly as the estrogen receptor levels increased. Similarly, previous studies have shown that the benefits of ET increase as estrogen and progesterone receptor expression increases [20]. The improvement in PFS duration with increasing ER levels may be attributed to the increased efficacy of ET used concomitantly with CDK4/6i. Similarly, because fulvestrant is generally used in cases of endocrine resistance, the PFS time of patients receiving fulvestrant was shorter than that of patients receiving letrozole. In our patients receiving palbociclib+fulvestrant had a mPFS of 7 months, similar to the PFS of 9.5 months in the PALOMA-3 study [21]. In our patients who received ribociclib+fulvestrant combination therapy, mPFS was 19 months, similar to the mPFS of 20.5 months in the MONALEESA-3 study [22].

In our study, the best response status of patients predicted the benefit of CDK4/6i+ET treatment (p=0.001) (Table-5). The ORR was 69% and the DCR was 90%; in PALOMA-2, a prospective study, the ORR was 56% and the DRC was 87%, with similar rates [23]. In our study, the use of CDK4/6 inhibitors in the first step resulted in a significant difference in mPFS compared to the use of CDK4-6 inhibitors in subsequent steps. In other studies, longer mPFS durations were observed with first-line CDK4/6i therapy [21, 22].

In an Austrian study, HER2-low expression was frequent in metastatic HR+ breast cancer and had no effect on prognosis [24]. HER2-low expression in early-stage breast cancer was considered a good prognostic biomarker in both HR positive and HR negative patients [7]. As tumor biology, resistance mechanisms, and mutations observedin the early and metastatic stages of breast cancer may differ, HER2 expression may have a different prognostic value. The CDK4/6i-dependent mOS of patients with recurrence was significantly shorter than that of patients with denovo metastasis. As patients with recurrence usually receive adjuvant ET before CDK4/6i and CDK4/6i therapy during the endocrine resistance period, the benefits provided by CDK4/6i are reduced. In our study, the CDK4/6i-dependent mOS duration was shorter in patients with primary endocrine resistance and those receiving fulvestrant treatment. In our study, the prognostic value of HER2-low expression for OS duration was not found. The survival times were consistent with those reported in previous studies [21].

The step in which CDK4/6i+ET was administered was significant for mPFS but not for mOS. Patients who progressed with CDK4/6I+ET treatment received ET or chemotherapy, and their mPFS was 9 months. In the DESTINYBreast-04 study, the mPFS was 10 months with trastuzumab deruxtecan treatment after 70% of the patients received CDK4/6i treatment [4]. Administration of trastuzumab deruxtecan after progression with CDK4/6i in HER2-low patients may prolong overall survival in this patient group. The best response to CDK4/6i+ET treatment was an independent parameter affecting OS duration in our study.

Table 5. Response rates, Endocrine resistance status, and mPFS duration of patients with the best response to CDK4-6i and endocrine therapy combination treatment

Best response status	n(%)	mPFS (95%CI) month	Endocrine resistance status		
			Endocrine sensitive, n(%)	primary resistance n(%)	secondary resistance n(%)
complete response	3(2.5%)	NR	3(100)	-	-
partial regression	73(60%)	23(18.2-27.8)	33(45.2)	28(38.4)	12(16.4)
stable disease	23(19%)	9(7.3-10.7)	14(60.9)	7(30.4)	2(8.7)
progressive disease	11(9%)	3(1.9-4)	2(18.2)	9(81.8)	-
Overall	110	20(17.7-22.3)	52(47.3)	44(40)	14(12.7)

mPFS: median Progression-free survival, NR: Not reached

Limitations

Retrospective design, the fact that CDK4/6i+ET treatment was applied at different steps, and relatively limited number of patients are the limitations of the study. Nevertheless, this is one of the few studies investigating the HER2-low expression in patients receiving CDK4/6i+ET treatment. Factors that may affect patient survival were comprehensively analyzed. The difference between this and other studies is that we also investigated the relationship between endocrine resistance and HER2-low status.

CONCLUSION

HER2-low expression is present in nearly half of the patients with breast cancer and may change during disease progression. HER2-low patients progressed more with CDK4/6i treatment, and their mPFS was lower than that of the HER2-zero group. Independent parameters affecting mPFS duration were HER2-low status, best response to CDK4/6i+ET treatment, and cytotoxic chemotherapy status. HER2-low expression was not a prognostic factor for OS but a predicted response to CDK4/6i treatment. Randomized studies are required to determine the predictive value of HER2-low expression in patients receiving CDK4/6i.

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Conflict of interests: The authors declare no conflicts of interest regarding the publication of this paper.

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Ethical Approval: This study was approved by Gaziantep University Faculty of Medicine Ethics Committee **(no.2023/48).**

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REFERENCES

[1] Siegel RL, Miller KD, Fuchs HE, Jemal A (2022) Cancer statistics, 2022. CA Cancer J Clin 72:7-33. https://doi.org/10.3322/caac.21708

[2] Prat A, Pineda E, Adamo B, Galvan P, Fernandez A, Gaba L, et al. (2015) Clinical implications of the intrinsic molecular subtypes of breast cancer. Breast 24 Suppl 2:S26-35. https://doi.org/10.1016/j.breast.2015.07.008

[3] Wolff AC, Hammond MEH, Allison KH, Harvey BE, Mangu PB, Bartlett JMS, et al. (2018) Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Focused Update. Arch Pathol Lab Med 142:1364-1382. https://doi.org/10.5858/arpa.2018-0902-SA

[4] Modi S, Jacot W, Yamashita T, Sohn J, Vidal M, Tokunaga et al. (2022) Trastuzumab Deruxtecan in Previously Treated HER2-Low Advanced Breast Cancer. N Engl J Med 387:9-20. https://doi.org/10.1056/NEJMoa2203690

[5] Gampenrieder SP, Dezentje V, Lambertini M, de Nonneville A, Marhold M, Le Du F, Cortes Salgado A, et al. (2023) Influence of HER2 expression on prognosis in metastatic triple-negative breast cancer-results from an international, multicenter analysis coordinated by the AGMT Study Group. ESMO Open 8:100747. https://doi.org/10.1016/j.esmoop.2022.100747

[6] Tan R, Ong WS, Lee KH, Lim AH, Park S, Park YH, et al. (2022) HER2 expression, copy number variation and survival outcomes in HER2-low non-metastatic breast cancer: an international multicentre cohort study and TCGA-METABRIC analysis. BMC Med 20:105. https://doi.org/10.1186/s12916-022-02284-6

[7] Ergun Y, Ucar G, Akagunduz B (2023) Comparison of HER2-zero and HER2-low in terms of clinicopathological factors and survival in early-stage breast cancer: A systematic review and meta-analysis. Cancer Treat Rev 115:102538. https://doi.org/10.1016/j.ctrv.2023.102538

[8] Giuliano M, Trivedi MV, Schiff R (2013) Bidirectional Crosstalk between the Estrogen Receptor and Human Epidermal Growth Factor Receptor 2 Signaling Pathways in Breast Cancer: Molecular Basis and Clinical Implications. Breast Care (Basel) 8:256-262. https://doi.org/10.1159/000354253

[9] Douganiotis G, Kesisis G, Lalla E, Korantzis I, Boukovinas I, Papazisis K (2022) Prognostic Significance of Low HER2 Expression in Patients With Metastatic Hormone Receptor-

positive Breast Cancer Treated With First Line CDK4/6 Inhibitors: A Greek Multicenter Real-world Data Analysis. Cancer Diagn Progn 2:585-591. https://doi.org/10.21873/cdp.10146

[10] Carlino F, Diana A, Ventriglia A, Piccolo A, Mocerino C, Riccardi F, et al. (2022) HER2-Low Status Does Not Affect Survival Outcomes of Patients with Metastatic Breast Cancer (MBC) Undergoing First-Line Treatment with Endocrine Therapy plus Palbociclib: Results of a Multicenter, Retrospective Cohort Study. Cancers (Basel) 14. https://doi.org/10.3390/cancers14204981

[11] Bao KKH, Sutanto L, Tse SSW, Man Cheung K, Chan JCH (2021) The Association of ERBB2-Low Expression With the Efficacy of Cyclin-Dependent Kinase 4/6 Inhibitor in Hormone Receptor-Positive, ERBB2-Negative Metastatic Breast Cancer. JAMA Netw Open 4:e2133132. https://doi.org/10.1001/jamanetworkopen.2021.33132

[12] Cardoso F, Senkus E, Costa A, Papadopoulos E, Aapro M, Andre F, et al. (2018) 4th ESO-ESMO International Consensus Guidelines for Advanced Breast Cancer (ABC 4) dagger. Ann Oncol 29:1634-1657. https://doi.org/10.1093/annonc/mdy192

[13] Baez-Navarro X, van Bockstal MR, Andrinopoulou ER, van Deurzen CHM (2023) HER2-Low Breast Cancer: Incidence, Clinicopathologic Features, and Survival Outcomes From Real-World Data of a Large Nationwide Cohort. Mod Pathol 36:100087. https://doi.org/10.1016/j.modpat.2022.100087

[14] Shi Q, Yu J, Liu D, Ren F, Wu J, Shen K (2023) Distribution, dynamic evolution, and clinical outcomes of patients with advanced breast cancer according to HER2 expression. BMC Cancer 23:173. https://doi.org/10.1186/s12885-023-10634-7

[15] Stanciu IM, Parosanu AI, Orlov-Slavu C, Iaciu IC, Popa AM, Olaru CM, et al. (2023) Mechanisms of Resistance to CDK4/6 Inhibitors and Predictive Biomarkers of Response in HR+/HER2-Metastatic Breast Cancer-A Review of the Literature. Diagnostics (Basel) 13. https://doi.org/10.3390/diagnostics13050987

[16] Mazumder A, Shiao S, Haricharan S (2021) HER2 Activation and Endocrine Treatment Resistance in HER2-negative Breast Cancer. Endocrinology 162. https://doi.org/10.1210/endocr/bqab153

[17] Hu Y, Gao J, Wang M, Li M (2021) Potential Prospect of CDK4/6 Inhibitors in Triple-Negative Breast Cancer. Cancer Manag Res 13:5223-5237. https://doi.org/10.2147/CMAR.S310649

[18] Buller W, Pallan L, Chu T, Khoja L (2023) CDK4/6 inhibitors in metastatic breast cancer, a comparison of toxicity and efficacy across agents in a real-world dataset. J Oncol Pharm Pract 29:1825-1835. https://doi.org/10.1177/10781552231163121

[19] Knudsen ES, Schultz E, Hamilton D, Attwood K, Edge S, O'Connor T, et al. (2022) Real-World Experience with CDK4/6 Inhibitors for Metastatic HR+/HER2- Breast Cancer at a Single Cancer Center. Oncologist 27:646-654. https://doi.org/10.1093/oncolo/oyac089

[20] Bartlett JM, Brookes CL, Robson T, van de Velde CJ, Billingham LJ, Campbell FM, et al. (2011) Estrogen receptor and progesterone receptor as predictive biomarkers of response to endocrine therapy: a prospectively powered pathology study in the Tamoxifen and Exemestane Adjuvant Multinational trial. J Clin Oncol 29:1531-1538. https://doi.org/10.1200/JCO.2010.30.3677

[21] Cristofanilli M, Turner NC, Bondarenko I, Ro J, Im SA, Masuda N, Colleoni M, et al. (2016) Fulvestrant plus palbociclib versus fulvestrant plus placebo for treatment of hormone-receptor-positive, HER2-negative metastatic breast cancer that progressed on previous endocrine therapy (PALOMA-3): final analysis of the multicentre, double-blind, phase 3 randomised controlled trial. Lancet Oncol 17:425-439. https://doi.org/10.1016/S1470-2045(15)00613-0

[22] Slamon DJ, Neven P, Chia S, Fasching PA, De Laurentiis M, Im SA, et al. (2018) Phase III Randomized Study of Ribociclib and Fulvestrant in Hormone Receptor-Positive, Human Epidermal Growth Factor Receptor 2-Negative Advanced Breast Cancer: MONALEESA-3. J Clin Oncol 36:2465-2472. https://doi.org/10.1200/JCO.2018.78.9909

[23] Finn RS, Crown JP, Lang I, Boer K, Bondarenko IM, Kulyk SO, et al. (2015) The cyclin-dependent kinase 4/6 inhibitor palbociclib in combination with letrozole versus letrozole alone as first-line treatment of oestrogen receptor-positive, HER2-negative, advanced breast cancer (PALOMA-1/

TRIO-18): a randomised phase 2 study. Lancet Oncol 16:25-35. https://doi.org/10.1016/S1470-2045(14)71159-3

[24] Gampenrieder SP, Rinnerthaler G, Tinchon C, Petzer A, Balic M, Heibl S, et al. (2021) Landscape of HER2-low metastatic breast cancer (MBC): results from the Austrian AGMT_MBC-Registry. Breast Cancer Res 23:112. https://doi.org/10.1186/s13058-021-01492-x

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Original Research

ECM Components are Essential for Proper i*n Vitro* Myogenesis

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ABSTRACT

Objective: *In vitro* models of skeletal muscle often utilize primary myoblast cells or myoblast cell lines. Myoblasts require adhesion to the extracellular matrix (ECM) to grow, proliferate, migrate, and differentiate in their natural environments *in vivo*. To meet the adhesion needs of adhesive cells under *in vitro* conditions, culture surfaces are coated with various biological or synthetic compounds. Within the scope of the study, the differentiation potential of H2K myoblasts, a cell line resembling primary myoblasts, were comparatively evaluated through morphological analysis on culture surfaces coated with various ECM and synthetic materials.

Methods: The culture surfaces were coated with fibronectin and laminin, the major adhesion proteins of ECM; gelatin, a molecular derivative of collagen; matrigel, an ECM extract; and PLL, a synthetic poly-amino acid. Cells were allowed to differentiate in each culture medium for 4 days and their capacity to adhere to the surface and differentiation rates from myoblast to myotube were evaluated by morphological analysis.

Results: In the uncoated culture environment, cells could only attach to 30-50% of the culture surface and myotube development was limited and not aligned with each other. On surfaces coated with PLL, no myotube development was observed and cells could only attach to 30-40% of the culture surface. Myotube development and alignment were similar on all surfaces coated with ECM components. On surfaces coated with ECM components laminin, fibronectin and matrigel, cells covered the entire culture surface and exhibited similar myotube development. However, on surfaces coated with gelatin, both cell adhesion to the surface and myotube development were limited compared to other ECM components. The mean myotube diameters of fibronectin, laminin, matrigel, PLL+laminin and gelatin were 49.71µm (±16.3µm), 52.31µm (±15.7µm), 51.9µm (±15.3µm), 53.06µm (±14.2µm) and 35.25µm (±11.4µm), respectively.

Conclusion: Within the scope of the study, it was revealed that coating the culture surface with only a cationic material such as PLL does not support myogenesis and ECM components are needed for cell viability and differentiation.

Keywords: Myogenesis, fibronectin, laminin, matrigel, gelatin, poly-l-lysine

INTRODUCTION

The human body is composed of approximately 650 skeletal muscles, which account for almost half of the entire body mass. Each skeletal muscle is composed of thousands of elongated, multinucleated cells, called muscle fibers, which are organized together through the extracellular matrix (ECM) to contract synchronously. The dynamic structure of this massive organization is kept under adaptive control by neural and physical stimulation, hormonal balance and nutrient availability [1-4]. Skeletal muscle performs many important functions, both mechanical and metabolic. It is connected to the bones by tendons and directs all movements of the body. It also maintains the posture of the body and keeps it in balance. It provides the power required for movement by converting chemical energy into mechanical energy during contractions. The heat generated during this process is used to maintain body temperature. Approximately 80% of circulating glucose is consumed by the muscles. Important compounds such as carbohydrates and amino acids are stored in the muscles. In case of starvation, they are able to utilize their stores for the energy the body needs for movement; they are also able to supply the amino acids needed to support protein synthesis in other tissues [5-7].

Deficiency of a protein in the skeletal muscle itself, as in Duchenne muscular dystrophy, or neurological diseases that prevent muscle innervation, as in amyotrophic lateral sclerosis, lead to chronic degeneration of skeletal muscles [8, 9]. Furthermore, metabolic diseases such as diabetes, sarcopenia and cachexia affect the normal availability of hormones, nutrients, metabolites and other important soluble modulators required for skeletal muscles, leading to skeletal muscle atrophy with a significant impact on quality of life [10-12]. For this reason, the molecules that both constitute the structure of skeletal muscle itself and provide its adaptive dynamism are the subject of intense research.

Main Points

- Myogenic cells need to interact with ECM in order to perform biological activities such as proliferation, migration and differentiation in their natural environment.
- Under in vitro conditions, a suitable adhesion environment must be provided to maintain myogenicity and to model the biological activities of myogenic cells.
- In order to mimic the adhesive environment of adhesion-dependent cells in vivo, the culture surface is usually coated with extracellular matrix components or synthetic polymers.
- This study well demonstrated that ECM components are necessary and sufficient for in vitro myogenesis.

Typically, myoblast cultures are utilized in investigating conditions that contribute to skeletal muscle pathophysiology. For this purpose, various myoblast cell lines such as C2C12, H2K, and L6, as well as primary myoblast cultures from humans or different animals, can be established [13-15]. In order to mimic muscle fibers in skeletal muscles, the differentiation of myoblast cells is stimulated to fuse with each other and form myotubes. Since myoblasts and myotubes are adhesion-dependent cells, they need to adhere to culture surfaces [16, 17]. In order to mimic the adhesive environment of adhesion-dependent cells *in vivo*, the culture surface is usually coated with ECM components or synthetic polymers [18, 19]. Although there is no generally accepted culture surface coating material for myogenic cells, culture surfaces are commonly coated with various materials in the myogenic differentiation process due to their low capacity to adhere only to physically treated culture surfaces. Within the scope of the study, the differentiation potential of H2K myoblasts, which have a very low capacity to adhere to the uncoated culture surface like primary myoblasts, on the culture surface coated with various ECM components and synthetic materials was evaluated comparatively by morphological analysis.

MATERIAL AND METHODS

Coating Procedures and Cell Culture Conditions

Before seeding cells, the well surfaces of 12-well plates were coated with 0.1 mg/ml poly-l-lysine (PLL) (Sigma, P5899), 1.5 µg/cm² laminin (Sigma, L2020), 1.5 µg/cm² fibronectin (Sigma, F2006), 0.1% gelatin (Sigma, G1393), and 100 µg/ml matrigel (Corning, CLS354234). To ensure adherence of the materials to the well surfaces, plates coated with laminin and gelatin were incubated for 2 hours and 45 minutes at 37°C, respectively; matrigel-coated plates were incubated for 1 hour at room temperature; and plates coated with fibronectin and PLL were air-dried for 2 hours. Additionally, to coat PLL-coated plates with laminin, after air-drying PLL-coated plates for 2 hours, 1.5 µg/cm² laminin was applied and incubated for 2 hours at 37°C.

The conditionally immortal mouse H2K myogenic cell line [20] was used for the experiments. A seeding density of 1 ×

10^5 myoblasts per well was used with growth medium (GM) containing high glucose DMEM (Dulbecco's modified Eagle's medium; Invitrogen, Gibco), 20% FBS (Fetal Bovine Serum; Biochrom), 0.5% chick embryo extracts (CEEs; US Biological), and 20 U/mL interferon (IFN)-γ (Roche). Cells were initially incubated in GM in a humidified incubator (33°C, 10% CO_2) for one day and then induced to differentiate using differentiation medium (DM) containing high glucose DMEM (Gibco) and 5% HS (Horse Serum, Biochrom) in a humidified incubator (37°C, 5% CO_2). Cells were allowed to differentiate for 4 days and were visualized using phase contrast brightfield microscopy. Images were captured at 100X magnification. At least 100 myotubes were randomly selected from 12 fields in triplicate wells for each experimental condition. The diameter of each myotube was measured at three approximately equidistant points along their length using Image J software (NIH) to calculate the mean diameter.

RESULTS

To evaluate the extent to which coating the culture surface with various biological and synthetic molecules affects the myogenesis performance of myoblasts, the H2K cell line was utilized. Since H2K cells are conditionally immortal, they are the cell line most similar to primary myoblasts directly cultured from animals, unlike cell lines such as C2C12 or L6 [20, 21]. Therefore, just as they need to adhere to ECM proteins under *in vivo* conditions, their need for attachment in the culture environment is very high for proliferation and differentiation. In order to make a comprehensive comparison, culture surfaces were coated with fibronectin and laminin proteins, which are the main components of the ECM; gelatin, a molecular derivative of collagen; matrigel, a direct ECM extract; and PLL, a synthetic poly-amino acid. PLL+laminin coating was also performed in order to see the effectiveness of synthetic and biological coating materials together.

The cells were left to differentiate in the culture environment for 4 days. It was observed that in the uncoated culture surface environment, cell density remained limited to 30-50%, and dead cells were found floating on the medium surface in clumped forms due to their inability to adhere to the culture surface. It was also observed that the limited number of developing myotubes were not aligned with each other and that the lengths were shorter and the diameters were narrower than the myotubes developing on the surface coated with ECM components. In the culture medium whose surface was coated only with PLL, cell density remained around 30-40%, there were dead cells clumped on the surface of the medium and no myotube development was observed. On all other surfaces coated with ECM components, it was observed that the cell density covering the surface and the development of myotubes were more effective. On the culture surface coated with gelatin, the number, diameter, length, and alignment of the formed myotubes showed more limited development compared to other ECM components, and the cells did not cover the entire culture surface. The culture surfaces coated with fibronectin, laminin, PLL+laminin, and matrigel were completely covered by the cells. Additionally, on these surfaces, it was determined that myotube development was more effective compared to the remaining surfaces, with myotube length, diameter, and alignment being similar to each other (Figure 1). Although it can be observed under the microscope that myotube development is more efficient on surfaces coated with ECM components than other coatings, myotube diameter analysis was performed to reveal which ECM component supports myotube maturation more efficiently. For this purpose, analyses of measuring the average myotube diameter and determining myotube contents based on their diameters were utilized. The mean myotube diameters of fibronectin, laminin, matrigel, PLL+laminin and gelatin were 49.71µm (±16.3µm), 52.31µm (±15.7µm), 51.9µm (±15.3µm), 53.06µm (±14.2µm) and 35.25µm (±11.4µm), respectively (Figure 2A). While the diameters of myotubes growing on culture surfaces coated with fibronectin, laminin, matrigel and PLL+laminin showed a similar distribution, the distribution of myotubes on surfaces coated with gelatin differed from the others, with a higher percentage of small diameter myotubes and a lower percentage of large diameter myotubes compared to other coatings (Figure 2B).

DISCUSSION

Skeletal muscle development and repair involve the differentiation of myoblasts originating from progenitor cells, which fuse with each other to form muscle fibers [4]. During this process, myoblasts need to migrate to reach other myoblasts if a new muscle fiber is being formed, or to reach the existing muscle fiber if a damaged muscle fiber is being repaired. For myoblasts to both migrate and differentiate, interaction with cell adhesion molecules on the cell surface and the ECM is necessary. The connections established by integrin proteins on the surfaces of myoblasts and muscle fibers with the major ECM proteins laminin and fibronectin regulate the processes involved in skeletal muscle development and repair [22-26]. Laminin, basal lamina's primary component, consists of three polypeptide

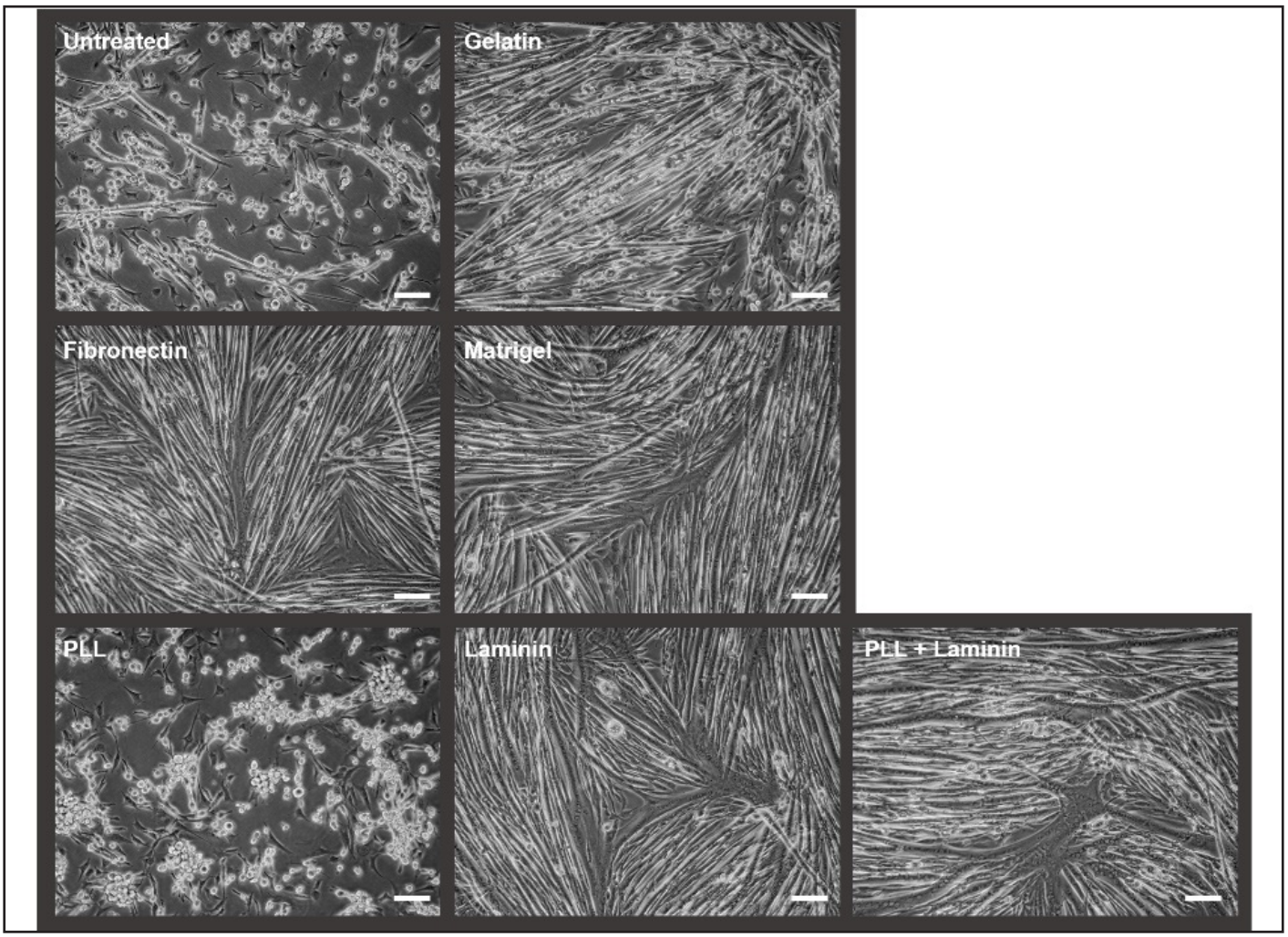

Figure 1. Morphological appearance of differentiated H2K myoblasts on different culture surfaces. Representative images showing morphological features of myogenic changes of H2K myoblasts differentiated on culture surfaces coated with gelatin, fibronectin, matrigel, PLL, laminin, and PLL+laminin after four days. The scale bar is 100 µm.

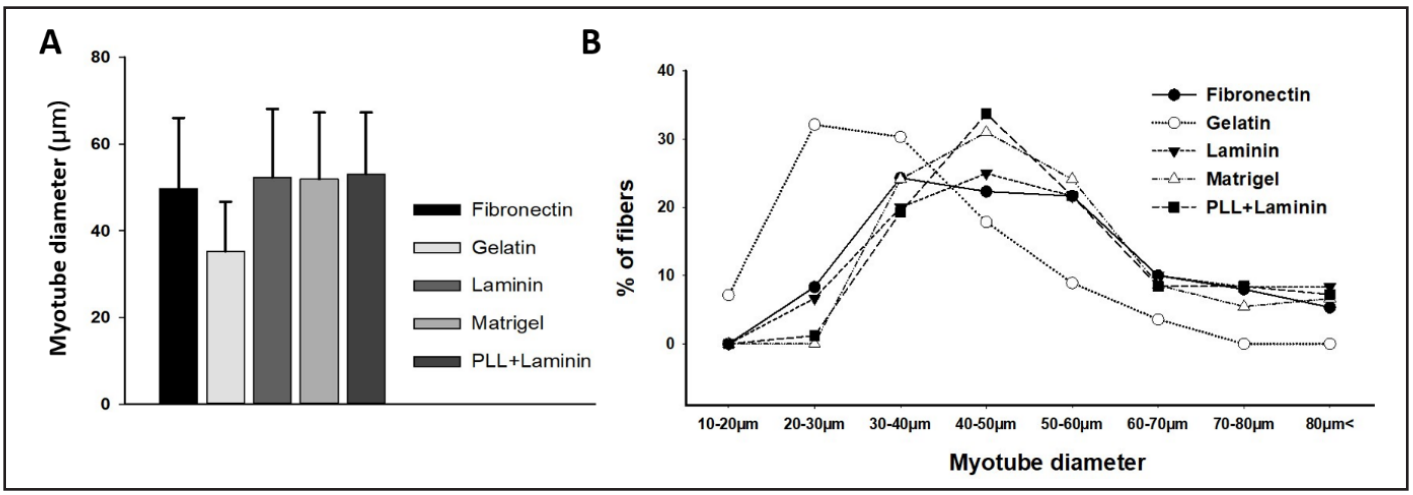

Figure 2. Myotube diameter analysis. (A) Mean myotube diameters. (B) Distribution of myotube diameters within each group. Data are means ± SEM.

chains held together by disulfide bonds and plays a major role in cell adhesion to the ECM [27-29]. Fibronectin, rich in RGD (Arg-Gly-Asp) motifs which serve as ligands for integrins, is an important ECM protein that facilitates cell binding to collagen and proteoglycans found in the ECM [30]. The presentation of these proteins, which support myoblast migration and muscle fiber differentiation *in vivo*, to H2K cells *in vitro* supported myoblast differentiation and myotube maturation. A similar differentiation profile was found on surfaces coated with matrigel. Matrigel is a basement membrane extract purified from murine Engelbreth-Holm-Swarm (EHS) tumors. It is high in laminin, entactin, collagen and proteoglycan levels but very low in fibronectin. Matrigel also contains many soluble factors that promote cell proliferation and growth such as transforming growth factor (TGF-beta), epidermal growth factor (EGF), insulin-like growth factor (IGF-1) and fibroblast growth factor (bFGF) [31]. Therefore, matrigel's ability to promote myoblast differentiation may be attributed to the presence of laminin and growth factors and partly to the presence of collagen. Gelatin is a hydrolyzed derivative of collagen. Myoblasts bind to collagen molecules via fibronectin, although there are some studies showing that muscle fibers can bind directly to collagen [32, 33]. Although the coating of the culture surface with gelatin supports myoblast differentiation, myogenesis efficiency seems to be limited compared to other ECM components included in the study. The PLL tested in this study did not promote differentiation of myoblasts into myotubes. PLL, a synthetic lysine polymer, mediates the cationic coating of the culture surface and thus the binding of cells to the culture surface due to their anionic membrane. The results of the study suggest that a cationic surface alone is not sufficient for myogenesis to occur.

CONCLUSIONS

As the largest tissue in the human body, skeletal muscle supports numerous vital activities, both mechanical and metabolic. Aging, cancer, metabolic diseases, and various genetic conditions often impair skeletal muscle function, significantly reducing quality of life. Therefore, the development and function of skeletal muscle are subjects of extensive research interest. *In vitro* skeletal muscle models, created for this purpose, particularly rely on primary myoblast cells that require adhesive conditions similar to those *in vivo*. Within this study, using H2K cells akin to primary myoblasts, it was demonstrated that merely coating the culture surface with a cationic material does not support myogenesis. Instead, it was shown that ECM components are necessary to maintain cell viability and enable differentiation, highlighting their critical role in this process.

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REFERENCES

[1] Brooks SV, Guzman SD, Ruiz LP (2023) Skeletal muscle structure, physiology, and function. Handb Clin Neurol 195:3-16. https://doi.org/10.1016/B978-0-323-98818-6.00013-3

[2] Frontera WR, Ochala J (2015) Skeletal muscle: a brief review of structure and function. Calcif Tissue Int 96:183-195. https://doi.org/10.1007/s00223-014-9915-y

[3] Lieber RL, Friden J (2000) Functional and clinical significance of skeletal muscle architecture. Muscle Nerve 23:1647-1666. https://doi.org/10.1002/1097-4598(200011)23:11<1647::aid-mus1>3.0.co;2-m

[4] Mukund K, Subramaniam S (2020) Skeletal muscle: A review of molecular structure and function, in health and disease. Wiley Interdiscip Rev Syst Biol Med 12:e1462. https://doi.org/10.1002/wsbm.1462

[5] Argiles JM, Campos N, Lopez-Pedrosa JM, Rueda R, Rodriguez-Manas L (2016) Skeletal Muscle Regulates Metabolism via Interorgan Crosstalk: Roles in Health and Disease. J Am Med Dir Assoc 17:789-796. https://doi.org/10.1016/j.jamda.2016.04.019

[6] Westerblad H, Bruton JD, Katz A (2010) Skeletal muscle: energy metabolism, fiber types, fatigue and adaptability. Exp Cell Res 316:3093-3099. https://doi.org/10.1016/j.yexcr.2010.05.019

[7] Lieber RL, Ward SR (2011) Skeletal muscle design to meet functional demands. Philos Trans R Soc Lond B Biol Sci 366:1466-1476. https://doi.org/10.1098/rstb.2010.0316

[8] Chang M, Cai Y, Gao Z, Chen X, Liu B, Zhang C, Yu W, Cao Q, Shen Y, Yao X, Chen X, Sun H (2023) Duchenne muscular dystrophy: pathogenesis and promising therapies. J Neurol 270:3733-3749. https://doi.org/10.1007/s00415-

023-11796-x

[9] Shefner JM, Musaro A, Ngo ST, Lunetta C, Steyn FJ, Robitaille R, De Carvalho M, Rutkove S, Ludolph AC, Dupuis L (2023) Skeletal muscle in amyotrophic lateral sclerosis. Brain 146:4425-4436. https://doi.org/10.1093/brain/awad202

[10] Cruz-Jentoft AJ, Bahat G, Bauer J, Boirie Y, Bruyere O, Cederholm T, Cooper C, Landi F, Rolland Y, Sayer AA, Schneider SM, Sieber CC, Topinkova E, Vandewoude M, Visser M, Zamboni M, Writing Group for the European Working Group on Sarcopenia in Older P, the Extended Group for E (2019) Sarcopenia: revised European consensus on definition and diagnosis. Age Ageing 48:16-31. https://doi.org/10.1093/ageing/afy169

[11] Martin A, Gallot YS, Freyssenet D (2023) Molecular mechanisms of cancer cachexia-related loss of skeletal muscle mass: data analysis from preclinical and clinical studies. J Cachexia Sarcopenia Muscle 14:1150-1167. https://doi.org/10.1002/jcsm.13073

[12] Espino-Gonzalez E, Dalbram E, Mounier R, Gondin J, Farup J, Jessen N, Treebak JT (2024) Impaired skeletal muscle regeneration in diabetes: From cellular and molecular mechanisms to novel treatments. Cell Metab 36:1204-1236. https://doi.org/10.1016/j.cmet.2024.02.014

[13] Romagnoli C, Iantomasi T, Brandi ML (2021) Available In Vitro Models for Human Satellite Cells from Skeletal Muscle. Int J Mol Sci 22. https://doi.org/10.3390/ijms222413221

[14] Carter S, Solomon TPJ (2019) In vitro experimental models for examining the skeletal muscle cell biology of exercise: the possibilities, challenges and future developments. Pflugers Arch 471:413-429. https://doi.org/10.1007/s00424-018-2210-4

[15] Chal J, Pourquie O (2017) Making muscle: skeletal myogenesis in vivo and in vitro. Development 144:2104-2122. https://doi.org/10.1242/dev.151035

[16] Taylor L, Wankell M, Saxena P, McFarlane C, Hebbard L (2022) Cell adhesion an important determinant of myogenesis and satellite cell activity. Biochim Biophys Acta Mol Cell Res 1869:119170. https://doi.org/10.1016/j.bbamcr.2021.119170

[17] Burkin DJ, Kaufman SJ (1999) The alpha7beta1 integrin in muscle development and disease. Cell Tissue Res 296:183-190. https://doi.org/10.1007/s004410051279

[18] Cooke MJ, Phillips SR, Shah DS, Athey D, Lakey JH, Przyborski SA (2008) Enhanced cell attachment using a novel cell culture surface presenting functional domains from extracellular matrix proteins. Cytotechnology 56:71-79. https://doi.org/10.1007/s10616-007-9119-7

[19] Wysotzki P, Gimsa J (2019) Surface Coatings Modulate the Differences in the Adhesion Forces of Eukaryotic and Prokaryotic Cells as Detected by Single Cell Force Microscopy. Int J Biomater 2019:7024259. https://doi.org/10.1155/2019/7024259

[20] Morgan JE, Beauchamp JR, Pagel CN, Peckham M, Ataliotis P, Jat PS, Noble MD, Farmer K, Partridge TA (1994) Myogenic cell lines derived from transgenic mice carrying a thermolabile T antigen: a model system for the derivation of tissue-specific and mutation-specific cell lines. Dev Biol 162:486-498. https://doi.org/10.1006/dbio.1994.1103

[21] Jat PS, Noble MD, Ataliotis P, Tanaka Y, Yannoutsos N, Larsen L, Kioussis D (1991) Direct derivation of conditionally immortal cell lines from an H-2Kb-tsA58 transgenic mouse. Proc Natl Acad Sci U S A 88:5096-5100. https://doi.org/10.1073/pnas.88.12.5096

[22] Thorsteinsdottir S, Deries M, Cachaco AS, Bajanca F (2011) The extracellular matrix dimension of skeletal muscle development. Dev Biol 354:191-207. https://doi.org/10.1016/j.ydbio.2011.03.015

[23] Mayer U (2003) Integrins: redundant or important players in skeletal muscle? J Biol Chem 278:14587-14590. https://doi.org/10.1074/jbc.R200022200

[24] Boppart MD, Mahmassani ZS (2019) Integrin signaling: linking mechanical stimulation to skeletal muscle hypertrophy. Am J Physiol Cell Physiol 317:C629-C641. https://doi.org/10.1152/ajpcell.00009.2019

[25] Crawley S, Farrell EM, Wang W, Gu M, Huang HY, Huynh V, Hodges BL, Cooper DN, Kaufman SJ (1997) The alpha7beta1 integrin mediates adhesion and migration of skeletal myoblasts on laminin. Exp Cell Res 235:274-286. https://doi.org/10.1006/excr.1997.3671

[26] Cachaco AS, Pereira CS, Pardal RG, Bajanca F, Thorsteinsdottir S (2005) Integrin repertoire on myogenic cells changes during the course of primary myogenesis in the mouse. Dev Dyn 232:1069-1078. https://doi.org/10.1002/dvdy.20280

[27] Gullberg D, Tiger CF, Velling T (1999) Laminins during muscle development and in muscular dystrophies. Cell Mol Life Sci 56:442-460. https://doi.org/10.1007/pl00000616

[28] Shibuya S, Wakayama Y, Inoue M, Kojima H, Oniki H (2003) Merosin (laminin-2) localization in basal lamina of normal skeletal muscle fibers and changes in plasma membrane of merosin-deficient skeletal muscle fibers. Med Electron Microsc 36:213-220. https://doi.org/10.1007/s00795-003-0227-y

[29] Yurchenco PD, McKee KK, Reinhard JR, Ruegg MA (2018) Laminin-deficient muscular dystrophy: Molecular pathogenesis and structural repair strategies. Matrix Biol 71-72:174-187. https://doi.org/10.1016/j.matbio.2017.11.009

[30] Snow CJ, Peterson MT, Khalil A, Henry CA (2008) Muscle development is disrupted in zebrafish embryos deficient for fibronectin. Dev Dyn 237:2542-2553. https://doi.org/10.1002/dvdy.21670

[31] Hughes CS, Postovit LM, Lajoie GA (2010) Matrigel: a complex protein mixture required for optimal growth of cell culture. Proteomics 10:1886-1890. https://doi.org/10.1002/pmic.200900758

[32] Wohlgemuth RP, Brashear SE, Smith LR (2023) Alignment, cross linking, and beyond: a collagen architect's guide to the skeletal muscle extracellular matrix. Am J Physiol Cell Physiol 325:C1017-C1030. https://doi.org/10.1152/ajpcell.00287.2023

[33] Kiss AA, Somlyai-Popovics N, Kiss M, Boldogkoi Z, Csiszar K, Mink M (2019) Type IV Collagen Is Essential for Proper Function of Integrin-Mediated Adhesion in Drosophila Muscle Fibers. Int J Mol Sci 20. https://doi.org/10.3390/ijms20205124

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Original Research

Changes in Plasma Amino Acid Levels in Crimean-Congo Hemorrhagic Fever Patients

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ABSTRACT

Objective: Crimean Congo Hemorrhagic Fever (CCHF) has an important place in viral hemorrhagic fever. Plasma amino acid (AA) levels of patients who were diagnosed with CCHF in the acute and convalescent period of the disease were investigated in this study.

Methods: 35 patients were included in the study specific polymerase chain reaction (PCR) and/or IgM antibody positivity for CCHF virus. AA levels were measured in the plasma derived from the blood samples of the patient and control groups, using liquid chromatography-mass spectrometry (LC-MS/MS) technique.

Results: In our study, we observed that plasma aspartate, glutamate, histidine, leucine, phenylalanine, tyrosine levels increased statistically significantly ($p<0.05$), while some AA levels decreased ($p<0.05$) in acute stage patients compared to the control groups. In addition, while there was an increase in plasma glutamate levels of convalescent patients compared to the control groups ($p<0.05$), there was a significant decrease in other AA levels ($p<0.05$).

Conclusion: Further studies to investigate the relationship between increased or decreased AAs in the plasma levels of these patients and the immune system are likely to contribute to a better understanding of the pathogenesis of the disease and to guide the approach to whether AA supplementation is necessary for treatment.

Keywords: biochemical analysis, Crimean-Congo hemorrhagic fever virus, metabolic analysis, research andanalysis methods

INTRODUCTION

Crimean-Congo Hemorrhagic Fever (CCHF) has an important place among the viral hemorrhagic fevers in the world. This disease occurs when infected with the CCHF Virus, which is classified in the Orthonairovirus genus of the Nairovirus family of the Bunyavirales group [1,2]. CCHF disease has a wide geographical distribution among tick-borne, medically important, viral diseases [2]. The disease is transmitted to humans through the attachment of ticks, unprotected contact with the blood, body fluids or tissues of infected people or

animals [3].

In healthy people, the AA concentration, which is in balance, is disrupted in various pathological conditions and many metabolic pathways are affected [4,5]. The need for AAs changes due to metabolic changes seen in infections or infectious diseases [4].

Currently, there is an increase in viral infections worldwide. CCHF disease is a viral infection that increases exponentially every year. It has been known for a long time that plasma AA levels are affected by the increase in catabolic and anabolic reactions in many diseases, especially infections [6,7]. In a study conducted with CCHF patients, Aydın and colleagues. showed that plasma glutamine levels decreased in patients [8]. Due to the relationship between AA level and distribution, infectious diseases, and immune response, we investigated the levels of different AAs in the plasma of CCHF patients in the acute and convalescent period in this study.

MATERIALS AND METHODS

A total of 70 people, 35 of whom were patients and 35 of whom were healthy individuals, were included in this study. The patient group consisted of adult patients who were followed up in the Infectious Diseases and Clinical Microbiology Service of Cumhuriyet University Medical Faculty Research Hospital with the diagnosis of CCHF and who wanted to participate in the study. The diagnosis of CCHF was made by detecting CCHF-specific polymerase chain reaction (PCR) and/or CCHF IgM antibody positivity in the blood of the patients. Approximately 2 ml of venous blood was taken from the patients (in the acute and convalescent periods) and the control group into hemogram tubes with EDTA. Blood samples were taken on the first day of hospitalization for the acute stage patient group and on the day of discharge for the convalescent stage patient group. A control group was formed from 35 volunteers who did not have any disease with similar characteristics to the patient group in terms of age and gender. For this study, permission was obtained from the *Ethics Committee of Sivas Cumhuriyet University Faculty of Medicine* (dated 02/04/2019 and numbered 2019-04/02). Informed consent forms were read to the patients and healthy volunteers and their signatures were obtained.

Main Points;

- In the article, the importance of plasma amino acid values in CCHF disease was discussed.
- In this study, we found that the plasma levels of some AAs decreased and some increased in CCHF patients during the acute and recovery periods.
- It is likely that it will contribute to future studies between increasing or decreasing plasma AA levels and the immune system.
- The article will contribute to a better understanding of the pathogenesis of the disease and will provide information for further research on whether AA supplementation is necessary for treatment.

Exclusion Criteria

Alcohol and substance use, autoimmune diseases and those with unusual eating habits (for example, those who eliminate certain food groups from the diet) were not included in our study. In addition, those with any acute or chronic disease (diabetes mellitus, hypertension, chronic renal failure, malignancy, hematological disorders, etc.) in the patient group, except CCHF disease, were not included in our study.

Amino Acid Analysis

The blood samples were taken with a centrifuge device at 4°C and 4000 rpm for 10 min. it was centrifuged. The obtained plasma was stored at -80°C until the eppendorf tubes were portioned and the analyzes were performed. When the number of patients required for the study was reached, AA levels were measured at once after all samples were brought to room temperature. Quantitative AA analysis kit was used with the LC-MS/MS device.

Statistical Analysis

Statistical analysis was performed by loading the data obtained in the study into the SPSS (version 23.0) program. Descriptive statistics for continuous variables in our study; mean, standard deviation, minimum and maximum; Categorical variables were expressed as numbers and percentages. Shapiro-Wilk ($n<50$) normality test was applied to numerical variables. Parametric tests were applied because the measurements were normally distributed as a result of the analysis. Independent T-test and F (ANOVA) were used to compare measurements according to patient groups. In our study, Wilcoxon Sign Test was used to compare two-category variables in groups with a correlation. Chi-square test was used to determine the relationship between categorical variables. The statistical significance level (α) was taken as 5% in the calculations.

RESULTS

In the study, the mean age of the patients was 44.00±20.171 (17-80 years), while the healthy volunteers were 44.60±12.793 (19-68 years). While 22 (63%) of the patient group were male and 13 (37%) were female, 23 (63%) of the control group were male and 12 (37%) were female. There was no significant difference between the patient and healthy individuals in terms of age and gender ($p>0.05$).

The average weight of the study group; was measured as 67.03±9.87 (50-88 kg) in the patient group in the acute period, and 66.11±10.09 (49-86 kg) in the patient group in the convalescent period. The amount of protein consumed daily was calculated based on the National Food Composition Database [9], the Turkish Ministry of Health Nutrition Guide 2015 (TUBER) [10], and the Bebis 7.1 program used in the Nutrition and Dietetics Department Research and Practice Hospital. Daily protein consumption was calculated as 55.97±8.63 gr in the control group, 39.12±12.29 gr in the acute period patient group, and 43.26±9.59 gr in the convalescent patient group (Table 1). The dietary habits of the patients before hospitalization were also questioned, and the average daily protein intake was calculated. The mean hospital stay of the patients was calculated as 7.69±1.92 days. The average amount of protein consumed daily in the patient and control groups is given in Table 1.

In this study, plasma AA aspartate, glutamate, histidine, leucine, phenylalanine, and tyrosine levels were significantly increased ($p<0.05$) in the acute period patient group compared to the control group, and the decrease in arginine, glutamine, proline and tryptophan levels was significant. ($p<0.05$). While an increase was observed in plasma glutamate levels in the convalescent group compared to the control group ($p<0.05$), the decrease in alanine, glutamine, asparagine, histidine, arginine, serine, tryptophan, tyrosine, methionine, and valine levels was statistically significant ($p<0.05$).

DISCUSSION

AAs are supplied from two sources, exogenous and endogenous. In a healthy person who is fed regularly, plasma AA concentration is in the balance depending on anabolic and catabolic reactions. However, this imbalance due to various pathological conditions affects metabolic pathways. Changes in metabolic pathways affect morbidity and mortality, especially immune response [4,5]. In infectious diseases, the effect of plasma AA levels of patients may be a clinical indicator of the magnitude of the immune response to the pathogen [6].

Exogenous sources are one of the most important factors determining the plasma level of branched-chain AAs (BCAA), which include leucine, isoleucine and valine AAs [11]. In our study, we found that the daily protein consumption of the acute period patient group was lower compared to the pre-disease period and the control group (table 1). Plasma levels of BCAAs were highest in the acute period (684,68), followed by the control group (667,66), followed by the convalescent patient group (589,44). The difference between the groups was found to be statistically significant ($p=8,57x10^{-30}$) (table 2). Although the daily protein consumption of the acute group was lower than that of the control and convalescent groups, the high plasma BCAA level in the acute group may be of endogenous origin due to the increase in catabolic reactions at the onset of the disease. The decrease in the convalescent period may have developed due to the decrease in the catabolic/anabolic reaction rate and its use as an energy source. Because studies have reported that anabolic reactions increase during the recovery period of viral and bacterial infections [7], and BCAAs are used as an energy source by the muscles during fasting [11,12]. The amino group released as a result of BCAA catabolism in skeletal muscle is converted to alanine if it gives pyruvate, to glutamate if it gives α-keto glutarate, and to glutamine if it gives glutamate and is given to the peripheral circulation [11,13]. In our study, we found the difference between plasma alanine levels ($p=0.0037$)

Table 1. Daily Average Protein consumption (gr)

	Mean(gr) ±SD	**Min.-Maks.**	**p**
Control Group (n=35)	55,97±8,64	36,0-72,0	$1,07x10^{-9}$
Acute Period Patient (n=35)	39,13±12,29	16,1-56,4	
Convalescent Period Patient (n=35)	43,26±9,59	20,6-58,3	
Before Illness (n=35)	47,17±08,32	28,0-60,0	

and glutamate levels ($p=2,60x10^{-18}$) statistically significant. When the groups are compared in pairs; There was an increase in plasma alanine ($p>0.05$) and glutamate ($p<0.05$) levels in the acute patient group compared to the control group, and the plasma glutamate level of the convalescent group increased compared to the control group ($p<0.05$), but the alanine level was lower ($p<0.05$). Plasma glutamine levels were highest in healthy subjects; When compared to the control group, a statistically significant decrease was observed in both the acute and covelling period patient groups (Table 2,3). Aydın et al. suggested that the plasma level of glutamine decreased due to the increase in the use of glutamine in CCHF patients [8]. Increases in alanine and glutamate levels may be due to BCAA catabolism as mentioned above. However, no increase in glutamine level; It may be due to excessive catabolism of BCAAs and increased use of glutamine. In a study of patients with sepsis, it was suggested that plasma alanine levels increased, which was related to BCAA metabolism [14]. This study supports our opinion.

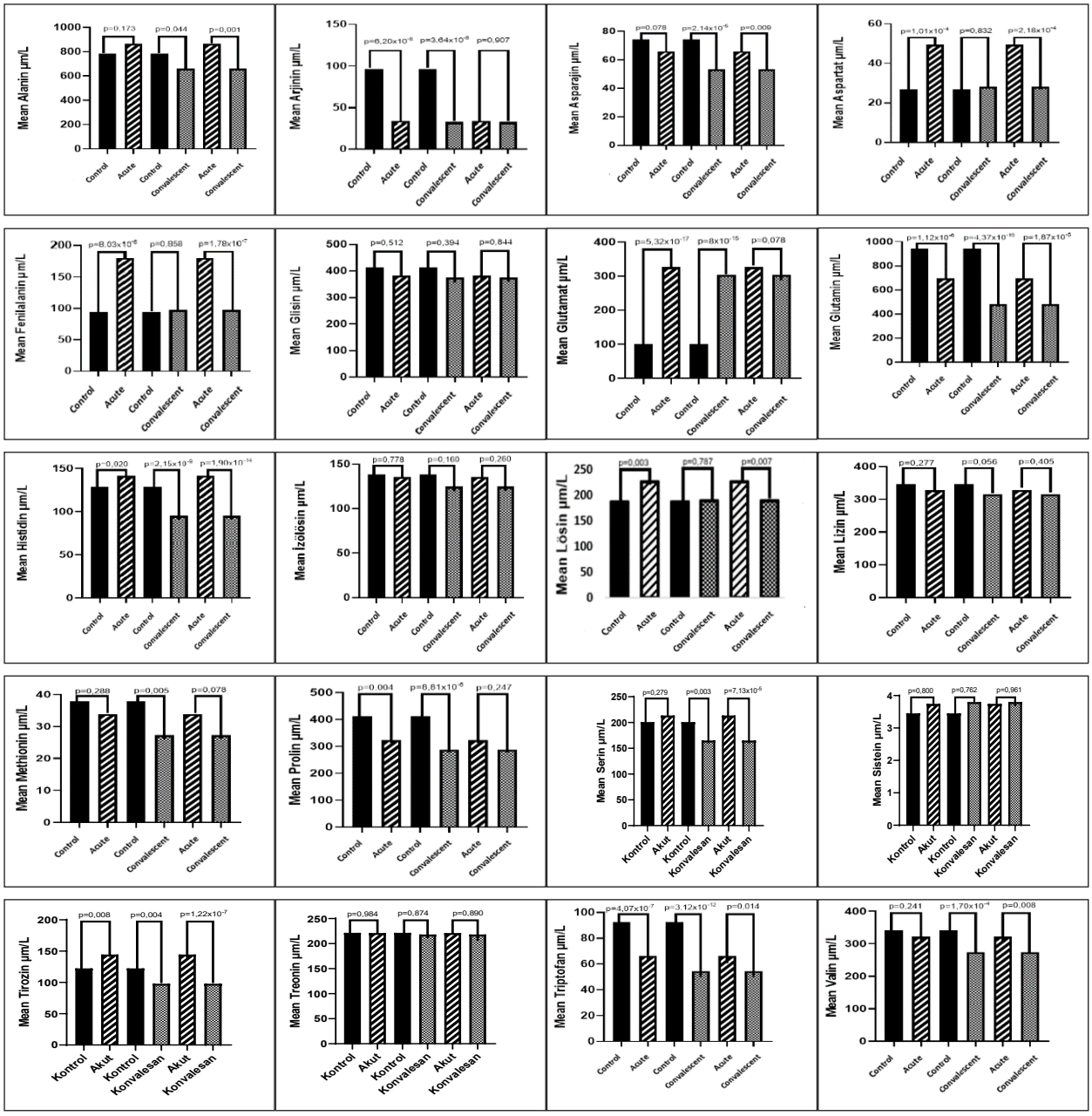
Aspartate and glutamate are non-essential AAs that play an important role in the metabolism and function of leukocytes. The AA aspartate contributes to the synthesis of purine and pyrimidine nucleotides by the proliferation of lymphocytes [15] and to the immune response with glutamate [16]. In addition to their role in leukocyte metabolism, these AAs are excitatory neurotransmitters in the central and peripheral nervous systems [15]. Glutamate is a substrate for GABA synthesis in both lymphocytes [17] and macrophages [18]. As a precursor of glutathione synthesis, it plays a role in the removal of oxidants and thus in the regulation of the immune response [19]. It has been reported that while plasma aspartate level increases in some viral diseases, it decreases in others [20,21,22]. We found that plasma aspartate levels of CCHF patients in the acute phase were significantly increased compared to healthy volunteers. We did not detect any difference between plasma aspartate levels in patients with convalescent periods and individuals in the control

Table 2. Plasma AA Levels of Control Group and CCHF Patients (Acute and Convalescent).

	Control (µmol/L)			Acute (µmol/L)			Convalescent (µmol/L)			
Amino acid	**Mean**	**SD±**	**Min.-Maks.**	**Mean**	**SD±**	**Min.-Maks.**	**Mean**	**SD±**	**Min.-Maks.**	**p**
Ala*	782,84	33,94	325-1144	864,38	51,44	331-2126	661,61	38,62	242-1212	0,0037
Arg*	96,3	10,18	17-302	33,71	6,63	7-190	32,45	4,95	3-135	$4,89x10^{-9}$
Asn*	73,83	2,89	44-108	65,94	3,86	16-144	53,41	3,11	10-95	$1,43x10^{-4}$
Asp*	26,99	2,42	10-84	49,46	5,76	8-185	26,16	2,69	9-64	$7.95x10^{-5}$
Phe*	94,65	2,25	69-124	179,89	17,30	83-614	97,30	4,60	59-190	$1.47x10^{-8}$
Glu*	99,35	4,27	61-148	326,69	19,46	198-589	304,50	19,11	140-607	$2.60x10^{-18}$
Gln*	939,63	23,45	655-1205	695,03	37,90	281-1162	482,99	36,85	65-902	$3.72x10^{-15}$
Gly	412,41	21,32	197-733	383,37	32,42	20-1151	374,65	37,61	17-1252	0,670
His*	129,11	3,37	97-176	141,19	4,05	92-182	95,44	3,40	60-126	$3.16x10^{-14}$
İle	138,60	7,77	83-319	135,82	6,97	59-245	124,71	5,93	63-224	0,330
Leu*	186,99	8,03	119-308	227,32	10,46	113-368	190,61	9,6	109-329	$4.49x10^{-3}$
Lys	346,43	11,83	214-519	328,67	9,92	183-462	315,06	12,57	209-481	0,159
Met*	37,83	1,57	21-60	33,88	3,64	0,5-87	27,29	2,19	6-51	0,019
Pro*	412,83	25,67	168-781	323,62	18,25	117-562	288,10	20,16	134-584	$0,28x10^{-4}$
Ser*	200,92	8,97	114-287	213,69	9,57	119-385	165,10	5,87	121-246	$2.08x10^{-4}$
Cys	3,45	0,97	0-25	3,74	0,67	0-21	3,80	0,78	0-18	0,94
Tyr*	122,35	4,35	75-173	144,50	7,52	81-243	98	4,97	60-211	$7.76x10^{-7}$
Thr	251,58	19,28	9-410	221,16	11,63	90-396	218,23	12,69	14-406	0,985
Trp*	92,40	3,21	46-128	66,44	4,14	17-123	54,46	2,64	24-82	$1.04x10^{-11}$
Val*	342,07	12,98	223-567	321,54	13,04	184-525	274,12	10,77	191-436	0,001
BCAA*	667,66	27,83	466-1186	684,68	28,94	357-1075	589,44	9,60	109-329	$8,57x10^{-30}$

* $p<0,05$

Table 3. Pairwise Comparison of Plasma AA Levels of Groups.



group (Table 3).

Although glutamin (Gln) and alanine constitute only 6-8% of the structural muscle protein, Gln and alanine constitute 70% of the AAs released by skeletal muscle during stress and sepsis [23]. Studies have reported that the plasma Gln level of HIV, Dengue Fever, and CCHF patients is decreased [8,21,22]. In another study, it was shown that oxidative stress increased in CCHF patients [24]. During stress and sepsis, serum and intracellular Gln concentration decrease, and under these conditions it becomes an essential AA [23]. In addition, Gln is an important energy source for immune system cells [25] and is very important for the synthesis of glutathione [26] and nucleotides (purine and pyrimidine) [27]. In our study, we found that plasma Gln level was statistically significantly lower in acute ($p=1,12x10^{-6}$) and convalescent period ($p=4,37x10^{-16}$) patients when compared to the control group consisting of healthy individuals. When we look at these results, it is seen that our results are compatible with the studies mentioned above. We thought that the endogenous synthesis of glutamine increased in our patient group, but the low plasma level could be due to oxidative stress, purine, pyrimine synthesis, and the glutaminolysis pathway.

Asparagine (Asn) is a non-essential AA synthesized from

aspartate and Gln by the ATP-dependent asparagine synthase enzyme. Petra et al. reported a decrease in plasma asparagine level in Dengue Fever [22]. In our study, plasma asparagine level in the acute period patient group was compared with the control group and the convalescent period; accordingly, while the decrease in acute period patients was not significant compared to the control group ($p=0.078$), it was significant compared to the convalescent period patients ($p<0.01$). When the plasma asparagine level in the convalescent period patients was compared with the control group; The decrease in patients with the convalescent period was found to be statistically significant ($p=2,14x10^{-5}$). While many non-essential AAs require intermediates of Gln metabolism for de-novo synthesis, Asn is dependent only on Gln [28]. It is possible that the low level of Asn is due to the decrease in the level of plasma Gln. It is also possible that the plasma Asn level is due to decreased activity of the enzyme. Because the enzyme Asparagine synthetase, which is ATP dependent, is the only enzyme that catalyzes the biosynthesis of Asn [28]. The need for ATP increases in viral diseases [29]. Therefore, the decrease in the Asn level may also have resulted from the energy deficit.

In the Wannemacher study, it was reported that plasma tryptophan and phenylalanine (Phe) levels increase in bacterial or viral infections [6]. Tryptophan, the precursor of serotonin and melatonin, is an essential AA [30]. It has been suggested that tryptophan metabolites affect both innate and acquired immunity [31]. Petra et al. reported that plasma tryptophan levels decreased in dengue fever [22]. Hortin and colleagues showed that there was a significant decrease in plasma tryptophan concentration in patients with HIV [32]. In our study, plasma tryptophan levels in CCHF patients in the acute phase of the disease were decreased compared to the control group ($p<0.05$). The results of Petra and colleagues [22] and Hortin and colleagues [32] are similar to our study results. Hortin et al. attributed the decrease in tryptophan to the response to HIV infection [32]. A similar situation is possible for CCHF patients. In other words, the low plasma tryptophan level we detected in CCHF patients may be related to the immune response of the host against infection.

The change in plasma AAs seen in infections is markedly different from the change seen in fasting. In many studies, it has been shown that plasma phenylalanine (Phe) level is affected in the presence of infection. Wannemacher et al. [6] reported increased levels of Phe in bacterial or viral infections, Yang et al. [20], in acute and chronic hepatitis, and Petra et al. [22] in dengue fever. Freund et al. reported that it increased significantly in patients with sepsis, and this rate decreased in patients who recovered [14]. Ziegler et al., in their study, found the plasma phenylalanine amount in HIV-infected individuals to be significantly lower than in healthy individuals [21]. In our study, plasma Phe levels in the control group and patients in the convalescent period were similar. However, plasma Phe levels in acute CCHF patients were increased by compared to the control group ($p=8,03x10^{-8}$). In addition, in our patient group, we found that the plasma Phe level in the convalescent period decreased compared to the acute period. In the studies mentioned above, it has been suggested that the increase in plasma phenylalanine level may be due to liver failure, muscle mass proteolysis, and changes in the activity of phenylalanine hydroxylase/dihydropteridine reductase enzymes. Powanda et al. suggested that phenylalanine hydroxylase activity decreased in mice infected with tularemia [33], while Wannemacher suggested that liver phenylalanine hydroxylase activity did not change in mice with pneumonia [6]. While our study results are consistent with the studies mentioned above [6,20,22], they show full parallelism with the study of Freund et al. [14] We did not look at muscle mass proteolysis and changes in the activity of phenylalanine hydroxylase/dihydropteridine reductase enzymes in CCHF patients, as this was not within the scope of our study. Further studies investigating how the expression or activity of phenylalanine hydroxylase/dihydropteridine reductase enzymes are affected in CCHF patients are likely to contribute to a better understanding of the pathogenesis of the disease.

Tyrosine ceases to be essential in the presence of sufficient phenylalanine in the organism. In the fasted state, only a small part of phenylalanine participates in tyrosine synthesis, while the majority participates in protein synthesis [6]. Tyrosine is the precursor of many biomolecules (catecholamines and thyroid hormones) [30]. Hortin and colleagues [32] and Ziegler and colleagues reported a decrease in plasma tyrosine levels in HIV [21], and Perte and colleagues in Dengue Fever [22]. Yang and colleagues found that plasma tyrosine levels increase in acute hepatitis, and increase in chronic hepatitis in proportion to the severity of the disease (moderate, severe) [20]. Freund and colleagues reported that plasma tyrosine levels increased in patients with sepsis, and this rate decreased in survivors after treatment [14]. In our study, we found that plasma Tyr levels in CCHF patients increased in the acute period compared to the healthy group ($p=0.008$), and decreased significantly ($p=0.004$) in patients who were discharged after treatment. While our

results were consistent with the study results of Yang and Freund's colleagues, they were not compatible with the results of other researchers.

Glycine is required for many metabolic pathways such as glutathione synthesis and purine nucleotides [34, 35]. Although it is generally known as a nonessential AA, it is also called a conditionally essential AA because it can be synthesized endogenously in a certain amount [32]. It is also a powerful antioxidant that scavenges free radicals [36]. Hortin and colleagues reported an increase in plasma glycine value in HIV [32]. Yang's team reported that plasma glycine levels decreased in acute and chronic hepatitis [20], and Petra in Dengue [22]. Ziegler and colleagues reported that there was no significant change in HIV [21]. In our study, acute and convalescent plasma glycine levels were decreased in our patient group compared to the control group, but this decrease was not statistically significant ($p>0.05$). The plasma glycine level in our study was in line with the results of Petra and Yang and their team, while not in agreement with the results of Hortin. It has been known for a long time that replication and oxidative stress increase in viral infection. Various studies have shown that oxidative stress is increased in CCHF patients and the antioxidant system is activated to prevent oxidative damage [24]. It is possible that the decrease in plasma glycine levels of CCHF patients is due to the activation of these metabolic pathways.

Proline and hydroxyproline make up one-third of the AAs in collagen, and they are important for the immune system [37,38]. In studies, it has been reported that plasma proline levels decrease in acute/chronic hepatitis [20] and HIV [21]. In our study, a significant decrease ($p<0.01$) was found in plasma proline levels in the patient group (in the acute and convalescent period) compared to the control group. However, no significant difference was found between the acute and convalescent patient groups ($p=0.247$). Our study results are similar to those mentioned above. Considering the increase in plasma glutamate level in the acute and convalescent period; It can be thought that proline is actively used to support immunity in the acute and convalescent period and the proline-P5C cycle is maintained in the direction of catabolisation. However, since the effect of this cycle on CCHF patients is not fully known, more detailed studies by looking at the concentrations of intermediates in this pathway will contribute to a better understanding of the pathogenesis of the disease. Collagen, in which proline is located, is an important structure that protects vascular structures. Damage to vascular structures may occur due to decreased collagen synthesis as a result of proline deficiency [38]. It is known that the main pathology in CCHF patients is endothelial damage. In our study, it is possible that the plasma proline level, which we found lower in CCHF patients compared to the control group, may contribute to the endothelial damage in the patients. Of course, further studies on this subject will be useful for our better understanding of the pathogenesis of the disease.

One of the important metabolic pathways in which arginine participates is nitric oxide (NO) synthesis. NO plays an important role in the immune response [39]. Freund et al. While in sepsis [14], Hortin reported a decrease in arginine level in HIV [32], Ziegler and colleagues reported no significant change in HIV [21]. In our study, we found a statistically significant decrease ($p<0.01$) in plasma arginine levels of CCHF patients in the acute and convalescent periods compared to the control group. From endogenous arginine sources; Despite the significant increase in glutamate ($p<0.01$), we saw a decrease in the amount of arginine; It may be due to the increased response to infectious pathogens in the acute and convalescent period in CCHF patients.

Plasma contains glycoproteins rich in histidine [40]. Hortin reported a statistically insignificant increase in plasma histidine levels in HIV [32]. Ziegler did not detect any changes [21]. Yang reported a significant decrease in plasma histidine levels in acute and chronic hepatitis [20], while Petra reported a significant decrease in dengue fever [22]. In our study, a statistically significant increase ($p=0.02$) in plasma histidine levels was observed in acute CCHF patients, while a decrease was found in the convalescent period ($p=2{,}15x10^{-9}$). In addition, histidine can be converted to histamine under the influence of histidine decarboxylase [38]. In our study, in CCHF patients, the amount of protein consumed during the convalescent phase of the disease was higher than in the acute phase. Despite the increased protein consumption in the convalescent period, the decrease in plasma histidine amount may be due to the increase in histamine production to support the immune system. Further studies are needed for the detailed regulation of the histidine-histamine pathway and a clearer understanding of its role in CCHF.

Serine is a non-essential AA. There are many ways in which the series is used; these; are purine and pyrimidine, ethanolamine, ceramide, and phosphatidylserine synthesis [4,34]. Hortin found that plasma serine level was increased in HIV [32], and Yang

in acute and chronic hepatitis [20]. However, Petra reported that plasma serine levels decreased in Dengue Fever [22]. In our study, we found that plasma serine levels increased in the acute phase (p=0.279) and decreased in the convalescent period (p=0.003) in CCHF patients. While our results were consistent with the results of HIV and hepatitis patients in the studies mentioned above, they were not compatible with those of Dengue Fever patients.

While more AAs increased in the acute period in CCHF patient plasmas, it was observed that they decreased more in the convalescent period. We thought that this may be due to the increase in catabolic reactions in the acute period and the increase in anabolic reactions in the convalescent period. It is thought that AAs that decrease in the acute period are used in cellular and humoral immune responses (such as cytokine, interleukin, acute phase reactant synthesis, purine, and pyrimidine synthesis). Since AAs participate in many metabolic pathways, it is difficult to discuss them individually. In this study, we determined the plasma AA profile of CCHF patients in the acute and convalescent periods.

As seen above, there is no consensus on the effects of AA supplementation on disease prognosis, pathophysiology, and mortality in many diseases. Since there were no patients who died in our study, we could not discuss the effect of plasma AA concentrations on mortality. In this study, we determined the increased/decreased plasma AA levels of CCHF patients and discussed their possible effects on metabolic pathways and the immune system. One of the weaknesses of our study is that we did not look at the levels of AAs and metabolites in the urine of the patients. However, it is an important study in terms of investigating the plasma levels of many AAs in the acute and convalescent period in CCHF patients. Considering our study results, there is a need for more comprehensive studies planned for this purpose to interpret the effect of AA supplementation on metabolic pathways, immune response, and mortality in CCHF patients.

It is known that AAs play an important role in immune response and the protection of energy metabolism. Protein loss occurs in the body due to catabolism caused by infection, and this loss can be balanced by increasing protein intake during recovery. Malnutrition can weaken the body's response to infection. While many studies have recently been found suggesting that protein/AA supplementation may help in the treatment of infectious diseases [7,12,14,21,35], studies showing which metabolic problems will result from excessive intake of protein/AAs are scarcely any.

CONCLUSION

In this study we did, we found that the plasma levels of some AAs decreased and some increased during the acute and convalescent period in CCHF patients. The differences we detected in plasma levels of AAs are likely to be due to different metabolic pathways during the acute and convalescent phases of the disease. Further studies, which will be planned to investigate the relationship between AAs with increasing or decreasing plasma levels and the immune system, will contribute to a better understanding of the pathogenesis of the disease and provide information on whether AA supplementation is necessary in terms of treatment.

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REFERENCES

[1] Adams MJ, Lefkowitz EJ, King AMQ, Harrach B, Harrison RL, Knowles NJ, Kropinski AM, Krupovic M, Kuhn JH, Mushegian AR, Nibert M, Sabanadzovic S, Sanfaçon H, Siddell SG, Simmonds P, Varsani A, Zerbini FM, Gorbalenya AE, Davison AJ (2017) Changes to taxonomy and the International Code of Virus Classification and Nomenclature ratified by the International Committee on Taxonomy of Viruses. Arch Virol.162(8):2505–2538. https://doi.org/10.1007/s00705-017-3358-5

[2] Ergönül Ö (2006) Crimean-Congo haemorrhagic fever. Lancet Infect Dis. 6(4):203-214.

[3] Vatansever Z (2007) Kırım Kongo Kanamalı Ateşi Epidemiyolojisinde Çevresel, Vektörel, İklimsel Değişikliklerin Rolü. 3.Ulusal Viroloji Kongre Kitabı, Bursa, s. 203-206.

[4] Li P, Yin YL, Li D, Kim SW, Wu G (2007) Amino acids and immune function. Br J Nutr. 98(2):237-252. https://doi.org/10.1017/S000711450769936X

[5] Dasgupta M, Sharkey JR, Wu G (2005) Inadequate intakes of indispensable amino acids among homebound older adults. J Nutr Elder. 24(3):85-99. https://doi.org/10.1300/J052v24n03_07

[6] Wannemacher RW (1977) Key role of various individual amino acids in host response to infection, Am J Clin Nutr. 30(8):1269-1280. https://doi.org/10.1093/ajcn/30.8.1269

[7] Kurpad AV (2006) The requirements of protein & amino acid during acute & chronic infections. Indian J Med Res. 124(2), 129-148.

[8] Aydın H, Engin A, Keleş S, Ertemür Z, Hekim SN (2020) Glutamine depletion in patients with Crimean-Congo hemorrhagic fever. J Med Virol. 92(12):2983-2991. https://doi.org/10.1002/jmv.25872

[9] Ulusal Gıda Kompozisyon Veri Tabanı (2017). Available from https://turkomp.tarimorman.gov.tr/database?type=foods (Accessed 12 Sep 2019)

[10] Türkiye Beslenme Rehberi (2019). Available from https://dosyasb.saglik.gov.tr/Eklenti/10915,tuber-turkiye-beslenme-rehberipdf.pdf (Accessed 12 Sep 2019)

[11] Holeček M (2018) Branched-chain amino acids in health and disease: metabolism, alterations in blood plasma, and as supplements. Nutr Metab (Lond). 15:33. https://doi.org/10.1186/s12986-018-0271-1

[12] Freund HR, Ryan JA Jr, Fischer JE (1978). Amino acid derangements in patients with sepsis: treatment with branched chain amino acid rich infusions. Ann Surg. 188(3):423-430. https://doi.org/10.1097/00000658-197809000-00017

[13] Holecek M, Siman P, Vodenicarovova M, Kandar R (2016) Alterations in protein and amino acid metabolism in rats fed a branched-chain amino acid- or leucine-enriched diet during postprandial and postabsorptive states. Nutr Metab (Lond). 13:12. https://doi.org/10.1186/s12986-016-0072-3

[14] Gluud LL, Dam G, Borre M, Les I, Cordoba J, Marchesini G, Aagaard NK, Risum N, Vilstrup H (2013) Oral branched-chain amino acids have a beneficial effect on manifestations of hepatic encephalopathy in a systematic review with meta-analyses of randomized controlled trials. J Nutr. 143(8):1263-1268. https://doi.org/10.3945/jn.113.174375

[15] Newsholme P, Procopio J, Lima MM, Pithon-Curi TC, Curi R (2003) Glutamine and glutamate--their central role in cell metabolism and function. Cell Biochem Funct. 21(1):1-9. https://doi.org/10.1002/cbf.1003

[16] Wu G, Meininger CJ (2002) Regulation of nitric oxide synthesis by dietary factors. Annu Rev Nutr. 22:61-86. https://doi.org/10.1146/annurev.nutr.22.110901.145329

[17] Tian J, Lu Y, Zhang H, Chau CH, Dang HN, Kaufman DL (2004) Gamma-aminobutyric acid inhibits T cell autoimmunity and the development of inflammatory responses in a mouse type 1 diabetes model. J Immunol. 173(8):5298-5304. https://doi.org/10.4049/jimmunol.173.8.5298

[18] Stuckey DJ, Anthony DC, Lowe JP, Miller J, Palm WM, Styles P, Perry VH, Blamire AM, Sibson NR (2005) Detection of the inhibitory neurotransmitter GABA in macrophages by magnetic resonance spectroscopy. J Leukoc Biol. 78(2):393-400. https://doi.org/10.1189/jlb.1203604

[19] Wu G, Bazer FW, Cudd TA, Meininger CJ, Spencer TE (2004) Maternal nutrition and fetal development. J Nutr.

134(9):2169-2172. https://doi.org/10.1093/jn/134.9.2169

[20] Yang J, He J, Cao H, Zhao X, Fu S, Lu H, Chen Y, Pan X, Li L (2012) Correlation between plasma amino acid profiles and the various stages of hepatitis B infection. Eur J Clin Microbiol Infect Dis. 31(8):2045-2052. https://doi.org/10.1007/s10096-011-1538-x

[21] Ziegler TR, Judd SE, Ruff JH, McComsey GA, Eckard AR (2017) Amino Acid Concentrations in HIV-Infected Youth Compared to Healthy Controls and Associations with CD4 Counts and Inflammation. AIDS Res Hum Retroviruses. 33(7):681-689. https://doi.org/10.1089/AID.2015.0369

[22] Klassen P, Fürst P, Schulz C, Mazariegos M, Solomons NW (2001) Plasma free amino acid concentrations in healthy Guatemalan adults and in patients with classic dengue. Am J Clin Nutr. 73(3):647-652. https://doi.org/10.1093/ajcn/73.3.647

[23] Kudsk KA (2006) Immunonutrition in surgery and critical care. Annu Rev Nutr. 26:463-479. https://doi.org/10.1146/annurev.nutr.26.061505.111230

[24] Aydin H, Guven FM, Yilmaz A, Engin A, Sarı I, Bakır D (2013) Oxidative stress in the adult and pediatric patients with Crimean-Congo haemorrhagic fever. J Vector Borne Dis. 50(4):297-301.

[25] Newsholme P, Curi R, Pithon Curi TC, Murphy CJ, Garcia C, Pires de Melo M (1999) Glutamine metabolism by lymphocytes, macrophages, and neutrophils: its importance in health and disease. J Nutr Biochem. 10(6):316-324. https://doi.org/10.1016/s0955-2863(99)00022-4

[26] Wu G, Fang YZ, Yang S, Lupton JR, Turner ND (2004) Glutathione metabolism and its implications for health. J Nutr. 134(3):489-492. https://doi.org/10.1093/jn/134.3.489

[27] Wu G, Field CJ, Marliss EB (1992) Enhanced glutamine and glucose metabolism in cultured rat splenocytes stimulated by phorbol myristate acetate plus ionomycin. Metabolism. 41(9):982-988. https://doi.org/10.1016/0026-0495(92)90124-S

[28] Zhang J, Fan J, Venneti S, Cross JR, Takagi T, Bhinder B, Djaballah H, Kanai M, Cheng EH, Judkins AR, Pawel B, Baggs J, Cherry S, Rabinowitz JD, Thompson CB (2014) Asparagine plays a critical role in regulating cellular adaptation to glutamine depletion. Mol Cell. 56(2):205-218. https://doi.org/10.1016/j.molcel.2014.08.018

[29] Soyöz M, Ayna TK, Pirim İ (2020) İmmünolojik Açıdan COVID-19 Enfeksiyonuna Bakış. İzmir Tepecik Eğitim Hastanesi Dergisi. 30(0):101-111 https://doi.org/10.5222/terh.2020.11298

[30] Altınışık M (2017) Protein ve Aminoasit Metabolizması. Available from https://www.mustafaaltinisik.org.uk/89-1-19.pdf (Accessed 19 Oct 2019)

[31] Platten M, Ho PP, Youssef S, Fontoura P, Garren H, Hur EM, Gupta R, Lee L. Y, Kidd BA, Robinson WH, Sobel RA, Selley ML, Steinman L (2005) Treatment of autoimmune neuroinflammation with a synthetic tryptophan metabolite. Science (New York, N.Y.) 310(5749):850-855. https://doi.org/10.1126/science.1117634

[32] Hortin GL, Landt M, Powderly WG (1994) Changes in plasma amino acid concentrations in response to HIV-1 infection. Clin Chem. 40(5):785-789.

[33] Powanda MC, Dinterman RE, Wannemacher RW Jr, Herbrandson GD (1974) Distribution and metabolism of phenylalanine and tyrosine during tualraemia in the rat. Biochem J. 144(1):173-176. https://doi.org/10.1042/bj1440173

[34] Kim SW, Mateo RD, Yin YL, Wu G (2007) Functional amino acids and fatty acids for enhancing production performance of sows and piglets. Asian-Aust J Anim Sci 20, 295–306.

[35] Ando T, Imamura H, Suzuki R, Aizaki H, Watanabe T, Wakita T, Suzuki T (2012) Visualization and measurement of ATP levels in living cells replicating hepatitis C virus genome RNA. PLoS Pathog. 8(3):e1002561. https://doi.org/10.1371/journal.ppat.1002561

[36] Fang YZ, Yang S, Wu G (2002) Free radicals, antioxidants, and nutrition. Nutrition. 2002;18(10):872-879. https://doi.org/10.1016/s0899-9007(02)00916-4

[37] Abumrad NN, Barbul A (2003) Metabolic and Therapeutic Aspects of Amino Acids in Clinical Nutrition 2nd Edition. In: The use of arginine in clinical practice. pp. 595-611

[38] Wang L, Liu S, Yang W, Yu H, Zhang L, Ma P, Wu P, Li X, Cho K, Xue S, Jiang B (2017) Plasma Amino Acid Profile in Patients with Aortic Dissection. Sci Rep. 7:40146.

Published 2017 Jan 10. https://doi.org/10.1038/srep40146

[39] Wu G, Flynn NE, Flynn SP, Jolly CA, Davis PK (1999) Dietary protein or arginine deficiency impairs constitutive and inducible nitric oxide synthesis by young rats. J Nutr. 129(7):1347-1354. https://doi.org/10.1093/jn/129.7.1347

[40] Jones AL, Hulett MD, Parish CR (2005) Histidine-rich glycoprotein: A novel adaptor protein in plasma that modulates the immune, vascular and coagulation systems. Immunol Cell Biol. 2005;83(2):106-118. https://doi.org/10.1111/j.1440-1711.2005.01320.x

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Original Research

Clinical and Radiological Predictors of Positive Microbiological Yield in Vertebral Osteomyelitis: A Retrospective Cohort Study

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ABSTRACT

Objective: To evaluate demographic, laboratory, biopsy, and imaging variables as potential predictors of microbial identification in patients with suspected vertebral osteomyelitis, aiming to enhance diagnostic accuracy and optimize patient care.

Methods: This retrospective cohort study analyzed 83 patients who underwent image-guided percutaneous disc-space sampling between June 2020 and December 2023. Inclusion criteria were adults aged ≥18 years with imaging-based evidence of vertebral osteomyelitis and clinical suspicion of infection. Exclusion criteria were presence of known malignancy and non-infective causes. Demographics, clinical history, imaging, biopsy, and microbiology data were collected. Logistic regression analysis was used to identify predictors of positive microbiological yield.

Results: Microorganisms were identified in 32 of 83 cases (38.6%), with *Mycobacterium tuberculosis* being the most common pathogen identified (21.9%). Elevated C-reactive protein (CRP) levels, erythrocyte sedimentation rate (ESR), and paravertebral signal changes on MRI were associated with a positive culture yield. Multivariate analysis identified CRP as an independent predictor of positive microbiological results. CT-guided percutaneous biopsy was found to be safe, with no major complications reported.

Conclusion: CT-guided percutaneous biopsy is a reliable and safe diagnostic tool for identifying the causative pathogens in vertebral osteomyelitis. Elevated CRP levels were independently associated with positive microbiological yield, highlighting its role as a crucial predictor in clinical practice. These findings underscore the importance of incorporating CRP levels into the diagnostic process, potentially guiding the selection of patients for biopsy to improve the detection of infection.

Keywords: biopsy; discitis; osteomyelitis; vertebra; spinal infection.

INTRODUCTION

Vertebral osteomyelitis with secondary discitis predominantly affects older men and accounts for a significant proportion (3-5%) of bone infections [1]. Although spondylodiscitis refers to intervertebral disc infections and vertebral osteomyelitis is associated with vertebral body invasion, the terms are often

used interchangeably due to their concurrent manifestation [2]. The incidence, estimated at approximately 2.4 cases per 100,000 individuals, rises with age and can be attributed to factors such as an aging population, an increase in immunocompromised states and intravenous drug usage. Left untreated, the condition can lead to severe complications, including spinal deformity, canal narrowing, paralysis, and a notable death rate of 2%-11% [3-5].

Diagnosing and managing vertebral osteomyelitis poses significant challenges due to the presence of nonspecific symptoms, often resulting in delayed diagnosis and worse clinical outcomes, including extended neurological deficits [4, 6]. Diagnosis involves a comprehensive approach that integrates clinical examination, laboratory tests, and imaging modalities. Although the laboratory tests, which may show the presence of elevated inflammatory markers, can aid in diagnosis, their specificity is limited, underscoring the need for improved diagnostic strategies [4, 7]. Additionally, blood cultures are positive in only 40-60% of the patients [8].

Contrast-enhanced magnetic resonance imaging (MRI) has emerged as the preferred imaging modality due to its high sensitivity in detecting discitis, although challenges remain, particularly in differentiating infectious from non-infectious conditions [4, 7, 9-12]. When clinical suspicion persists despite negative blood cultures, a direct biopsy is often carried out to confirm the presence of infection; yet, its variable sensitivity (31-91%) necessitates the identification of predictive factors to optimize culture yield [13-15].

Main Points

- CT-guided percutaneous biopsy is a safe diagnostic tool for vertebral osteomyelitis.
- Elevated CRP levels serve as a significant independent predictor of positive microbiological yield.
- MRI-based paravertebral signal changes correlate with higher culture positivity rates.
- Mycobacterium tuberculosis is the most commonly identified pathogen, stressing the need for routine TB cultures in endemic regions.

The current study aimed to comprehensively evaluate demographic, laboratory, biopsy, and imaging variables as potential predictors of microbial detection in specimens from patients with suspected vertebral osteomyelitis, with the aim of improving diagnostic accuracy and patient care through consolidated and validated results.

MATERIALS AND METHODS

A retrospective cohort study was conducted on patients who underwent image-guided percutaneous disc-space sampling for suspected vertebral discitis and/or osteomyelitis between June 2020 and December 2023, following the acquisition of ethical approval.

The inclusion criteria consisted of patients aged 18 years or older with imaging-based evidence of vertebral osteomyelitis, the presence of edema and contrast enhancement of the intervertebral disc, vertebrae, or paravertebral soft tissues. Additional criteria were the presence of an abscess or facet joint effusion, along with clinical suspicion of osteomyelitis as evidenced by manifestations such as back pain, fever, and/or neurological deficits. Exclusion criteria comprised of known or established malignancy and other non-infective causes (e.g., reactive osteitis) with clinical or radiological features. Cases where imaging results were indeterminate for infection or where neoplasm was a major consideration, as well as cases with incomplete procedural notes, pathology reports, or clinical records, were excluded. Additionally, cases where the biopsy was prematurely terminated or led to no specimen submission, and cases where the final pathology indicated the presence of noninfectious mass lesions, were also excluded. Based on these criteria, only one patient out of the 84 initially recruited was excluded due to the presence of diffuse B-cell lymphoma, resulting in the inclusion of 83 patients in the study.

All relevant patient data, including baseline demographic information, clinical history, histopathology, and microbiology data, were retrieved from the hospital's electronic medical record system. Imaging data were reviewed on the Radiology Department's electronic Picture Archiving and Communication System (PACS) by a musculoskeletal interventional radiologist, who was blinded to microbiological or histological results. The pre-procedural MRI for all patients was reviewed, with the radiologist noting specific imaging features such as disc hyperintensity, the presence of paraspinal or paravertebral abscess or fluid, epidural abscess, paravertebral signal changes,

vertebral endplate signal changes, disc height reduction, disc degeneration, and narrowing of the disc space.

Data retrieved from the databases included patient age, sex, procedure level, specimen culture/staining result, presence or absence of prior antibiotic treatment, procedural details, needle size, and blood cultures, as well as pre-procedural CRP, ESR, and WBC levels. The history of antibiotic usage was obtained from multiple sources, including patient-reported use of medications at home, documentation from outside hospital transfers, infectious diseases consultation notes, and inpatient pharmacy records. Patients with any intravenous antibiotic exposure 6 weeks prior to undergoing disc aspiration were considered positive for antecedent antibiotic therapy [1].

All biopsies were carried out by the same interventional radiologist with 8 years of experience, under local anesthesia and CT guidance. The CT-guided biopsy was carried out on a Somatom Definition AS plus (Philips, Netherlands), with thin-slice planning CT scans obtained in the prone position, and multiplanar reconstructions that were used for non-traumatic biopsy needle positioning. Abscess samples were collected with a Chiba® needle (18G). For the discal space, a Geotek® needle (11G, 13G or 16G) was used (Figures 1, 2).

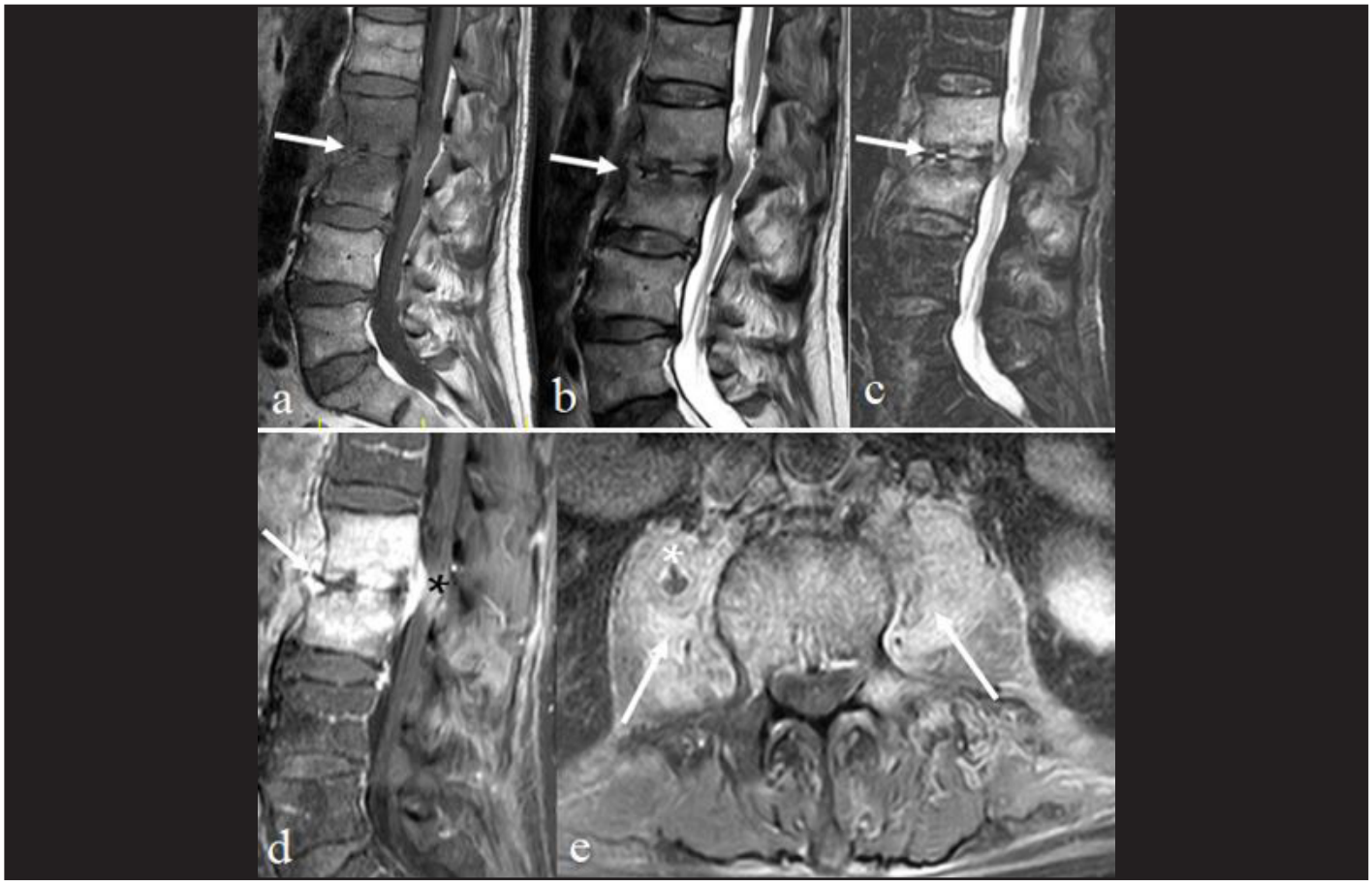

Figure 1. A 46-year-old male patient presented with severe back pain and fever. The patient had a 3-week history of fevers and increasing back pain. In the sagittal T1-weighted (A), T2-weighted (B), and STIR (C), fat-suppressed T1 gadolinium-enhanced (D) MR images of the thoracic spine, loss of height and increased T2 signal intensity and enhancement of the L2-3 disk are observed (white arrow). Cortical erosions with low T1 signal intensity, high T2 signal intensity, enhancement of adjacent endplates, and essentially the entirety of each vertebral body are evident. Additionally, there is high T2 signal intensity and enhancing anterior and posterior paravertebral soft tissue centered at this level, which is a concern for discitis-osteomyelitis. An anterior epidural abscess (black asterisk) spans L2-3. In the axial T1-weighted fat-suppressed gadolinium-enhanced MR image of the thoracic spine, marked enhancement of the L3 vertebral body and overlying anterior paravertebral soft tissues (white abscess) and a right paraspinal abscess (white asterisk) are visible.

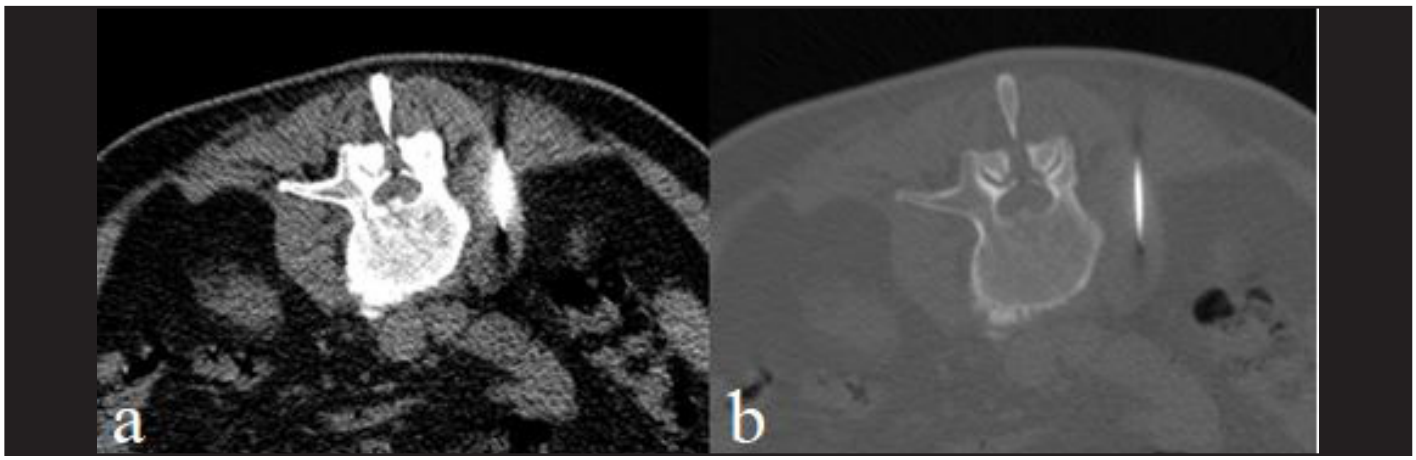

Figure 2. A: The sample (same patient as Figure 1) is depicted in the soft tissue window. B: The sample shows the bone window. An axial unenhanced CT image, taken with the patient in the prone position during percutaneous biopsy, reveals the needle (white arrow) in the paravertebral soft tissues adjacent to the L3 vertebral body. The pathology report noted the presence of acute and chronic osteomyelitis. Cultures from the biopsy showed the presence of *Staphylococcus aureus* infection.

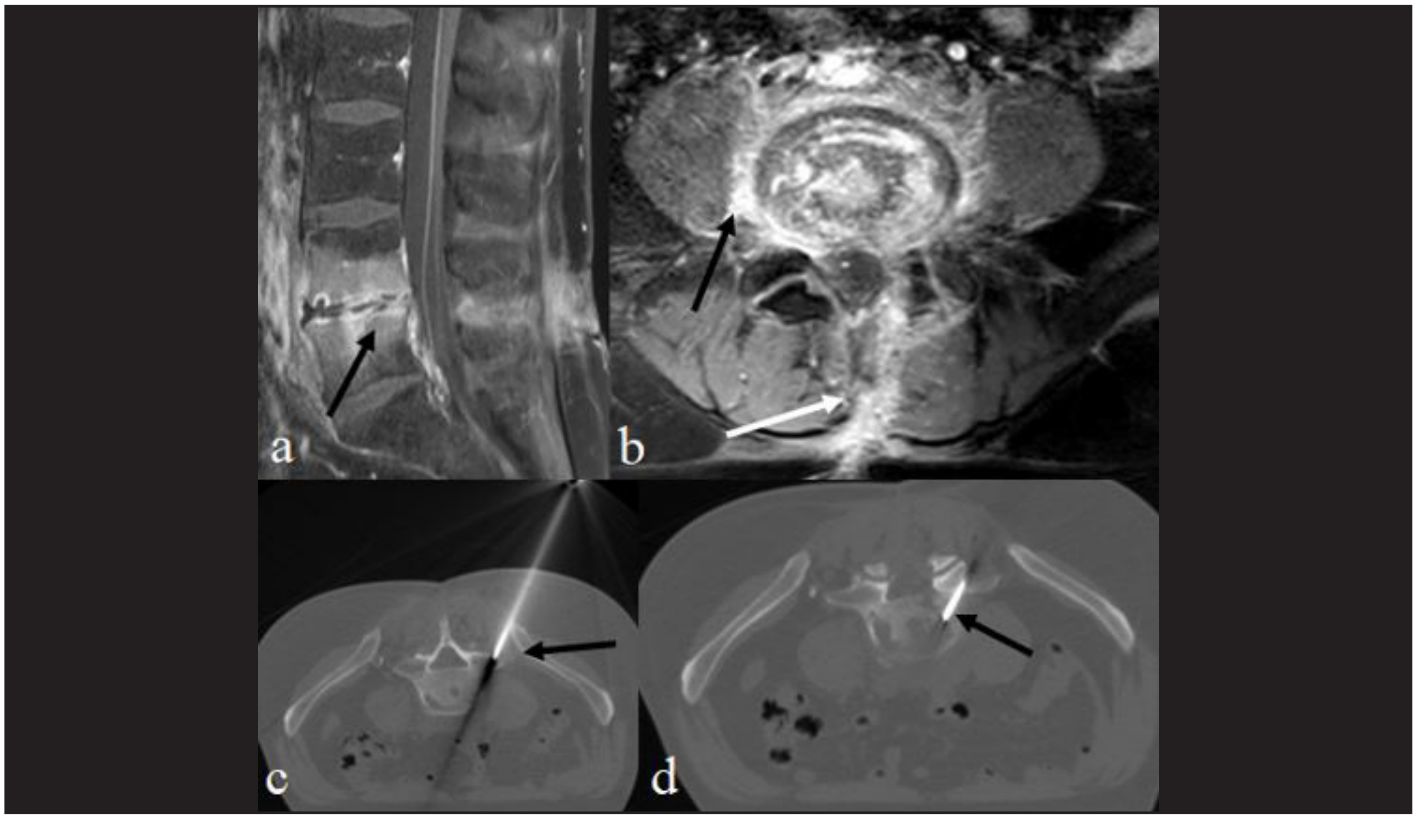

Figure 3. A 75-year-old male patient with a history of severe back pain for three months. A: Sagittal post-contrast lumbar spine image shows loss of the L4-5 intervertebral disk, erosions of adjacent endplates, narrowing in the intervertebral disk space, and contrast enhancement of vertebral bone (black arrow). B: Post-contrast axial image reveals paravertebral signal changes, contrast enhancement (black arrow), and a fistulation tract to the dermal area (white arrow), which is a concern for discitis-osteomyelitis. C and D: Axial unenhanced CT images with the patient in the prone position during percutaneous biopsy, taken 6 days after A, show a biopsy needle in the L4-5 intervertebral disk (black arrow). The pathology report noted fragments of fibrocollagenous tissue and acute and chronic inflammation. Cultures from the biopsy showed the presence of Viridans Group Streptococci**.**

The microbiological evaluation of the samples included in the study was performed by a microbiology specialist. Materials such as abscess, tissue/biopsy taken from patients during the procedure were placed in a sterile container and peripheral venous blood samples were sent to the microbiology laboratory in blood culture bottles. Abscess and tissue/biopsy samples were microscopically examined by Gram staining and inoculated onto sheep blood agar, eosin-methylene agar and chocolate agar. Blood culture samples were placed in the blood culture unit and, if the signal was positive, the bottles were microscopically examined by Gram staining and inoculated onto appropriate media. Microorganisms growing in samples incubated for 2-3 days under appropriate environmental conditions were identified by matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF Microflex LT/SH Smart MS, Bruker Daltonics).

Molecular rapid diagnostic tests have been used for *Mycobacterium tuberculosis*. The BD MAX MDR-TB assay is an automated, qualitative molecular assay for the direct detection of *M. tuberculosis* DNA from clinical specimens and was used in this study for abscess, tissue/biopsy specimens. Solid samples, such as tissue/biopsy samples, are cut into small pieces in a sterile mortar. Approximately 2 mL of sterile phosphate buffered saline is added to the abscess, tissue/biopsy sample. The sample and PBS solution were mixed until a homogeneous suspension was obtained. One ml of the prepared sample was mixed with 2 ml of BD Sample Processing Reagent. Further steps, according to the manufacturer's instructions, took approximately 30 minutes, and the samples were loaded into the instrument. The BD MAX multiplex PCR platform automatically performed DNA extraction, target PCR amplification, detection, and result interpretation.

The data were analyzed with SPSS software (version 23, IBM, Armonk, NY, USA). The normal distribution of all quantitative variables was assessed using the Shapiro–Wilk test, with normalizing transformations carried out as necessary. Data were expressed as mean ± standard deviation (SD) unless otherwise stated. Two-group comparisons utilized Student's t test for continuous variables or the chi-squared test for categorical variables. Logistic regression analysis was carried out to determine univariable predictors of positive microbial yield, with predictors having $P < 0.1$ included in a multivariable model to identify independent predictors of yield. P-values less than 0.05 were considered as statistically significant**.**

RESULTS

A total of 83 vertebral biopsies conducted on 83 patients over a period of 3 years were included in the study. All patients underwent MR imaging prior to the biopsy. The mean age of the patients was 57.63 ± 17.03 years (range: 16 to 86 years) and 36 were male. Table 1 shows detailed demographics and baseline characteristics. Eleven patients (13.2%) presented with fever, while two patients (2.4%) experienced acute paraparesis. The predominant symptom observed among the majority of patients was back pain, accounting for 84.3% of the cases.

The 83 biopsies were conducted on 83 individual patients, with no instances of repeat biopsies at our center. All 83 biopsies (100%) were successfully carried out without any complications. Notably, there were no instances of major or minor hemorrhage, infection, fistula formation, or pneumothorax associated with the biopsies. Core biopsy procedures were carried out in 74 (89.1%) patients and aspiration biopsy was carried out in 9 (10.9%) patients. Microorganisms were detected by PCR or culture in 32 cases (38.6%). *Mycobacterium tuberculosis* was the most common microorganism, accounting for 21.9% of the cases, followed by *Staphylococcus aureus* (18.8%) and viridans group Streptococci (15.6%) (Table 2).

In our patient cohort, 34 individuals (41%) had received antibiotic therapy prior to biopsy. Remarkably, microbiological findings from the biopsy significantly impacted antibiotic management in 43 cases (51.8%). For instance, microbial detection results led to changes in antibiotic therapy in line with the antibiotic susceptibility profiles obtained. In addition, in cases where empiric antibiotic treatment was initiated, microbiological identification results and antibiotic susceptibility, if any, influenced subsequent treatment protocols.

Our study also showed that in 8 out of 32 cases (25%) where microorganisms were detected, there was a match between the organisms identified in blood cultures and those isolated from biopsy samples. In four cases within our cohort, patients required surgery due to negative biopsy results. Post-surgery, two patients were diagnosed with *M. tuberculosis*, one with *Staphylococcus aureus*, and one with *Actinomyces* spp., leading to a revision of their treatment plans based on surgical findings. The pathology results and biopsy procedural details are summarized in Table 3. Of the total biopsies performed, 18 (21.7%) were thoracic, 65 (78.3%) were lumbar, and none were cervical. Biopsy locations ranged from T4-5 to T12 and L1-S1, with varying frequencies

and percentages across different levels. For example, the most common biopsy location was L4-5, accounting for 25.3% of all biopsies, followed by L3-4 (14.5%) and L5-S1 (13.3%).

The biopsy locations included the disc, paraspinal paravertebral fluid, vertebra endplate, and paravertebral soft tissue. Disc biopsy was the most prevalent (49.4%), followed by vertebra endplate (34.9%) and paraspinal paravertebral fluid (10.8%). The different techniques used for biopsies included transpedicular, trans costovertebral, posterior, and posterolateral with transpedicular being the most common (59%). Needle sizes varied, with 74.7% of biopsies using a needle size of 11. Needle sizes 18 and 13 accounted for 10.9%, and size 16 for 3.6% of the biopsies.

Pathology results included the presence of inflammation, spondylodiscitis osteomyelitis, granulomatous lesions, and fungal pathogens along with non-specific findings. Non-specific findings were the most common pathology result, accounting for 49.4% of all biopsies, followed by inflammation (37.3%) and spondylodiscitis osteomyelitis (9.6%). Patient characteristics and technical details regarding a positive microbiological yield are delineated in Table 1. CRP (p=0.003), sedimentation rate (p=0.027), and paravertebral signal changes (p=0.049) were significantly associated with a positive culture yield.

Univariate and multivariate analysis results are presented in Table 4. Univariate regression analysis was performed to identify independent variables that may have an impact on the results of microbial detection. We identified that a one-unit increase in the CRP value increased the probability of a positive result by 1.02 times (95% CI: 1.004-1.039; p=0.014). All variables examined in univariate analysis and with a p value below 0.1 were included in a multivariate logistic regression using the backward logistic regression method. The multivariate analysis indicated that a one-unit increase in CRP increased the probability of a positive microbiological result by 1.01 times (95% CI: 1.003-1.036; p=0.021). The Nagelkerke R Square value for the multivariate model was 0.168. Other variables, such as age, gender, biopsy level, presence of disc hypersensitivity, presence of paraspinal or epidural abscesses, paravertebral signal changes, endplate signal changes, disc thinning, sedimentation rate, white blood cell count and antibiotic use did not show statistically significant associations with a positive microbiology result in either univariate or multivariate analyses.

Table 1. Biopsy characteristics associated with a positive microbiological yield.

	Microorganism detection		
	Negative (n=51)	**Positive (n=32)**	**p**
Age/mean±SD	59.11±14.19	58.68±16.39	0,900[a]
CRP/median (IQR)	6.80 (2.60-21.40)	23.55 (9.55-53.77)	**0.003**[b]
ESR/median (IQR)	23.00 (13.00-43.00)	38.00 (22.00-60.50)	**0.027**[b]
WBC/median (IQR)	7.34 (5.90-9.92)	7.89 (6.60-9.20)	0.674[c]
	n (%)	**n (%)**	
Biopsy level			
Thoracic	8 (15.7)	10 (31.2)	0.081[c]
Lumbar	43 (84.3)	22 (68.8)	
Sex			
Female	29 (56.9)	18 (56.3)	0.956[c]
Male	22 (43.1)	14 (43.8)	
Biopsy localization			
Disc			
Yes	45 (88.2)	24 (75.0)	0.117[c]
No	6 (11.8)	8 (25.0)	

Paraspinal paravertebral fluid			
Yes	4 (7.8)	6 (18.8)	0.128 [c]
No	47 (92.2)	26 (81.3)	
Vertebra endplate			
Yes	21 (41.2)	15 (46.9)	0.610 [c]
No	30 (58.8)	17 (53.1)	
Paravertebral soft tissue			
Yes	3 (5.9)	4 (12.5)	0.254 [c]
No	48 (94.1)	28 (87.5)	
Biopsy types			
Tru-cut	48 (94.1)	26 (81.3)	0.072 [c]
Aspiration	3 (5.9)	6 (18.8)	
MRI findings			
Discitis, hyperintensity in the disk			
Yes	40 (78.4)	28 (87.5)	0.296 [c]
No	11 (21.6)	4 (12.5)	
Paraspinal paravertebral abscess-fluid			
Yes	17 (33.3)	17 (53.1)	0.074 [c]
No	34 (66.7)	15 (46.9)	
Epidural abscess			
Yes	3 (5.9)	2 (6.3)	0.644 [c]
No	48 (94.1)	30 (93.8)	
Paravertebral signal changes			
Yes	19 (37.3)	19 (59.4)	**0.049** [c]
No	32 (62.7)	13 (40.6)	
Vertebral endplate signal changes and irregularities			
Yes	45 (88.2)	25 (78.1)	0.217 [c]
No	6 (11.8)	7 (21.9)	
Disk thinning, disc degeneration or narrowing of the disc space			
Yes	24 (47.1)	17 (53.1)	0.591 [c]
No	27 (52.9)	15 (46.9)	
Pre-existing antibiotic therapy			
Yes	30 (58.8)	19 (59.4)	0.960 [c]
No	21 (41.2)	13 (40.6)	

[a] Independent sample t test; [b] Mann-Whitney U test; [c] Chi square test

CRP:C reactive protein, ESR: Erythrocyte sedimentation rate, WBC: White blood cell

Table 2. Microorganisms identified from the samples included in the study

Microorganisms	**n**	**%**
Mycobacterium tuberculosis	7	21.9
Staphylococcus aureus	6	18.8
Viridans group Streptococci	5	15.6
Brucella spp.	4	12.5
Coagulase-negative staphylococci	3	9.4
Actinomyces spp.	3	9.4
Candida spp.	2	6.3
Morganella morganii	1	3.1
Bacillius mageterium	1	3.1
Total	**32**	**100**

Table 3. Technical and procedural details of the biopsies.

	Microorganisms detected n=32 (38.6%)		**No microorganism detected n=51 (61.4%)**	
Biopsy location				
0 T4-5	2	6.3%	0	
1 T6-7	1	3.1%	0	
2 T 8-9	3	9.4%	2	3.9%
3 T10-11	2	6.3%	4	7.8%
4 T12-L1	2	6.3%	1	2.0 %
5 L1	2	63%	1	2.0 %
6 L1-2	5	15.6%	4	7.8 %
7 L2-3	3	9.4%	5	9.8 %
8 L3-4	1	3.1%	11	21.6 %
9 L4-5	8	25.0%	13	25.5 %
10 L5-S1	2	6.3%	9	17.6 %
11 T9-10	1	3.1%	0	
12 T12	0		1	2%
Biopsy localization				
1 disc	12	37.5	29	56.9
2 paraspinal paravertebral fluid	6	18.8	3	5.9
3 vertebra end plate	12	37.5	17	33.3
4 paravertebral soft tissue	2	6.3	2	3.9
Technique				
1 Transpedincular	15	46.9	34	66.7
2 Transcostovertebral	5	15.6	3	5.9
3 Posterior	4	12.5	6	11.8
4 Posterolateral	8	25.0	8	15.7

Needle size				
11	20	62.5	42	82.4
13	4	12.5	5	9.8
16	2	6.3	1	2.0
18	6	18.8	3	5.9
Pathology results				
1 Non-specific	15	46.9	26	51.0
2 Inflammatory Processes	12	37.5	19	37.3
3 Spondylodiscitis Osteomyelitis	3	9.4	5	9.8
4 Granulomatous Lesions	1	3.1	1	2.0
5 Fungal Pathogens	1	3.1	0	

Table 4. Univariate and multivariate logistic regression analysis results in determining variables effective on microbiology results.

Variables	Univariate Analysis		Multivariate Analysis	
	OR (95% CI)	p	OR (95% CI)	p
Age	0.998 (0.969-1.028)	0.898		
Sex; Female (Ref: Male)	1.025 (0.420-2.501)	0.956		
Biopsy level; Thoracic (Ref: Lumbar)	2.815 (0.986-8.042)	**0.053**		
Disc hyperintensity No (Ref: Yes)	1.925 (0.556-6.666)	0.301		
Paraspinal abscess (No) (Ref: Yes)	2.267 (0.916-5.609)	**0.077**		
Epidural abscess (No) (Ref: Yes)	1.067 (0.168-6.760)	0.945		
Paravertebral signal changes (No) (Ref: Yes)	2.462 (0.995-6.088)	**0.051**	2.307 (0.893-5.963)	0.084
Endplate signal changes (Yes) (Ref: No)	2.100 (0.636-6.938)	0.224		
Thinning of disc (No) (Ref: Yes)	1.275 (0.526-3.091)	0.591		
CRP	1.021 (1.004-1.039)	**0.014**	1.019 (1.003-1.036)	0.021
ESR	1.017 (0.998-1.037)	**0.085**		
WBC	0.999 (0.998-1.000)	**0.068**		
Antibiotic (no) (Ref: yes)	1.053 (0.893-1.242)	0.539		

Nagelkerke R Square: 0.168

DISCUSSION

In this retrospective study, we investigated the diagnostic efficacy of CT-guided percutaneous biopsies and aspirates in 83 suspected cases of vertebral osteomyelitis. Our findings revealed that microorganisms were detected in 38.6% (32 patients) of the samples included in the study. At the species level, *M. tuberculosis* was the predominant microorganism (21.9%) followed by *Staphylococcus aureus* (18.8%). At the genus level, *Staphylococcus* spp. were detected in 32.3% of the positive cases, together with *Staphylococcus aureus* and coagulase-negative Staphylococci.

Several factors were found to be associated with microbial detection, including CRP levels (p=0.003), sedimentation rate (p=0.027), and paravertebral signal changes (p=0.049). However, in the multivariate analysis, only CRP levels emerged as an independent factor with a significant association with a positive microbiology result. A one-unit increase in CRP levels was associated with a 1.019 times increase in the likelihood of a positive result (p = 0.021).

The literature reports a wide range of CT-guided vertebral

biopsy yields, spanning from 31% to 91% [13, 14, 16]. Several factors may contribute to this variability in diagnostic yield. These factors include differences in the imaging modalities used for biopsy guidance (CT versus fluoroscopy), the type of causative organism (e.g., *M. tuberculosis* versus *S. aureus*), prior antimicrobial therapy administered before biopsy, needle size used for the biopsy procedure, the number of specimens obtained per patient, experience of the pathologist, and criteria utilized for histopathological diagnosis of vertebral osteomyelitis.

Moreover, inconsistencies in reference standards across different studies and the potential for bacterial contamination may lead to either overestimation or underestimation of the accuracy of percutaneous spinal biopsy results [17]. Despite efforts to address these factors, the reasons behind the comparatively lower diagnostic yield of percutaneous spinal biopsy compared to other specimen acquisition methods remain unclear [6].

Understanding the pathophysiology of vertebral osteomyelitis and spondylodiscitis may shed light on this discrepancy. Infections in adults usually start with a hematogenous spread, affecting the subchondral bone before advancing into the intervertebral discs. Ideally, samples should be taken from the subchondral bone since paravertebral abscesses and intervertebral fluid may be sterile [18]. Chronic inflammation in vertebral bodies may increase sclerosis and reduce blood supply, potentially lowering diagnostic yields even in infected osseous samples [19]. These complexities emphasize the need for further research to improve diagnostic accuracy [6].

Another significant finding in our study population is the prevalence of *M. tuberculosis* as the most frequently encountered infectious agent. This differs from pyogenic spinal osteomyelitis frequently seen in Western countries, where *Staphylococcus* is typically the primary infecting organism, accounting for over 50% of cases [11, 20-24]. We observed a much lower frequency of *Staphylococcus* infections, with only 6 cases out of the 32. One potential explanation for this variation compared to other studies is the endemic presence of *M. tuberculosis* in Türkiye and the high awareness of tuberculosis (TB).

The heightened incidence of TB in certain geographical regions, driven by socioeconomic factors, the HIV epidemic, and increasing immunosuppression, underscores the critical need to include TB-specific cultures when testing specimens. However, it is concerning that only 45% of However, it is concerning that only 45% of the studies reviewed identified *M. tuberculosis [6]*. With the recent rise in HIV and immunosuppression-associated cases of TB and the projected increase in the incidence of TB, it is imperative to consider TB during the diagnosis of vertebral osteomyelitis and ensure that TB-specific diagnostic methods are integrated into protocols. The Infectious Diseases Society of America (IDSA) recommends additional serological tests in cases suspected of atypical infections such as tuberculosis or brucellosis [25]. Suspicion for these atypical organisms may be manifested as multilevel involvement on MRI or with thoracic involvement, which is frequently observed in tuberculous discitis-osteomyelitis [21].

In this study, we observed that the inflammatory markers CRP and ESR were significantly higher in the group where microorganisms were detected, which is consistent with previous studies [6, 15, 26-29]. A logistic regression analysis identified CRP at admission as the sole independent predictor of a positive yield from CT-guided biopsy, corroborating the data by Ahuja et al. [26]. Specifically, each unit increase in CRP was associated with a 1.01-fold increase in the odds of microbial detection (95% CI: 1.002-1.036; p=0.021; Table 4). These findings underscore the significance of elevated inflammatory markers in patients with microbial infections, as it generally indicates a potentially greater disease burden or a more aggressive disease course [6].

We found that paravertebral signal changes were significantly associated with a positive yield (p=0.049) in the current study. While some studies have reported similar findings, others have suggested that increased paravertebral soft tissue thickness correlates with positive microbiological results [30, 31]. These data suggest that paravertebral soft tissue changes in cases of discitis-osteomyelitis are not merely reactive; rather, it is indicative of actual bacterial infection. Therefore, such changes are reliable targets for biopsy and are determinants of microbial detection results. Compared to endplate and disc paravertebral soft tissues often have a richer vascular supply that can potentially foster bacterial growth. This could explain the observed association between paravertebral signal changes and microbial detection.

Patients exhibiting paravertebral soft tissue changes suggestive of aggressive infections may harbor a higher pathogen burden, thereby increasing the likelihood of a positive microbiological yield from percutaneous needle biopsy. Conversely, patients without paravertebral signal changes may have less aggressive

or less advanced infections, possibly resulting in a smaller pathogen burden and may not benefit from a percutaneous needle biopsy [31]. Future prospective studies can be designed to assess the severity of infection and paravertebral soft-tissue changes at the time of biopsy and explore their correlation with microbial detection efficiency.

We observed no association between prior antibiotic usage and microbiological yield in the current study, aligning with findings from the literature [6, 16]. This underscores the importance of carrying out biopsies even in cases with prior exposure to antibiotics [29]. However, the guidance from the IDSA remains consistent, recommending the withholding of antimicrobial therapy for 1-2 weeks before biopsy to optimize microbiologic yield, unless the patient is severely ill. Yet, evidence on the impact of antecedent antimicrobial therapy on biopsy yield remains limited and controversial [11, 31-34]. A recent meta-analysis failed to demonstrate a significant difference in microbiological yield whether or not the patient was exposed to antimicrobial therapy before the biopsy [16]. Nevertheless, the interpretation of these findings poses challenges due to variations in study methodologies and definitions of antimicrobial exposure. Since the publication of the meta-analysis of McNamara, several studies have reported no disparity in microbiological yield with antecedent antimicrobial exposure [35, 36]. Despite such conflicting data, the IDSA maintains its recommendation to withhold antimicrobial therapy before biopsy to maximize yield, thus facilitating the initiation of targeted antimicrobial therapy and minimizing the need for empirical treatment.

The present study has several limitations that need to be considered. First, its retrospective design and small sample size impose inherent constraints on the robustness and generalizability of the findings. However, given the rarity of vertebral osteomyelitis, conducting a prospective study with a large patient cohort presents significant challenges. Second, antibiotic usage by the patients was assessed on the basis of medical records. The retrospective nature of the study limited our ability to ascertain the regularity and duration of antibiotic use accurately. This may have introduced variability in the results. Third, we did not collect data on the volume of the biopsy specimens, which prevents a quantitative analysis of the impact of specimen volume on diagnostic yield. Lastly, data on long-term outcomes were not collected, preventing an assessment of the durability of the treatment and patient prognosis.

CONCLUSION

In summary, CT-guided percutaneous biopsy is a safe diagnostic tool for vertebral osteomyelitis; nonetheless, isolation of the causative pathogen remained limited at 38.6%. Elevated CRP and ESR values, along with paravertebral signal changes in MRI correlated with higher rates of positive microbiology samples, with CRP serving as a significant independent predictor for microbiological yield.

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REFERENCES

[1] Sobottke R, Seifert H, Fätkenheuer G, Schmidt M, Gossmann A, Eysel P (2008) Current diagnosis and treatment of spondylodiscitis. Dtsch Arztebl Int 105:181-187. https://doi.org/10.3238/arztebl.2008.0181

[2] Duarte RM, Vaccaro AR (2013) Spinal infection: state of the art and management algorithm. Eur Spine J 22:2787-2799. https://doi.org/10.1007/s00586-013-2850-1

[3] Schinkel C, Gottwald M, Andress HJ (2003) Surgical

treatment of spondylodiscitis. Surg Infect (Larchmt) 4:387-391. https://doi.org/10.1089/109629603322761445

[4] Mylona E, Samarkos M, Kakalou E, Fanourgiakis P, Skoutelis A (2009) Pyogenic vertebral osteomyelitis: a systematic review of clinical characteristics. Semin Arthritis Rheum 39:10-17. https://doi.org/10.1016/j.semarthrit.2008.03.002

[5] Cottle L, Riordan T (2008) Infectious spondylodiscitis. J Infect 56:401-412. https://doi.org/10.1016/j.jinf.2008.02.005

[6] Sertic M, Parkes L, Mattiassi S, Pritzker K, Gardam M, Murphy K (2019) The Efficacy of Computed Tomography-Guided Percutaneous Spine Biopsies in Determining a Causative Organism in Cases of Suspected Infection: A Systematic Review. Can Assoc Radiol J 70:96-103. https://doi.org/10.1016/j.carj.2018.09.003

[7] Gouliouris T, Aliyu SH, Brown NM (2010) Spondylodiscitis: update on diagnosis and management. J Antimicrob Chemother 65 Suppl 3:iii11-24. https://doi.org/10.1093/jac/dkq303

[8] Renfrew DL, Whitten CG, Wiese JA, el-Khoury GY, Harris KG (1991) CT-guided percutaneous transpedicular biopsy of the spine. Radiology 180:574-576. https://doi.org/10.1148/radiology.180.2.2068332

[9] Agarwal V, Wo S, Lagemann GM, Tsay J, Delfyett WT (2016) Image-guided percutaneous disc sampling: impact of antecedent antibiotics on yield. Clin Radiol 71:228-234. https://doi.org/10.1016/j.crad.2015.10.031

[10] Modic MT, Feiglin DH, Piraino DW, Boumphrey F, Weinstein MA, Duchesneau PM, Rehm S (1985) Vertebral osteomyelitis: assessment using MR. Radiology 157:157-166. https://doi.org/10.1148/radiology.157.1.3875878

[11] de Lucas EM, Gonzalez Mandly A, Gutierrez A, Pellon R, Martin-Cuesta L, Izquierdo J, Sanchez E, Ruiz E, Quintana F (2009) CT-guided fine-needle aspiration in vertebral osteomyelitis: true usefulness of a common practice. Clin Rheumatol 28:315-320. https://doi.org/10.1007/s10067-008-1051-5

[12] Enoch DA, Cargill JS, Laing R, Herbert S, Corrah TW, Brown NM (2008) Value of CT-guided biopsy in the diagnosis of septic discitis. J Clin Pathol 61:750-753. https://doi.org/10.1136/jcp.2007.054296

[13] Sehn JK, Gilula LA (2012) Percutaneous needle biopsy in diagnosis and identification of causative organisms in cases of suspected vertebral osteomyelitis. Eur J Radiol 81:940-946. https://doi.org/10.1016/j.ejrad.2011.01.125

[14] Chew FS, Kline MJ (2001) Diagnostic yield of CT-guided percutaneous aspiration procedures in suspected spontaneous infectious diskitis. Radiology 218:211-214. https://doi.org/10.1148/radiology.218.1.r01ja06211

[15] Lee HT, Sebro R (2020) Predictors of positive bone cultures from CT-guided bone biopsies performed for suspected osteomyelitis. J Med Imaging Radiat Oncol 64:313-318. https://doi.org/10.1111/1754-9485.13012

[16] McNamara AL, Dickerson EC, Gomez-Hassan DM, Cinti SK, Srinivasan A (2017) Yield of Image-Guided Needle Biopsy for Infectious Discitis: A Systematic Review and Meta-Analysis. AJNR Am J Neuroradiol 38:2021-2027. https://doi.org/10.3174/ajnr.A5337

[17] Pupaibool J, Vasoo S, Erwin PJ, Murad MH, Berbari EF (2015) The utility of image-guided percutaneous needle aspiration biopsy for the diagnosis of spontaneous vertebral osteomyelitis: a systematic review and meta-analysis. Spine J 15:122-131. https://doi.org/10.1016/j.spinee.2014.07.003

[18] Michel SC, Pfirrmann CW, Boos N, Hodler J (2006) CT-guided core biopsy of subchondral bone and intervertebral space in suspected spondylodiskitis. AJR Am J Roentgenol 186:977-980. https://doi.org/10.2214/ajr.05.0109

[19] Heyer CM, Al-Hadari A, Mueller KM, Stachon A, Nicolas V (2008) Effectiveness of CT-guided percutaneous biopsies of the spine: an analysis of 202 examinations. Acad Radiol 15:901-911. https://doi.org/10.1016/j.acra.2008.01.020

[20] Torda AJ, Gottlieb T, Bradbury R (1995) Pyogenic vertebral osteomyelitis: analysis of 20 cases and review. Clin Infect Dis 20:320-328. https://doi.org/10.1093/clinids/20.2.320

[21] Colmenero JD, Jiménez-Mejías ME, Sánchez-Lora FJ, Reguera JM, Palomino-Nicás J, Martos F, García de las Heras J, Pachón J (1997) Pyogenic, tuberculous, and brucellar vertebral osteomyelitis: a descriptive and comparative study of 219 cases. Ann Rheum Dis 56:709-715. https://doi.org/10.1136/ard.56.12.709

[22] Mellado JM, Pérez del Palomar L, Camins A, Salvadó E, Ramos A, Saurí A (2004) MR imaging of spinal

infection: atypical features, interpretive pitfalls and potential mimickers. Eur Radiol 14:1980-1989. https://doi.org/10.1007/s00330-004-2310-8

[23] Sharif HS, Morgan JL, al Shahed MS, al Thagafi MY (1995) Role of CT and MR imaging in the management of tuberculous spondylitis. Radiol Clin North Am 33:787-804

[24] Ross PM, Fleming JL (1976) Vertebral body osteomyelitis: spectrum and natural history. A retrospective analysis of 37 cases. Clin Orthop Relat Res:190-198

[25] Berbari EF, Kanj SS, Kowalski TJ, Darouiche RO, Widmer AF, Schmitt SK, Hendershot EF, Holtom PD, Huddleston PM, 3rd, Petermann GW, Osmon DR, Infectious Diseases Society of A (2015) 2015 Infectious Diseases Society of America (IDSA) Clinical Practice Guidelines for the Diagnosis and Treatment of Native Vertebral Osteomyelitis in Adults. Clin Infect Dis 61:e26-46. https://doi.org/10.1093/cid/civ482

[26] Ahuja N, Sharma H (2017) The effectiveness of computed tomography-guided biopsy for the diagnosis of spondylodiscitis: an analysis of variables affecting the outcome. Eur Rev Med Pharmacol Sci 21:2021-2026

[27] Chong BSW, Brereton CJ, Gordon A, Davis JS (2018) Epidemiology, Microbiological Diagnosis, and Clinical Outcomes in Pyogenic Vertebral Osteomyelitis: A 10-year Retrospective Cohort Study. Open Forum Infect Dis 5:ofy037. https://doi.org/10.1093/ofid/ofy037

[28] Ang MT, Wong GR, Wong DR, Clements W, Joseph T (2019) Diagnostic yield of computed tomography-guided biopsy and aspiration for vertebral osteomyelitis. J Med Imaging Radiat Oncol 63:589-595. https://doi.org/10.1111/1754-9485.12923

[29] Marschall J, Bhavan KP, Olsen MA, Fraser VJ, Wright NM, Warren DK (2011) The impact of prebiopsy antibiotics on pathogen recovery in hematogenous vertebral osteomyelitis. Clin Infect Dis 52:867-872. https://doi.org/10.1093/cid/cir062

[30] Diffre C, Jousset C, Roux AL, Duran C, Noussair L, Rottman M, Carlier RY, Dinh A (2020) Predictive factors for positive disco-vertebral biopsy culture in pyogenic vertebral osteomyelitis, and impact of fluoroscopic versus scanographic guidance. BMC Infect Dis 20:512. https://doi.org/10.1186/s12879-020-05223-z

[31] Chang CY, Simeone FJ, Nelson SB, Taneja AK, Huang AJ (2015) Is Biopsying the Paravertebral Soft Tissue as Effective as Biopsying the Disk or Vertebral Endplate? 10-Year Retrospective Review of CT-Guided Biopsy of Diskitis-Osteomyelitis. AJR Am J Roentgenol 205:123-129. https://doi.org/10.2214/ajr.14.13545

[32] Peckham ME, Shah LM, Johnson SM, Ryals E, Noda G, Hutchins TA (2021) Defining Disc Biopsy Timing in Relation to Blood Culture Results for Inpatients with Suspected Discitis-Osteomyelitis. J Vasc Interv Radiol 32:121-127. https://doi.org/10.1016/j.jvir.2020.08.020

[33] Lopes Floro K, Munckhof W, Coucher J (2018) Retrospective review of CT-guided intervertebral disc biopsies performed at a tertiary referral centre for suspected osteodiscitis. J Med Imaging Radiat Oncol 62:307-312. https://doi.org/10.1111/1754-9485.12686

[34] Kuo AH, Cho CH, Huang RY, Kim CJ, Lee TC (2020) Target-specific yield rate and clinical utility of percutaneous tissue sampling in spinal infection. Clin Imaging 68:257-262. https://doi.org/10.1016/j.clinimag.2020.08.025

[35] Hoang D, Fisher S, Oz OK, La Fontaine J, Chhabra A (2019) Percutaneous CT guided bone biopsy for suspected osteomyelitis: Diagnostic yield and impact on patient's treatment change and recovery. Eur J Radiol 114:85-91. https://doi.org/10.1016/j.ejrad.2019.01.032

[36] Schirò S, Foreman SC, Bucknor M, Chin CT, Joseph GB, Link TM (2020) Diagnostic Performance of CT-Guided Bone Biopsies in Patients with Suspected Osteomyelitis of the Appendicular and Axial Skeleton with a Focus on Clinical and Technical Factors Associated with Positive Microbiology Culture Results. J Vasc Interv Radiol 31:464-472. https://doi.org/10.1016/j.jvir.2019.08.013

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Original Research

Evaluation of Water Displacement Method in Estimating Mandibular Ramus Autograft Volume

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ABSTRACT

Objective: This study aims to identify the most reliable method for measuring graft volumes comparable to those harvested from the ramus region using 3D-printed models.

Methods: Using a cross-sectional design in an in vitro setting, CBCT images from 20 individuals who met the inclusion criteria for ramus grafting were examined. Volumetric evaluations were conducted on these images, and 3D-printed graft models were created. Two blinded raters assessed the graft volumes using the displacement method (with beakers of 10 cc, 25 cc, 50 cc capacity and a 100 cc biopsy cup) and the overflow liquid method (with beakers of 10 cc, 25 cc, and 50 cc capacity). The intraclass correlation coefficient and t tests were applied for statistical validation of intra- and inter-rater reliability.

Results: High levels of both intra- and interrater reliability were observed, particularly for the 10 cc rise and overflow methods. These methods exhibited not only exceptionally high ICC values but also statistically meaningful p values. Furthermore, most of these methods strongly correlated and agreed with the CBCT measurements, except for the 50 cc overflow method, which showed significant divergence.

Conclusion: The findings of this study validate the 10 cc beaker methods for reliable 3D printed ramus graft volume measurement and recommend a narrow-diameter syringe for optimal accuracy. These findings have crucial implications for both clinical practice and future research.

Keywords: alveolar ride augmentation, three-dimensional image, organ size

INTRODUCTION

Volume measurement is often crucial, especially in research-oriented studies [1]. The basic application of 3D surface volume measurement in a clinical setting can be traced back to 1967, as outlined by Burke and Beard [2]. Subsequent technological advancements have facilitated the 3D rendering of human tissues through various scanning techniques [3]. Nonetheless, such methodologies carry substantial limitations owing to their complex application procedures [4].

For irregularly shaped objects such as harvested bone grafts, the current method for volume measurement stems from

Archimedes' principle, established over two millennia ago. According to this principle, the volume of an object submerged in fluid is equal to the volume of the displaced fluid. Currently, this method serves as the reference test for tracking lymphedema in extremities [5,6], and is also utilized for volumetric calculations postprostatectomy [7], and mastectomy [8], as well as for tumor specimens [9]. In the field of oral and maxillofacial surgery, it is useful in measuring the volume of the temporomandibular joint condyle [10,11], in vitro extracted teeth [12], and bone grafts [13].

While foundational, Archimedes' principle is not without its limitations, and ongoing research continues to explore its intricacies [14]. The principle neglects factors such as surface tension, which can, in practical terms, compromise its premises [15]. Complex liquids can also confound its estimates [16]. In a clinical scenario, the immersion of the graft in a saline solution could influence bone graft volume assessment. Additionally, the method mandates that the diameter of the measurement container be at least as wide as the object, potentially compromising accuracy [14]. Further studies are warranted to determine whether the gold standard 'water displacement method', known for its efficacy in measuring larger limbs, retains its validity and reliability for smaller volumes.

The objective of this study was to identify the most reliable method for the in vitro measurement of graft volume equivalent to that harvested from the autogenous ramus donor site. To achieve this, we used a cross-sectional design to assess the intrarater and interrater reliability of water displacement methods with various containers for measuring the volume of 3D-printed models simulating bone grafts from the ramus region. The ultimate aim is to propose a straightforward, cost-effective method with established validity and reliability for graft volume estimation. The null hypothesis (H0) for this study was that there is no significant difference in the reliability of volume measurements of 3D-printed graft models between the CBCT method and the water displacement methods using various containers.

Main Points

It has been determined that using the smallest possible container and syringe for volume measurements to be performed in a clinical environment using water displacement methods increases the reliability of the results.

MATERIALS AND METHODS

Sample Size Calculation

A priori power analysis was conducted using G*Power software (version 3.1) to determine the required sample size for detecting a significant difference between the CBCT and water displacement methods. An effect size (dz) of 0.7 was chosen to represent a medium-to-high effect size, ensuring the detection of a clinically meaningful difference. Assuming an alpha level of 0.05 and a desired power of 0.80, the analysis indicated that a total sample size of 19 subjects was necessary to achieve adequate statistical power (actual power = 0.822). Therefore, 20 subjects were included in the study.

Study Design

The study included 20 consecutive patients who underwent ramus grafting in the Department of Oral and Maxillofacial Surgery Clinic at Marmara University during the year 2022. Inclusion criteria specified that participants must be at least 18 years of age, have a cone beam computed tomography (CBCT) scan, have undergone bone augmentation utilizing the mandibular ramus as the donor site, and provided written consent for the academic use of their radiological data. Patients were excluded if they had systemic diseases impacting bone metabolism, previous mandibular ramus donations, bone-related lesions or surgeries in the ramus area, recent radiotherapy or chemotherapy treatments, or inadequate quality of imaging.

CBCT Measurements

Volumetric CBCT measurements were conducted by an observer who was trained and calibrated in CBCT evaluations. CBCT images were obtained from the institutional healthcare system using a uniform machine and settings: Planmeca Promax 3D Mid (Helsinki, Finland), 90 kVp, 10 mA, 10.08 s, 0.20 mm voxel size, and 160x160 mm field of view (FOV). These images were then imported into Slicer 5.2.2 software (Slicer Community) in DICOM format [12,17]. The mandible was segmented based on a threshold value that optimally defined the bone boundaries for each patient [18]. Four osteotomy lines were strategically placed in the mandibular ramus: two vertical lines in the proximal area, one horizontal line apically, and one crestal line, marking the boundaries for ramus graft segments. After osteotomy, the volumes of the graft segments were recorded in cubic millimeters (mm^3). These segments were exported as STL files and imported into Chitubox 1.9.4 software for 3D printing. The 3D models were created with a Phrozen Sonic Mini 4K 3D printer (Phrozen Tech Co., Ltd, Taiwan) using UV-sensitive

resin (3D Printing UV Sensitive Resin, Anycubic Technology Co., Ltd, Hongkong). The finished graft models were assigned random numbers for identification.

Measurement & Evaluation

One investigator (G.G.) presented the models for assessment. Two blinded raters, unaware of the 3D models they were evaluating and each other's evaluations, carried out two sets of measurements each. The sequence of 3D models examined was randomized using Excel's RAND() function. The primary rater (S.A.E.) conducted an initial measurement and repeated it one week later to calculate intrarater variability; all specimens were reassessed on both occasions. A second rater (F.B.) also executed two separate sets of measurements across the sample to gauge interrater variability.

Volume assessments of the ramus models were performed using the Archimedes principle. Four distinct containers with varying diameters and designs were used: beakers with capacities of 10 cc, 25 cc, 50 cc, and a 100 cc biopsy container. Sterile saline served as the medium for both measurement methods to more closely approximate clinical conditions. Two techniques were used for each container: measuring the volume of displaced liquid and measuring that of overflow liquid. For the biopsy container, only the displacement method was applied. In total, fourteen unique measurements were taken for each 3D-printed graft model, as each of the two raters carried out measurements twice.

For the overflow group, the container was initially filled to excess, allowing surplus saline to drain until equilibrium was reached. A secondary empty container was placed underneath to collect the overflow upon submersion of the ramus model. The overflow volume was precisely gauged using a 1 cc syringe calibrated to a 0.01 cc scale. A second syringe was used when the ramus model volume exceeded 1 cc. In contrast, the displaced group involved filling the selected containers to a predefined level. The ramus model was then submerged, and the displaced liquid volume was meticulously noted. Consistency in the observer's eye level was maintained pre- and postimmersion. The volume was also measured using a syringe.

The recorded volumes in cc were converted to mm^3 by multiplying by 1000 and compared with values ascertained using Slicer software for additional validation.

Statistical Analysis

SPSS (Statistical Package for the Social Sciences) program was used to conduct the statistical analysis (version 22.0, IBM Corp., Armonk, NY, USA). Descriptive statistics were analyzed to summarize the graft volume data obtained from various methods and containers, shedding light on the dataset's central tendencies and spread.

For intrarater reliability, two separate sets of measurements were collected by the first and second raters with a 15-day interval between them. The intraclass correlation coefficient (ICC) was used to measure the level of agreement between these two sets of measurements for each method, providing insight into the repeatability and internal consistency of each rater's assessments. To assess interrater reliability, both the initial and follow-up measurements from all types of containers were included in the analysis. The degree of consistency and agreement between the first and second raters was evaluated using ICC values, which were categorized according to the classification system outlined by Portney and Watkins: an ICC below 0.50 indicates poor reliability, between 0.51 and 0.75 suggests moderate reliability, between 0.75 and 0.90 indicates good reliability, and above 0.90 signifies excellent reliability.

Scatter plots were created for each pair of measurement methods, starting with the 10 cc container and CBCT, to conduct an initial investigation into the relationships between variables. For each pair, a t test was performed to determine the statistical significance of the differences between the measurement methods. The null hypothesis (H0) assumed that there were no significant differences between the two methods. Bland–Altman plots were used to visually examine the level of agreement between the methods. The mean difference and 95% limits of agreement were calculated. To identify any proportional biases between the pairs of methods, linear regression analyses were conducted. A p value greater than 0.05 led to the retention of the null hypothesis, indicating no proportional bias.

RESULTS

Intrarater Consistency

Table 1 provides an overview of the ICC values, offering insight into the consistency and agreement across the two measurements. The first evaluator's assessments of both the 10 cc rise and 10 cc overflow methods yielded an exceptionally high ICC value of 0.975, supported by p values less than 0.001, signifying strong agreement. Likewise, the first evaluator demonstrated

significant agreement in the 25 cc rise and 25 cc overflow methods, with ICC values of 0.746 and 0.864, respectively, each with p values less than 0.001. Evaluation of the 50 cc rise and 50 cc overflow methods also indicated good levels of agreement, manifested by ICC values of 0.876 and 0.580, respectively, each with p values less than 0.02. In contrast, the biopsy container rise method displayed average agreement levels, as indicated by an ICC value of 0.518 and a p value of 0.01 (Table 1).

The second evaluator, assessing the identical set of methods, also observed high ICC values and statistically meaningful p values, denoting strong agreement. For the 10 cc rise and 10 cc overflow methods, the second evaluator reported ICC values of 0.975 and 0.968, respectively, each with p values less than 0.001. The 25 cc rise and 25 cc overflow methods also showed substantial agreement levels, as indicated by ICC values of 0.934 and 0.769, respectively, and p values less than 0.001. The evaluation of the 50 cc rise method yielded a high ICC value of 0.917, with a p value less than 0.001. However, the 50 cc overflow method presented a somewhat lower, yet still noteworthy, ICC value of 0.592 and a p value of 0.001. Finally, the biopsy container rise method, as evaluated by the second evaluator, displayed average agreement with an ICC value of 0.482 and a p value of 0.015 (Table 1).

Table 1. Intrarater reliability between the first (T0) and second measures (15 days later)

	ICC Coefficient	P value
First rater		
10 cc rise	0.975	<0.001
10 cc overflow	0.854	<0.001
25 cc rise	0.746	<0.001
25 cc overflow	0.864	<0.001
50 cc rise	0.876	<0.001
50 cc overflow	0.580	0.02
Biopsy container rise	0.518	0.01
Second rater		
10 cc rise	0.975	<0.001
10 cc overflow	0.968	<0.001
25 cc rise	0.934	<0.001
25 cc overflow	0.769	<0.001
50 cc rise	0.917	<0.001
50 cc overflow	0.592	0.001
Biopsy container rise	0.482	0.015

Interrater Consistency

For the 10 cc rise technique, both the initial and subsequent evaluations indicated high ICC values of 0.961 and 0.897, respectively, each supported by p values less than 0.001. In a similar manner, the 10 cc overflow approach also showed significant agreement across evaluators, with ICC values of 0.897 and 0.952 for the initial and subsequent evaluations, respectively, and p values less than 0.001. For the 25 cc beaker, the ICC values indicated substantial agreement across most metrics. For both the rise and overflow methods, the ICC values for the initial and subsequent evaluations exceeded 0.8, each substantiated by p values less than 0.001 (Table 2).

The 50 cc beaker displayed varying degrees of interrater consistency. The initial evaluation using the rise technique yielded an exceptionally high ICC value of 0.962, substantiated by a p value less than 0.001. The subsequent evaluation revealed an ICC value of 0.936, which was also supported by a p value less than 0.001. In contrast, the overflow method manifested relatively lower ICC values for both evaluations, albeit statistically significant (0.438 and 0.763, with p values of 0.043 and 0.001, respectively). In terms of the biopsy container rise technique, moderate interrater consistency was evident. The initial and subsequent evaluations displayed ICC values of 0.676 and 0.628, respectively, each supported by p values of 0.008 and 0.020 (Table 2).

Table 2. Interrater reliability between the first and second raters

	ICC Coefficient	P value
First measurement		
10 cc rise	0.961	<0.001
10 cc overflow	0.897	<0.001
25 cc rise	0.862	<0.001
25 cc overflow	0.898	<0.001
50 cc rise	0.962	<0.001
50 cc overflow	0.438	0.043
Biopsy container rise	0.676	0.008
Second measurement		
10 cc rise	0.897	<0.001
10 cc overflow	0.952	<0.001
25 cc rise	0.883	<0.001
25 cc overflow	0.816	<0.001
50 cc rise	0.936	<0.001
50 cc overflow	0.763	0.001
Biopsy container rise	0.628	0.628

Comparison with CBCT Measurements

The current investigation showed scatter diagrams that revealed significant correlations between various volume measurement techniques—specifically, 10 cc rise, 10 cc overflow, 25 cc rise, 25 cc overflow, and 50 cc rise—and CBCT measurements, excluding the 50 cc overflow method (Figure 1). T tests indicated that the null hypothesis could not be dismissed for all comparisons except the 50 cc overflow versus CBCT, where a p value less than 0.05 led to its rejection. Bland–Altman plots further validated these findings; the majority of data points were within the 95% limits of agreement for all evaluated pairs, except for the 50 cc overflow method. Additionally, linear regression analysis did not reveal any proportional biases across any of the pairs examined, supported by p values greater than 0.05 (Figure 2).

DISCUSSION

The water displacement method is the reference standard for lymphedema monitoring [5,6], postsurgical volumetric assessments [7], and tumor specimen measurements [9] and has applications in oral and maxillofacial surgery, including temporomandibular joint [10,11], extracted teeth [12], and bone graft volume [13] measurements. This study represents the first comprehensive evaluation of both intrarater and interrater reliability for various volume measurement methods using water displacement in the context of 3D-printed models that simulate bone grafts harvested from the ramus region. We found high degrees of reliability, particularly with the 10 cc beaker methods (both rising and overflow), thus validating their internal consistency and dependability. However, it is crucial to note that as the volume of the container increases, the reliability correspondingly diminishes, indicating potential inaccuracies in larger containers.

The study's data reveal significant levels of intrarater and interrater reliability across a range of methods employed for measuring the volume of 3D-printed grafts. Intrarater reliability, as illustrated by ICC values that range from moderate to excellent, emphasizes the internal reliability of each measurement approach. Specifically, the 10 cc Beaker (Rising and Overflow) method showed the highest level of intrarater reliability, positioning it as a particularly reliable method for volume measurement. On the other hand, the Biopsy container rise method showed only moderate agreement, indicating inherent limitations or inconsistencies. An observable trend emerged, showing a decrease in reliability as the size of the container increased, which calls for further investigation into potential errors in larger containers.

Patterns in interrater reliability also became evident across varying container sizes and measurement techniques. Exceptionally high ICC values were observed for both the rising and overflow methods in the 10 cc container, confirming the stability of these methods. This consistency is particularly remarkable, with ICC values exceeding 0.9 for most samples, highlighting significant agreement between evaluators. The results for the 25 cc cup supported the reliability of both the rising and overflow techniques. However, the 50 cc container revealed different outcomes: while the rising method maintained a high level of agreement, the overflow method evidenced lower levels of interrater reliability. This observation aligns with our earlier finding that reliability tends to decline with larger containers. Consistent with intrarater findings, the Biopsy container method showed only moderate levels of interrater reliability, suggesting caution is advised until further validation. The high level of interrater reliability, especially with smaller container sizes, underscores the suitability of these methods for scenarios requiring precise data collection.

Comparisons with cone-beam computed tomography (CBCT) generally demonstrated good agreement for several methods, such as the 10 cc and 25 cc rising methods, which were also substantiated by high intrarater and interrater reliability figures. However, the 50 cc overflow method did not align well with CBCT and displayed lower reliability, indicating that this method may require further evaluation.

Limitations

One limiting factor for the use of the water displacement method is surface tension. In a clinical setting, the surface tension increases when saline is used instead of water. While the addition of ethanol to water has been suggested to reduce this surface tension, such a practice is not feasible for calculating the volume of autogenous ramus in clinical applications [19]. Another consideration is related to the materials used in this study. The measurements were conducted on 3D-printed models made of acrylic, which may not perfectly simulate the characteristics of autogenous ramus grafts, thus necessitating caution when interpreting these findings to a clinical context. Additionally, the markings on the side of the container are also of importance; a wider range can decrease the reliability of measurements. As the needed area of the container expands—

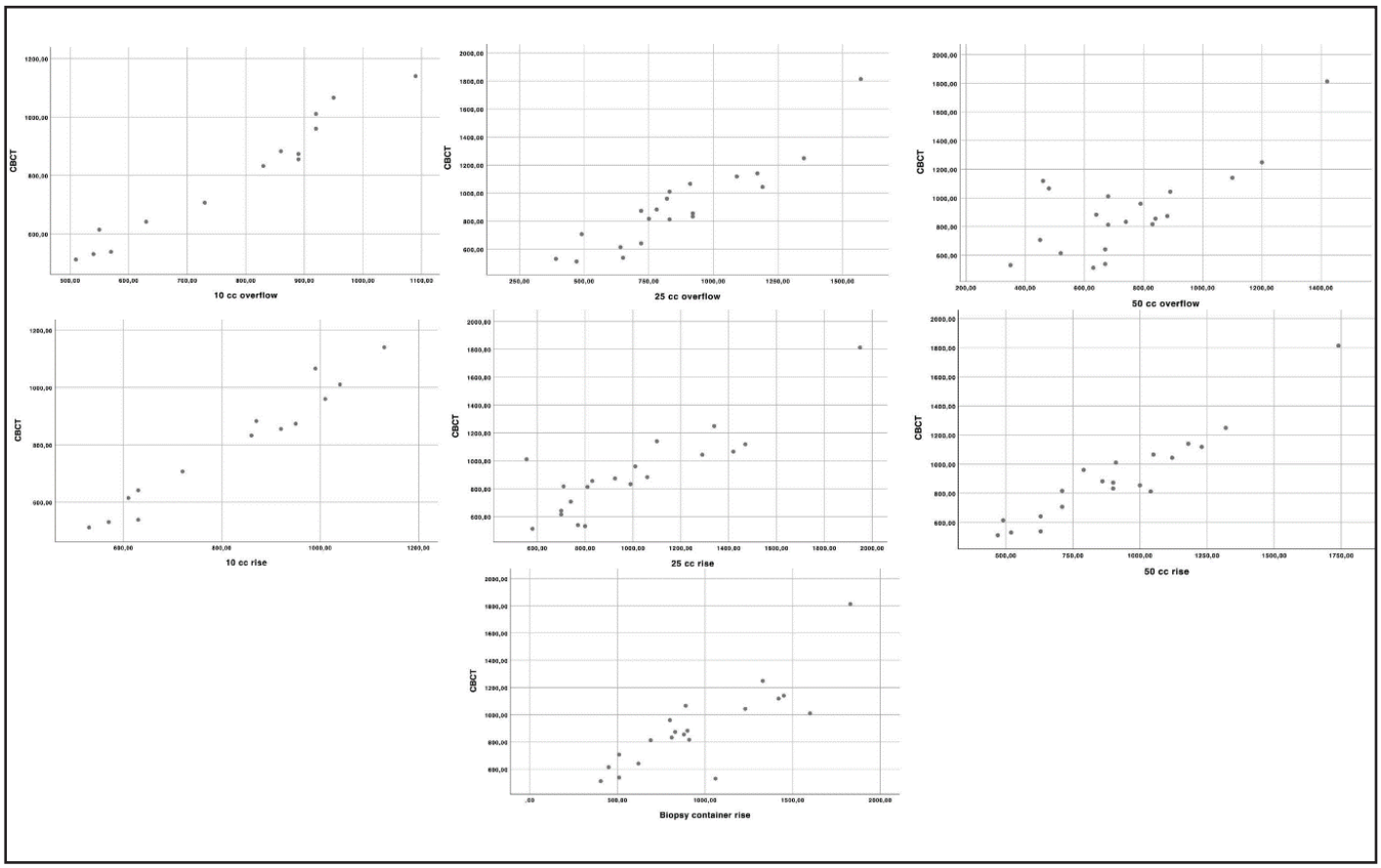

Figure 1. Scatter plots illustrating the relationship between CBCT and various volume measurement methods.

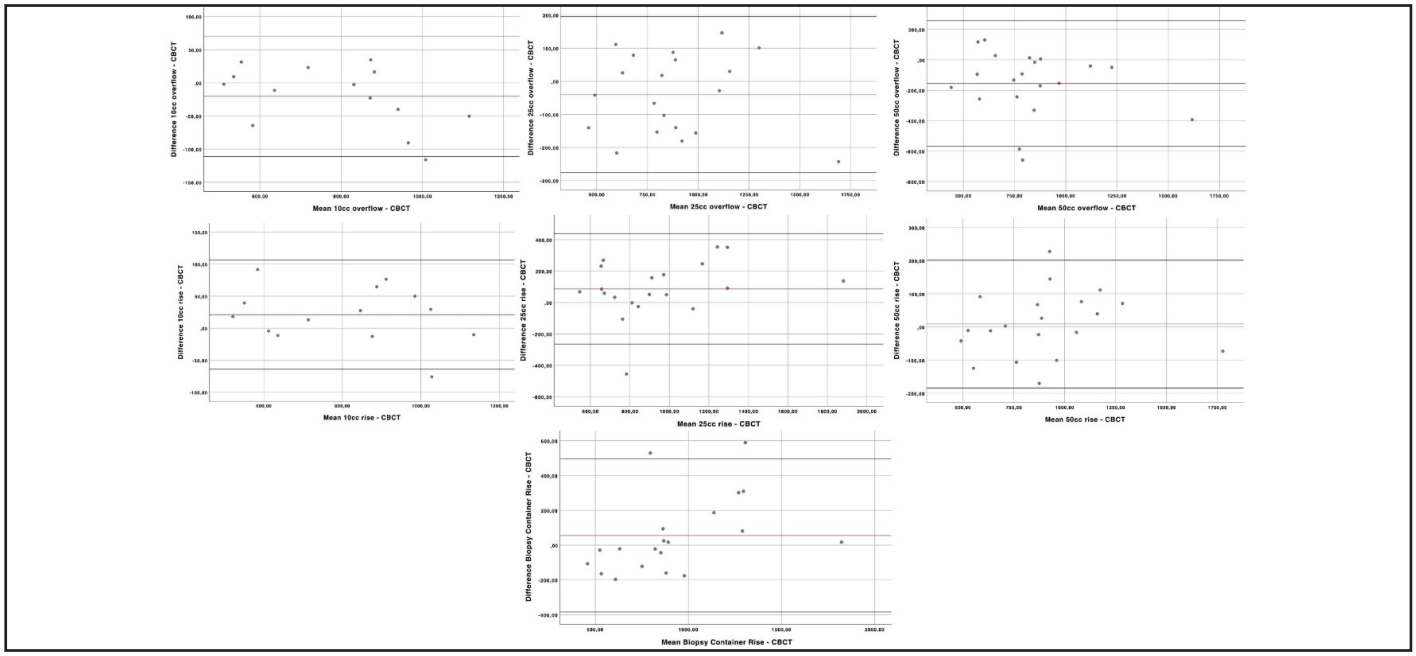

Figure 2. Bland–Altman plots evaluating the agreement between CBCT and various volume measurement methods. The dashed lines represent the 95% limits of agreement.

necessary to accommodate the ramus model—a need for containers with larger levels may arise. However, an alternative approach involving the use of a narrow-diameter syringe to draw fluid has demonstrated a positive effect on result reliability.

CONCLUSIONS

In summary, this study supports the reliability and validity of using a 10 cc beaker with water displacement methods for assessing 3D printed autogenous ramus graft model volumes from the mandibular ramus. We recommend the use of the smallest possible container that can accommodate the graft and the use of a narrow-diameter syringe for extracting the displaced liquid to ensure the most accurate and reliable measurements.

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REFERENCES

[1] Misch CM (1997) Comparison of intraoral donor sites for onlay grafting prior to implant placement. Int J Oral Maxillofac Implants. 12(6):767-776.

[2] Burke PH, Beard FH (1967) Stereophotogrammetry of the face. A preliminary investigation into the accuracy of a simplified system evolved for contour mapping by photography. Am J Orthod. 53(10):769-782. https://doi.org/10.1016/0002-9416(67)90121-2

[3] Chan KK, Feng CJ, Shih ZC, Tsai YF, Huang CC, Lin YS, Hsiao FY, Yu WC, Tseng LM, Perng CK (2024) Automatic segmentation of MRI in prospective breast volume evaluation: Comparison of different assessments for immediate breast reconstruction. J Plast Reconstr Aesthet Surg. 95:273-282. https://doi.org/10.1016/j.bjps.2024.05.029

[4] Kovacs L, Eder M, Hollweck R, Zimmermann A, Settles M, Schneider A, Udosic K, et al. (2006) New aspects of breast volume measurement using 3-dimensional surface imaging. Ann Plast Surg. 57(6):602-610. https://doi.org/10.1097/01.sap.0000235455.21775.6a

[5] Karlsson K, Johansson K, Nilsson-Wikmar L, Brogardh C (2022) Tissue Dielectric Constant and Water Displacement Method Can Detect Changes of Mild Breast Cancer-Related Arm Lymphedema. Lymphat Res Biol. 20(3):325-334. https://doi.org/10.1089/lrb.2021.0010

[6] Karlsson K, Nilsson-Wikmar L, Brogårdh C, Johansson K (2020) Palpation of increased skin and subcutaneous thickness, tissue dielectric constant, and water displacement method for diagnosis of early mild arm lymphedema. Lymphat Res Biol. 18:219–225. https://doi.org/10.1089/lrb.2019.0042

[7] Guo S, Zhang J, Jiao J, Li Z, Wu P, Jing Y, et al. (2023) Comparison of prostate volume measured by transabdominal ultrasound and MRI with the radical prostatectomy specimen volume: a retrospective observational study. BMC Urol. 23(1):62. https://doi.org/10.1186/s12894-023-01234-5

[8] Huang DW, Chou YY, Liu HH, Dai NT, Tzeng YS, Chen SG (2022) Is 3-Dimensional Scanning Really Helpful in Implant-Based Breast Reconstruction?: A Prospective Study. Ann Plast Surg. 88(1s Suppl 1):S85-S91. https://doi.org/10.1097/SAP.0000000000003088

[9] West CT, Tiwari A, Matthews L, Drami I, Mai DVC, Jenkins JT, Yano H, West MA, Mirnezami AH (2024) Eureka: objective assessment of the empty pelvis syndrome to measure volumetric changes in pelvic dead space following pelvic exenteration. Tech Coloproctol. 28(1):74. https://doi.org/10.1007/s10151-024-02952-0

[10] Kim JJ, Lagravere MO, Kaipatur NR, Major PW, Romanyk DL (2021) Reliability and accuracy of a method for measuring temporomandibular joint condylar volume. Oral

Surg Oral Med Oral Pathol Oral Radiol. 131(4):485-493. https://doi.org/10.1016/j.oooo.2020.08.014

[11] Garcia-Sanz V, Bellot-Arcis C, Hernandez V, Serrano-Sanchez P, Guarinos J, Paredes-Gallardo V (2017) Accuracy and Reliability of Cone-Beam Computed Tomography for Linear and Volumetric Mandibular Condyle Measurements. A Human Cadaver Study. Sci Rep. 7(1):11993.

[12] Shetty H, Shetty S, Kakade A, Shetty A, Karobari MI, Pawar AM, et al. (2021) Three-dimensional semi-automated volumetric assessment of the pulp space of teeth following regenerative dental procedures. Scientific Reports. 11(1):21914. https://doi.org/10.1038/s41598-021-01489-8

[13] Jensen J, Kragskov J, Wenzel A, Sindet-Pedersen S (1998) In vitro analysis of the accuracy of subtraction radiography and computed tomography scanning for determination of bone graft volume. J Oral Maxillofac Surg. 56(6):743-748. https://doi.org/10.1016/s0278-2391(98)90811-4

[14] Mohazzab P (2017) Archimedes' Principle Revisited. Journal of Applied Mathematics and Physics. 5(4):836-843.

[15] Falkovich G, Weinberg A, Denissenko P, Lukaschuk S (2005) Floater clustering in a standing wave. Nature. 435(7045):1045-1046. https://doi.org/10.1038/4351045a

[16] Wilson RM (2012) Archimedes's principle gets updated. Physics Today. 65(9):15-17. https://doi.org/10.1063/PT.3.1701

[17] Fedorov A, Beichel R, Kalpathy-Cramer J, Finet J, Fillion-Robin JC, Pujol S, et al. (2012) 3D Slicer as an image computing platform for the Quantitative Imaging Network. Magn Reson Imaging. 30(9):1323-1341. https://doi.org/10.1016/j.mri.2012.05.001

[18] Otsu N (1979) A threshold selection method from Gray-level histograms. IEEE Trans Syst Man Cybern 9:62–6. https://doi.org/10.1109/TSMC.1979.4310076

[19] Hargens AR, Kim JM, Cao P (2014) Accuracy of water displacement hand volumetry using an ethanol and water mixture. Aviat Space Environ Med. 85(2):187-90. https://doi.org/10.3357/asem.3485.2014

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Original Research

Comparative Analysis of Large Language Models in Simplifying Turkish Ultrasound Reports to Enhance Patient Understanding

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ABSTRACT

Objective: To evaluate and compare the abilities of Language Models (LLMs) in simplifying Turkish ultrasound (US) findings for patients.

Methods: We assessed the simplification performance of four LLMs: ChatGPT 4, Gemini 1.5 Pro, Claude 3 Opus, and Perplexity, using fifty fictional Turkish US findings. Comparison was based on Ateşman's Readability Index and word count. Three radiologists rated medical accuracy, consistency, and comprehensibility on a Likert scale from 1 to 5. Statistical tests (Friedman, Wilcoxon, and Spearman correlation) examined differences in LLMs' performance.

Results: Gemini 1.5 Pro, ChatGPT-4, and Claude 3 Opus received high Likert scores for medical accuracy, consistency, and comprehensibility (mean: 4.7–4.8). Perplexity scored significantly lower (mean: 4.1, $p<0.001$). Gemini 1.5 Pro achieved the highest readability score (mean: 61.16), followed by ChatGPT-4 (mean: 58.94) and Claude 3 Opus (mean: 51.16). Perplexity had the lowest readability score (mean: 47.01). Gemini 1.5 Pro and ChatGPT-4 used significantly more words compared to Claude 3 Opus and Perplexity ($p<0.001$). Linear correlation analysis revealed a positive correlation between word count of fictional US findings and responses generated by Gemini 1.5 Pro (correlation coefficient = 0.38, $p<0.05$) and ChatGPT-4 (correlation coefficient = 0.43, $p<0.001$).

Conclusion: This study highlights strong potential of LLMs in simplifying Turkish US findings, improving accessibility and clarity for patients. Gemini 1.5 Pro, ChatGPT-4, and Claude 3 Opus performed well, highlighting their effectiveness in healthcare communication. Further research is required to fully understand the integration of LLMs into clinical practice and their influence on patient comprehension and decision-making.

Keywords: Large Language Models, ChatGPT, Claude 3 Opus, Ultrasound, Simplify

INTRODUCTION

Large Language Models (LLMs) are AI systems designed to comprehend and generate human language [1]. ChatGPT, developed by OpenAI and launched in November 2022, stands out as a prominent example of LLMs [2]. Alongside ChatGPT, there exist various other LLMs such as Google's Gemini, Microsoft's Copilot, Anthropic's Claude, and Perplexity. Studies across different medical specialties have evaluated the performance of LLMs [3-5].

Notably, in radiology, multiple studies have shown that large language models (LLMs) can effectively structure and simplify radiology reports, as well as educate patients about interventional radiology procedures [6-8].

Ultrasound (US) is among the most frequently utilized modalities in radiology, with reports heavily reliant on medical terminology [9]. Barrat et al.'s systematic review highlighted the negative impact of medical terminology on patient anxiety and treatment perceptions [10]. This highlights the need to provide patients with imaging reports that are easy to comprehend, especially as these reports become more readily available [11].

Main Points

- This study found that large language models (LLMs9 can effectively simplify Turkish ultrasound findings for non-medical individuals, achieving high Likert scores for accuracy and comprehensibility.
- Gemini 1.5 Pro and ChatGPT 4 emerged as top performers in terms of accuracy, comprehensibility, and readability, while Claude 3 Opus performed reasonably well but with slightly lower readability. Perplexity lagged behind in accuracy and readability.
- Transforming complex medical terminology into clear, accessible language, which can empower patients with a deeper understanding of their health status and treatment choices, highlights the potential of LLMs to facilitate patient-centered communication in healthcare, as demonstrated in this study.

Simplified reports have the potential to significantly benefit patients by enhancing their understanding of their condition and promoting adherence to treatment plans [12]. Among the various readability indices for texts, Ateşman's Readability Index specifically measures the readability of Turkish texts, assessing how easily the target audience can understand them [13].

While studies comparing the performance of LLMs in simplifying radiology reports exist for different languages, there is currently no literature available on this topic for Turkish ultrasound reports. Therefore, the aim of this study is to assess and compare the performance of different LLMs in simplifying Turkish ultrasound reports into languages understandable by patients.

MATERIALS AND METHODS

Study Design

The study conducted an assessment and comparison of several LLMs including ChatGPT 4 (https://chat.openai.com), Gemini 1.5 Pro (https://gemini.google.com), Claude 3 Opus (https://claude.ai), and Perplexity (https://perplexity.ai) to simplify Turkish US findings. Our study exclusively utilized fictional US findings and did not involve actual radiology reports, thereby exempting it from the need for ethical board approval due to the absence of real patient information. The study adhered to the Standards for Reporting of Diagnostic Accuracy Studies (STARD) guidelines for its design and implementation [14].

Data Collection and Prompt Design

Radiologist 1 (Y.C.G.) created 50 fictional Turkish US findings used in radiology reports. Care was taken to ensure these findings were common in daily practice and portrayed realistically. The designed findings were entered into each LLM via their respective websites following the prompt, "I will write the findings from the MRI report below. Please explain them in a way that someone without a medical background can understand." in Turkish (Figure 1). Each finding was processed in a new window with default settings used for each model. The study was conducted in April 2024. All findings are shown in Table 1. The study's workflow is illustrated in Figure 2.

Table 1. Common Fictional Ultrasound Report Sentences

The liver parenchymal echo pattern appears coarse and granular.
An echo increase consistent with grade 2 steatosis is observed in the liver.
A hyperechoic lesion, primarily suggestive of a hemangioma, measuring 10 mm in segment 4 of the liver, is noted.
The gallbladder appears contracted.
Multiple stones, with the largest measuring 1 cm in diameter, are observed in the gallbladder.
The gallbladder is hydropic.
The gallbladder is hydropic, with increased wall thickness diffusely. Millimetric stones and biliary sludge are present within the gallbladder.
Biliary sludge is observed within the gallbladder.
Diffuse, millimetric hyperechoic lesions causing posterior comet-tail artifacts on the gallbladder wall are noted. The findings are suggestive of diffuse adenomyomatosis.
Phrygian cap appearance is observed in the gallbladder.
Intrahepatic bile ducts are dilated in both lobes.
Features consistent with acute cholecystitis are observed in the gallbladder.
A primarily polyp-like lesion measuring 7 mm in diameter, immobile with patient movement and non-vascular on ultrasound, is noted in the gallbladder, suggesting evaluation for acute appendicitis.
Pancreas and midline structures cannot be evaluated due to gas.
A cystic lesion measuring 7 mm in diameter, without solid components or septa, is observed in the pancreatic head.
The pancreatic parenchyma appears diffusely edematous, with fluid collections in the peripancreatic area.
The spleen measures 14 cm in size and appears enlarged.
An accessory spleen measuring 7 mm in diameter is observed at the splenic hilum.
A nodular lesion measuring 8 mm in diameter, demonstrating arterial vascularity on ultrasound, is observed at the splenic hilum, suggesting a splenic artery aneurysm.
Collateral vascular structures are observed at the splenic hilum.
Grade 2 increase in echogenicity is noted in the parenchyma of both kidneys.
Both kidneys appear decreased in size and parenchymal thickness.
Multiple simple cortical cysts measuring less than 1 cm in diameter are observed in both kidneys.
Fusion of the lower poles of both kidneys to the midline of the abdomen anterior to the abdominal aorta is observed, suggesting a horseshoe kidney.
A hyperechoic nodular lesion, primarily suggestive of an angiomyolipoma, measuring 8 mm in the mid-pole of the right kidney, is observed.
An isoechoic solid mass lesion with internal vascularity measuring 27x24 mm, extending exophytically in the lower segment of the right kidney, is observed. Dynamic upper abdominal CT/MRI is recommended for lesion characterization.
Grade 2 dilation of the pelvicalyceal system is noted in both kidneys.
A hyperechoic solid lesion measuring 17x12 mm, demonstrating vascularity on ultrasound, with papillary extensions into the lumen, is observed in the posterolateral wall of the bladder. Histopathological correlation is recommended.
There is diffuse thickening of the bladder wall.
There is increased trabeculation of the bladder wall.
Widespread millimetric echogenicities are observed within the bladder lumen. Clinical and laboratory evaluation is recommended for cystitis.
A submucosal edema measuring 9 mm in diameter, originating from the terminal ileum in the right lower quadrant, with a blind-ending tubular segment unresponsive to compression, is observed. Clinical and laboratory evaluation is recommended for acute appendicitis.
Omental fat tissue and bowel loops demonstrating herniation from a fascial defect measuring approximately 13 mm in the right inguinal region with valsalva maneuver are observed.
Omental fat tissue and bowel loops demonstrating herniation from a fascial defect measuring approximately 13 mm in the right inguinal region with valsalva maneuver are observed. There is no return of herniated material following the valsalva maneuver. Thickening of up to 7 mm is measured at the thickest point of the herniated bowel loops, with loss of vascularity on ultrasound examination. Fluid measuring 5 mm in depth is observed within the hernia sac.

Multiple simple cysts measuring less than 5 mm in diameter are observed in both breasts.
A solid lesion measuring 13x14 mm, parallel to the skin with regular contours and no vascularity on ultrasound examination, is observed approximately 24 mm from the nipple at 3 o'clock in the right breast (BI-RADS 3).
A hypoechoic solid lesion demonstrating spiculated margins and vascularity on ultrasound examination, measuring 13x14 mm, is observed approximately 33 mm from the nipple at 6 o'clock in the right breast (BI-RADS 5).
An echogenic hilum lymph node measuring 13 mm in diameter is observed in the right axilla.
An LAP with asymmetric cortical thickening measuring 17 mm in diameter, with a cortex thickness of 3.4 mm at the thickest point, is observed in the right axilla.
The thyroid parenchyma appears heterogeneous with scattered hypoechoic areas and echogenic fibrous septa.
An isoechoic nodule with microcystic degeneration areas measuring 16 mm in diameter is observed in the mid-section of the right thyroid lobe.
Multiple isoechoic nodules, with the largest measuring 8 mm in the mid-section of the right lobe and 11 mm in the lower section of the left lobe, are observed in both thyroid lobes.
A hypoechoic solid nodule with irregular margins measuring 13x13 mm in the lower section of the right thyroid lobe is observed, with microcalcifications within.
Multiple lymph nodes with echogenic hilum, measuring less than 1 cm in short diameter and with thick cortex, are observed in both cervical chains.
A cystic appearance primarily suggestive of a dominant follicle, measuring 23 mm, is observed in the right ovary.
A heterogeneous lesion with thick septa and a solid component demonstrating vascularity on ultrasound examination, measuring 23x24 mm, is observed in the left ovary. Contrast-enhanced pelvic MRI is recommended for lesion characterization.
An intramural myoma measuring 13 mm in diameter is observed in the right half of the uterine corpus.
Endometrial thickness, measured as double-wall thickness, is 17 mm and significantly increased.
A tubular structure measuring 14 mm in diameter in the right adnexal region, suggestive of a tubal structure consistent with hydrosalpinx, is observed.
A lesion measuring 17 mm in diameter with multiple septa within, primarily suggestive of a hemorrhagic cyst, is observed in the right ovary.

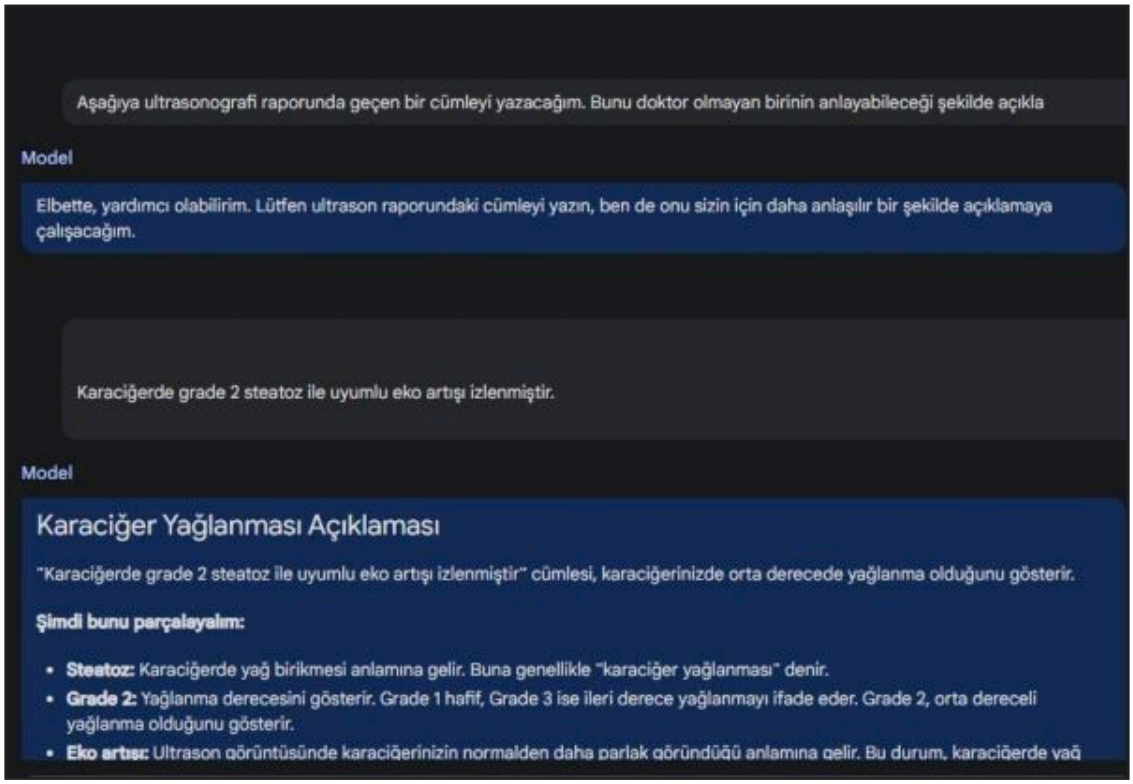

Figure 1. Demonstrates the Turkish input and output process for Turkish ultrasound findings in the study through Gemini 1.5 Pro.

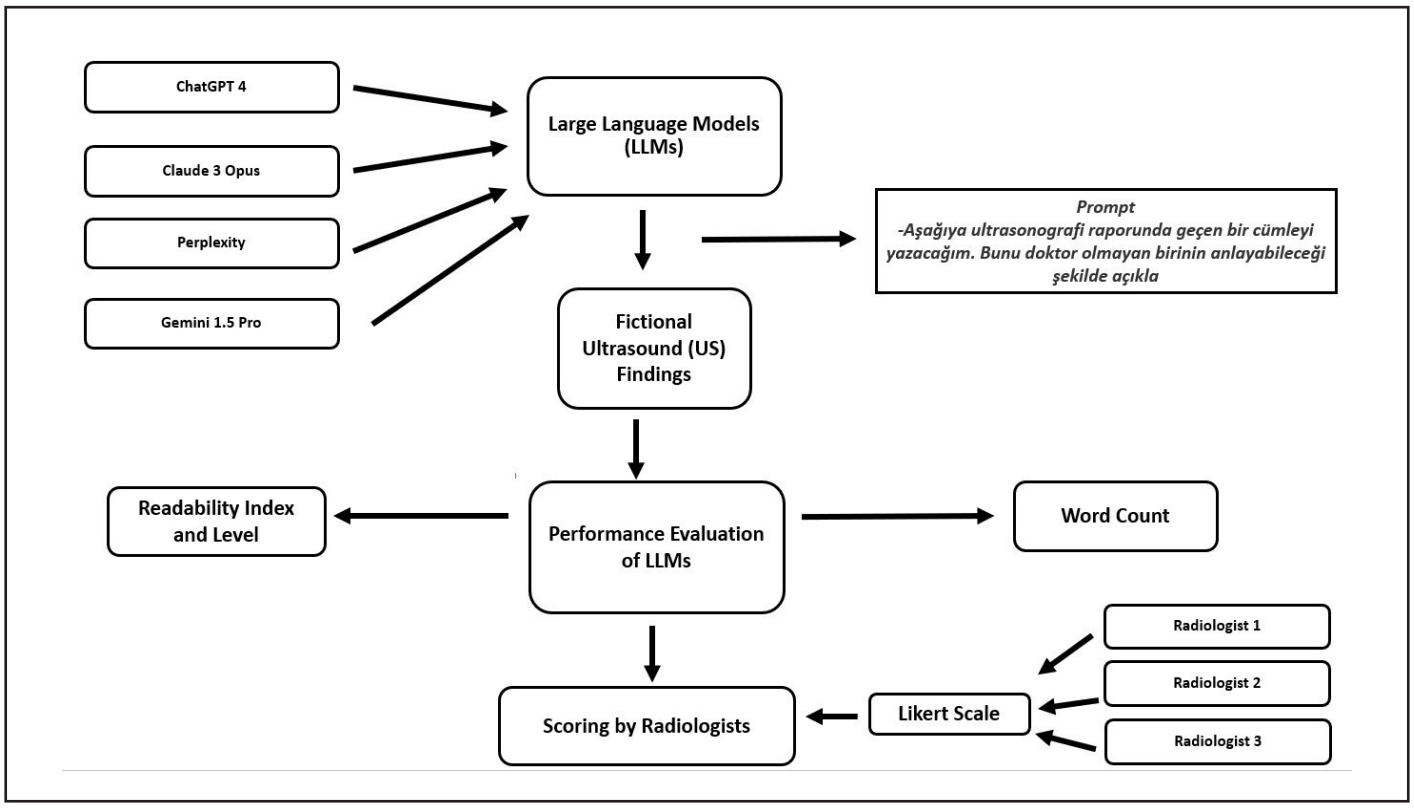

Figure 2. The study's workflow is illustrated in Figure 2.

Performance Evaluation of LLMs

The responses from the LLMs were analyzed using the Ateşman's Readability Index [198,825 - (40,175 x number of syllables/number of words) - (2,610 x number of words/number of sentences)] to determine readability levels in Turkish which has been introduced in 1997 by Ender Ateşman (Table 2) [13]. The word length used in the formula is calculated in syllables and sentence length is calculated in words. The publicly available and free website "http://okunabilirlikindeksi.com" was used for this analysis. The responses were rated jointly by three radiologist [Radiologist 1 (Y.C.G.), Radiologist 2 (T.C.), Radiologist 3 (E.Ç.), each with 6 years of experience in general radiology and certified by the European Diploma in Radiology (EDiR)] using a Likert Scale from 1 to 5 based on medical accuracy, consistency of recommendations, and comprehensibility. The number of words in each response was recorded. Additionally, the word count and Ateşman's Readability Index of the fictional US findings were compared to the responses generated by each LLM.

Statistical Analysis

Data distribution was examined using the Kolmogorov-Smirnov and Shapiro-Wilk tests, while the Levene test was utilized to assess data variance. Descriptive statistics, comprising measures such as minimum, maximum, average, median, standard deviation, interquartile range, and percentages, were then calculated. Subsequently, to identify significant relationships among quantitative data within dependent groups, both the Friedman and Wilcoxon tests were employed. Additionally, Spearman correlation analysis was conducted to investigate the linearity of correlations between quantitative data. Statistical analyses were performed using IBM SPSS Version 26.

RESULTS

Likert Scale

No statistically significant difference was found between the scores of ChatGPT 4 (mean: 4.82; median: 5.0), Gemini 1.5 Pro (mean: 4.78; median: 5.0), and Claude 3 Opus (mean: 4.68; median: 5.0) based on the Likert scale ($p>0.05$). However, the scores of ChatGPT 4, Gemini 1.5 Pro, and Claude 3 Opus were significantly higher than those of Perplexity (mean: 4.08; median: 4.0) ($p<0.001$). No difference was found between the scores of Claude 3 Opus and Gemini 1.5 Pro ($p=0.39$) (Figure 3) (Table 3).

Table 2. The Ateşman Readability Index and Its Corresponding Readability Level

Index	Readability Level
90 - 100	Easily understood by 4th grade and below students
80 - 89	Easily understood by 5th or 6th graders
70 - 79	Easily understood by 7th or 8th graders
60 - 69	Easily understood by 9th or 10th graders
50 - 59	Easily understood by 11th or 12th graders
40 - 49	Easily understood by 13th or 15th-year (associate degree) students
30 - 39	Easily understood by bachelor's degree
< 30	Easily understood by postgraduates

Table 3. Descriptive Findings of the Responses of the Large Language Models.

	Gemini 1.5 Pro	Claude 3 Opus	ChatGPT 4	Perplexity
Likert Scores*				
Minimum-Maximum	2.0-5.0	3.0-5.0	3.0-5.0	1.0-5.0
Mean ± SD	4.8 ± 0.5	4.7 ± 0.6	4.8 ± 0.5	4.1 ± 0.6
Median (IQR)	5.0 (0)	5.0 (0)	5.0 (0)	4.0 (1.0)
Ateşman Readability Index				
Minimum-Maximum	45.0 - 72.0	28.0 - 66.0	29.0-73.0	26.0 – 71.0
Mean ± SD	61.16 ± 6.66	51.16 ± 7.23	58.94 ± 9.61	46.25 ± 11.98
Median (IQR)	54.1 (14.2)	47.2 (10.3)	37.8 (7.6)	37.3 (6.2)
Readability Level				
Minimum	7.-8. Grade	9.-10. Grade	7.-8. Grade	7.-8. Grade
Maximum	Associate's Degree	Postgraduate	Postgraduate	Postgraduate
Median	11.-12. Grade	11.-12. Grade	11.-12. Grade	13.-15. Grade
Word Count				
Minimum-Maximum	77-283	69-197	57-292	40-187
Mean ± SD	166.9 ± 46.9	99.24 ± 36.3	154.9 ± 39.8	88.4 ± 26.5
Median (IQR)	171.5 (24.5)	79.0 (47.0)	157.0 (72.0)	91.5 (35.0)

*Likert Scores: In our study, the accuracy of the explanations, consistency and comprehensibility of the suggestions made by the big language models were rated on a scale of 1 to 5.

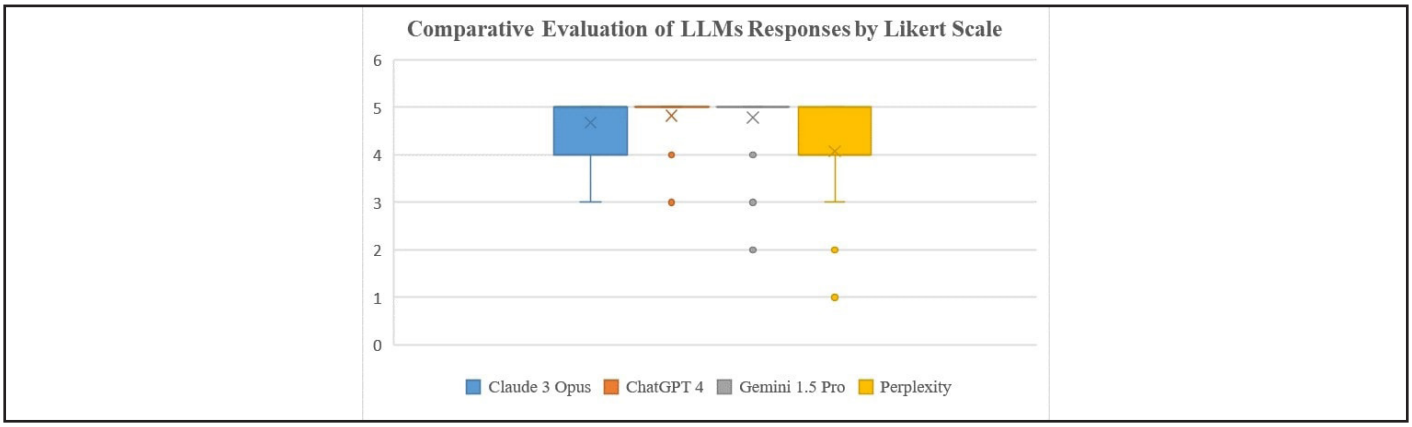

Figure 3. A box-plot displays the radiologists' consensus scores for the Large Language Models' answers, with the median score denoted by 'x' and outlying scores marked by dots.

SD: Standard Deviation, IQR: Interquartile range.

Ateşman's Readability Index

A significant difference was found between the mean scores of the LLMs based on the Ateşman's Readability Index ($p<0.001$). The highest mean score was 61.16 for Gemini 1.5 Pro, and the second-highest score was 58.94 for ChatGPT 4, which did not show a significant difference ($p=0.208$). Additionally, both Gemini 1.5 Pro and ChatGPT 4 had significantly higher Ateşman's Readability Index scores than Claude 3 Opus (51.16) and Perplexity (47.01) ($p<0.001$). Claude 3 Opus's Ateşman's Readability Index score was also significantly higher than Perplexity's ($p=0.006$) (Table 3).

Word Counts

No significant difference was found between the mean word count of Gemini 1.5 Pro (166.2) and ChatGPT 4 (154.38) ($p=0.213$). However, both Gemini 1.5 Pro and ChatGPT 4 used significantly more words than Claude 3 Opus (99.24) and Perplexity (93.48) ($p<0.001$). No significant difference was found between the word count of Claude 3 Opus and Perplexity ($p>0.05$) (Table 3).

Correlation Analysis

A linear correlation was observed between the word count of fictional US findings and the word count of responses generated by Gemini 1.5 pro (correlation coefficient = 0.38, $p<0.05$) and ChatGPT 4 (correlation coefficient = 0.43, $p<0.001$). Conversely, no correlation was found between the word count of Claude 3 Opus ($p>0.05$) and Perplexity ($p\geq0.05$).

A significant correlation was identified between the Ateşman's Readability Index of fictional US findings and the Ateşman's Readability Index of responses from Claude 3 Claude 3 Opus (correlation coefficient = 0.42, $p<0.001$), ChatGPT 4 (correlation coefficient = 0.51, $p<0.001$), and Gemini 1.5 pro (correlation coefficient = 0.45, $p<0.001$). However, no correlation was detected between the Ateşman's Readability Index of fictional US findings and Perplexity ($p>0.05$).

DISCUSSION

Our study found that LLMs effectively simplified commonly used Turkish US findings for non-medical individuals. Gemini 1.5 Pro, Claude 3 Opus, and ChatGPT 4 received high Likert scores (4.68 to 4.82 out of 5), indicating their effectiveness in conveying US findings clearly. However, Perplexity scored lower (4.1), suggesting less accuracy and comprehensibility. Gemini 1.5 Pro had the highest readability score (61.16), while ChatGPT 4 (58.94) and Claude 3 Opus (51.16) followed closely. Perplexity had the lowest average score (46.25). These results suggest that Gemini 1.5 Pro produced the easiest to understand responses, while Perplexity generated the least readable responses.

In terms of readability, all LLMs predominantly produced responses suitable for readers with at least an associate degree or higher educational background. However, there were variations in the readability levels produced by each model. Gemini 1.5 Pro and ChatGPT 4 mainly generated responses at a 7th to 8th-grade reading level, while Claude 3 Opus and Perplexity tended to produce responses at a slightly higher reading level, ranging from 9th to 12th grade.

Gemini 1.5 Pro and ChatGPT 4 provided more detailed explanations with higher word counts, while Claude 3 Opus and Perplexity had lower word counts. Overall, Gemini 1.5 Pro and ChatGPT 4 emerged as top performers in accuracy, comprehensibility, and readability, while Claude 3 Opus performed reasonably well but with slightly lower readability. Perplexity lagged behind in accuracy and readability.

The variations in performance among LLMs could be attributed to their differing design architectures. For example, Perplexity's lower accuracy and readability scores might result from its unique feature of having internet access, potentially incorporating less reliable online sources [15].

Previous studies have also demonstrated the effectiveness of LLMs in simplifying radiology reports [16-21]. Doshi et al. conducted a study showcasing the efficacy of ChatGPT 4, ChatGPT 3.5, Google Bard, and Microsoft Copilot in simplifying 750 radiology reports. These reports, spanning various modalities like ultrasound (US), CT, MRI, mammography, and X-ray, were subjected to three distinct prompts [16]. The first prompt sought a general simplification of the report, while the second prompt required the report to be simplified from a patient's perspective. The final prompt mandated the report to be simplified to a 7th-grade reading level [16]. In our study, a prompt similar to the second prompt used by Doshi was successfully employed to simplify ultrasound reports.

Haver et al. [17] demonstrated that ChatGPT could simplify responses to 25 breast cancer questions to a sixth-grade reading level. They evaluated the original and simplified responses

using the Flesch Reading Ease Index and five readability scales. Ninety-two percent of the simplified responses were deemed clinically appropriate, showing significant improvements in reading ease, readability, and word count reduction.

Chung et al. [18] utilized ChatGPT to summarize MRI reports of prostate cancer patients, customizing them for patient comprehension levels. Their study yielded fifteen summarized reports from five full MRI reports, revealing a noteworthy decrease in the median Flesch-Kincaid Grade Level (FKGL) score from 9.6 to 5.0.

Li et al. [19] showcased ChatGPT's efficacy in simplifying radiology reports to below an 8th-grade reading level across various modalities, including radiographs, ultrasound (US), computed tomography (CT), and magnetic resonance imaging (MRI), demonstrating its versatility in different imaging contexts. Their study revealed that the mean report length was 164 words, accompanied by a Flesch reading ease score of 38.0 and a FKGL of 10.4.

In Lyu et al.'s study [20], radiologists evaluated ChatGPT's output for chest CT and brain MRI scans, acknowledging some missing and incorrect information. Despite these discrepancies, the overall quality score was 4.268 out of 5 on a five-point Likert scale, with minor discrepancies of 0.08 and 0.07 for missing and misinformation, respectively.

Tepe et al. [21] compared the effectiveness of ChatGPT 4, Google Bard, and Microsoft Copilot in translating CT and MRI reports into patient-friendly language, with all models achieved understandability scores above 70%. but Bard showing superior readability scores.

Our findings align with these studies, showing that ChatGPT 4, Gemini 1.5 Pro, and Claude 3 Opus effectively simplified Turkish US findings, achieving high Likert scores for accuracy and comprehensibility. However, Perplexity scored lower in these areas. Gemini 1.5 Pro and ChatGPT 4 also produced the highest readability scores, indicating their superior ability to simplify medical language.

In contrast to previous studies, our research focused specifically on Turkish US reports, providing new insights into the performance of LLMs in this context rather than English radiological reports. Therefore, we used the Ateşman's Readability Index for the Turkish language, rather than the Flesch Reading Ease Index and other readability scales.

Our study also indicates the potential of LLMs to facilitate patient-centred communication in healthcare. By transforming complex medical terminology into clear, accessible language, these systems have the potential to empower patients with a deeper understanding of their health status and treatment choices. This enhanced comprehension can facilitate more confident decision-making, heightened patient involvement, and ultimately, improved health outcomes.

Limitations

Although our study represents the first investigation into the simplification of Turkish US findings by LLMs for individuals without medical backgrounds, it has several limitations. Firstly, although the US sentences utilized in our study are commonly encountered in daily medical practice, they are synthetic in nature. To provide a more accurate assessment of LLMs' performance, real-world reports from hospital settings should be incorporated into future studies. Secondly, the sample size of sentences used in our study was limited, and they primarily focused on straightforward cases. To enhance the applicability of our findings, future research should expand the scope to include a broader range of complex sentences tailored to different anatomical regions. Thirdly, our study employed a single prompt, which may not fully capture the range of scenarios encountered in clinical practice. Further investigation into the impact of different prompts on LLM performance is warranted to better understand how varying contexts influence their effectiveness. Lastly, our study lacked data on real-life patients' satisfaction and comprehension of simplified reports. Prospective multicenter studies utilizing simplified reports generated by LLMs and assessing patients' understanding and satisfaction levels are essential for validating the practical utility of these systems in healthcare communication.

CONCLUSIONS

In conclusion, our study demonstrates that LLMs successfully simplify Turkish US findings, emphasizing their role in improving accessibility and understandability of radiological information for patients. Further research and implementation efforts are needed to fully harness the potential of LLMs in facilitating effective communication between healthcare

providers and patients.

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Ethical Approval: Our study only included fictional ultrasound findings and did not use actual radiology reports or patient information, so it did not require ethical board approval.

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REFERENCES

[1] Aydin Ö, Karaarslan E (2023) Is ChatGPT Leading Generative AI? What is Beyond Expectations? Academic Platform Journal of Engineering and Smart Systems 11:118-134. https://doi.org/10.21541/apjess.1293702

[2] Lee H (2023) The rise of ChatGPT: Exploring its potential in medical education. Anatomical sciences education. https://doi.org/10.1002/ase.2270

[3] Kuang Y-R, Zou M-X, Niu H-Q, Zheng B-Y, Zhang T-L, Zheng B-W (2023) ChatGPT encounters multiple opportunities and challenges in neurosurgery. International Journal of Surgery 109:2886-2891. https://doi.org/10.1097/JS9.0000000000000571

[4] Griewing S, Gremke N, Wagner U, Lingenfelder M, Kuhn S, Boekhoff J (2023) Challenging ChatGPT 3.5 in senology—an assessment of concordance with breast cancer tumor board decision making. Journal of Personalized Medicine 13:1502. https://doi.org/10.3390/jpm13101502

[5] Suthar PP, Kounsal A, Chhetri L, Saini D, Dua SG (2023) Artificial intelligence (AI) in radiology: a deep dive into ChatGPT 4.0's accuracy with the American Journal of Neuroradiology's (AJNR)" Case of the Month". Cureus 15. https://doi.org/10.7759/cureus.43958

[6] Jeblick K, Schachtner B, Dexl J, Mittermeier A, Stüber AT, Topalis J, Weber T, Wesp P, Sabel BO, Ricke J (2023) ChatGPT makes medicine easy to swallow: an exploratory case study on simplified radiology reports. Eur Radiol:1-9. https://doi.org/10.1007/s00330-023

[7] Scheschenja M, Viniol S, Bastian MB, Wessendorf J, König AM, Mahnken AH (2024) Feasibility of GPT-3 and GPT-4 for in-depth patient education prior to interventional radiological procedures: a comparative analysis. Cardiovasc Intervent Radiol 47:245-250. https://doi.org/10.1007/s00270-023-03563-2

[8] Elkassem AA, Smith AD (2023) Potential use cases for ChatGPT in radiology reporting. American Journal of Roentgenology 221:373-376. https://doi.org/10.2214/AJR.23.29198

[9] Chan V, Perlas A (2011) Basics of ultrasound imaging. Atlas of ultrasound-guided procedures in interventional pain management:13-19. https://doi.org/10.1007/978-1-4419-1681-5_2

[10] Barratt A, Copp T, McCaffery K, Moynihan R, Nickel B (2017) Words do matter: a systematic review on how different terminology for the same condition influences management preferences. https://doi.org/10.1136/bmjopen-2016-014129

[11] Johnson AJ, Frankel RM, Williams LS, Glover S, Easterling D (2010) Patient access to radiology reports: what do physicians think? Journal of the American College of Radiology 7:281-289. https://doi.org/10.1016/j.jacr.2009.10.011

[12] Amin K, Khosla P, Doshi R, Chheang S, Forman HP (2023)

Focus: Big Data: Artificial Intelligence to Improve Patient Understanding of Radiology Reports. The Yale Journal of Biology and Medicine 96:407. https://doi.org/10.59249/NKOY5498

[13] Ateşman E (1997) Türkçede okunabilirliğin ölçülmesi. Dil Dergisi 58

[14] Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig L, Lijmer JG, Moher D, Rennie D, De Vet HC (2015) STARD 2015: an updated list of essential items for reporting diagnostic accuracy studies. Radiology 277:826-832. https://doi.org/10.1136/bmj.h5527

[15] Khan R, Gupta N, Sinhababu A, Chakravarty R (2023) Impact of Conversational and Generative AI Systems on Libraries: A Use Case Large Language Model (LLM). Science & Technology Libraries:1-15. https://doi.org/10.1080/0194262x.2023.2254814

[16] Doshi R, Amin KS, Khosla P, Bajaj SS, Chheang S, Forman HP (2024) Quantitative evaluation of large language models to streamline radiology report impressions: a multimodal retrospective analysis. Radiology 310:e231593. https://doi.org/10.1148/radiol.231593

[17] Haver HL, Gupta AK, Ambinder EB, Bahl M, Oluyemi ET, Jeudy J, Yi PH (2024) Evaluating the Use of ChatGPT to Accurately Simplify Patient-centered Information about Breast Cancer Prevention and Screening. Radiology: Imaging Cancer 6:e230086. https://doi.org/10.1148/rycan.230086

[18] Chung EM, Zhang SC, Nguyen AT, Atkins KM, Sandler HM, Kamrava M (2023) Feasibility and acceptability of ChatGPT generated radiology report summaries for cancer patients. Digital Health 9:20552076231221620. https://doi.org/10.1177/20552076231221620

[19] Li H, Moon JT, Iyer D, Balthazar P, Krupinski EA, Bercu ZL, Newsome JM, Banerjee I, Gichoya JW, Trivedi HM (2023) Decoding radiology reports: potential application of OpenAI ChatGPT to enhance patient understanding of diagnostic reports. Clin Imaging 101:137-141. https://doi.org/10.1016/j.clinimag.2023.06.008

[20] Lyu Q, Tan J, Zapadka ME, Ponnatapura J, Niu C, Myers KJ, Wang G, Whitlow CT (2023) Translating radiology reports into plain language using ChatGPT and GPT-4 with prompt learning: results, limitations, and potential. Visual Computing for Industry, Biomedicine, and Art 6:9. https://doi.org/10.1186/s42492-023-00136-5

[21] Tepe M, Emekli E (2024) Decoding medical jargon: The use of AI language models (ChatGPT-4, BARD, microsoft copilot) in radiology reports. Patient Educ Couns:108307. https://doi.org/10.1016/j.pec.2024.108307

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Original Research

A Study on Diaphyseal Nutrient Foramen of Humerus

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ABSTRACT

Objective: The aim of this study is to provide detailed data about the nutrient foramen (NF) of the humerus, the entry point of the nutrient artery, used in order to avoid damaging the nutrient artery, which has an important role in the nutrition of the humerus, during surgical approaches to the humeral diaphysis.

Methods: This study was performed on 113 (58 right, 55 left) humeri. The number, direction, location, position, size and foraminal index of the nutrient foramina (NFs) were evaluated. In addition, total length of the humerus and distance between NF and proximal end of the humerus were measured.

Results: One NF was observed on 86 of 113 (76.11%) humeri, two NFs on 17 of 113 (15.05%) humeri, 3 NFs on the one right sided humerus and no NF was observed on 9 of 113 (7.96%) humeri. While 122 (99.19%) of all NFs were directed distally, one (0.81%) NF was directed horizontally. Eighty-six (69.92%) NFs were located on the anteromedial surface, while 18 (14.63%) NFs were found to be located on the medial border, 12 (9.76%) NFs on the posterior surface, 4 (3.25%) NFs on the anterolateral surface and 3 (2.44%) NFs on the lateral border. 20G sized NF was detected on 50 of 123 (40.65%) NFs. 14G and 16G sized NFs were not detected. 5 (4.07%) NFs were located on the proximal 1/3, 113 (91.86%) NFs were located on middle 1/3 and 5 (4.07%) NFs were located on distal 1/3 of the humerus. The mean values of total length of the humerus, distance between NF and proximal end of the humerus and foraminal index were found out to be 301.68±**20.61** mm, 166.70±**32.50** mm and 55.33±**9.48%, respectively.**

Conclusion: It is found out that there is usually one NF on the humerus and that this foramen is directed distally, localized on the anteromedial surface and the middle 1/3 of the humerus and sized at 20G. And also, we observed horizontally directed NF on the humerus. To our knowledge, there is no study which observed horizontally directed NF. There may be differences between populations about the morphology and morphometry of NF. Therefore, being aware for the morphology, morphometry and variations of the NF is important for the orthopaedic surgeons in surgeries such as fracture repairing and vascularized bone graft in order to avoid damaging the nutrient arteries.

Keywords: Humerus, nutrient foramen, morphology, morphometry, anatomy

INTRODUCTION

Arterial supply of the long bones is provided by the nutrient, epiphyseal, metaphyseal and periosteal arteries [1]. Nutrient foramina (NFs) are found on the bones, providing passage for nutrient arteries. These foramina are located on the diaphysis in long bones and elsewhere in irregular bones [2-4] and enable

nutrient arteries to enter the medullary space and supply the bone marrow and inner 2/3 of the cortex [1]. Directions of the nutrient foramina are usually facing away from the dominant growing ends of the long bones [5]. The nutrient artery of the humerus is a branch of the brachial artery. During surgical procedure such as bone repair, bone grafting, vascularized bone microsurgery, detailed information about the nutrition of the bones will be helpful for the orthopaedic surgeons to minimize damage to the nutrient artery of the humerus [6]. The nutrient artery is particularly important during the active growth period of the embryo and foetus and early ossification stage [7]. Fracture of the long bones is a common condition. Delayed union is one of the most common complication of fractures, and poor bone nutrition occurs to be one of the outcomes of such complication. In this respect, the nutrient artery is important for fracture healing [8]. The nutrient foramen (NF) or its nearby regions are also central to the development of longitudinal stress fractures [9].

According to our literature survey, there are few studies about the NF of the humerus within the Turkish population [10, 11]. The aim of this study is to provide detailed data about the NF, the entry point of the nutrient artery, to be used in order to avoid damaging the nutrient artery, which has an important role in nutrition of the humerus, during surgical approaches to the humeral diaphysis.

MATERIALS AND METHODS

Data Collecting

This study was performed on 113 (58 right, 55 left) humeri of adult Turkish population in the Department of Anatomy, Faculty of Medicine, Hacettepe University. The age and sex of the humeri were unknown. The humeri with fracture or deformation were excluded from the study. Ethic approval was taken from the Hacettepe University Non-Invasive Clinical Research Ethics Committee (date: 18/04/2023 number: 2023/07-08).

The following parameters were evaluated;

1. Total length (TL)
2. Number of NF
3. Localization of the NF on the humeral surfaces or borders
4. Size of NF
5. Position of NF on the humerus
6. Direction of NF
7. Distance between NF and proximal end of the humerus (DNF)
8. Foraminal index (FI) = (DNF/TL) x 100

Initially, NFs were defined using a magnifying glass on the surfaces and borders of the humerus, number, and direction (towards the proximally, distally and horizontally) of the NFs were recorded. Size of the NFs were evaluated respectively by 14, 16, 18, 20, 22, and 24 gauge sized hypodermic needles, and which needle fits the foramens's diameter was determined; the needle with the accurate gauge size was noted as size of examined NF. We evaluated diaphyseal NFs on the humerus, epiphyseal NFs were excluded from the study.

The total length of the humerus (TL) was measured using a tape measure from the most proximal end of the head of the humerus to the most distal end of the humerus. The distance between NF and proximal end of the humerus (DNF) was measured between the NF and most proximal end of the head of the humerus (Figure 1.). Foraminal index was calculated using the Hughes formula: FI=(DNF/TL)*100 [12]. The position of the NF was divided into three parts according to FI: proximal 1/3 (FI up to 33.33%), middle 1/3 (FI from 33.33% to 66.66%) and distal 1/3 (FI above 66.66%).

Statistical Analysis

Statistical analysis of the data was evaluated using the SPSS software v23 (Statistical Package for the Social Sciences– SPSS Inc.). The descriptive statistics of the data were given as percentage, mean, standard deviation, minimum and maximum values. The conformity of the data to the normal distribution was examined using histogram graphics and Kolmogorov-Smirnov/Shapiro-Wilk tests. Independent samples t-test was used to compare the differences between sides. A 5% type-1 error level was used to statistical significance.

Main Points;

- Nutrient foramen is found on the diaphysis of the long bones, providing passage for nutrient arteries.
- The nutrient artery is particularly important during the active growth period of the embryo and foetus and early ossification stage.
- During surgical approaches to the humeral diaphysis, protecting the nutrient foramen is important to reduce possible complications.

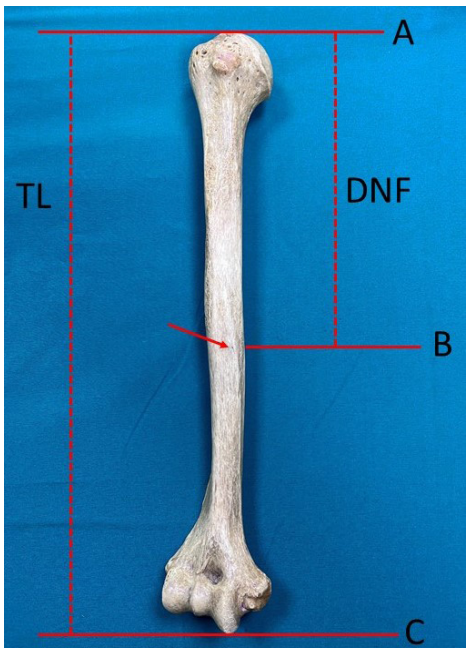

Figure 1. Demonstration of the measurements. A: the most proximal end of the head of the humerus, B: line passing through the nutrient foramen, C: the most distal end of the humerus. TL: total length, DNF: distance between nutrient foramen and proximal end of humerus. Red arrow indicates the nutrient foramen

Figure 2. Demonstration of the two nutrient foramina on humerus, a) anterior view of humerus and anteromedial location of nutrient foramen, b) posterior view of humerus and posterior location of nutrient foramen. Hypodermic needles show the entrance points of nutrient foramina.

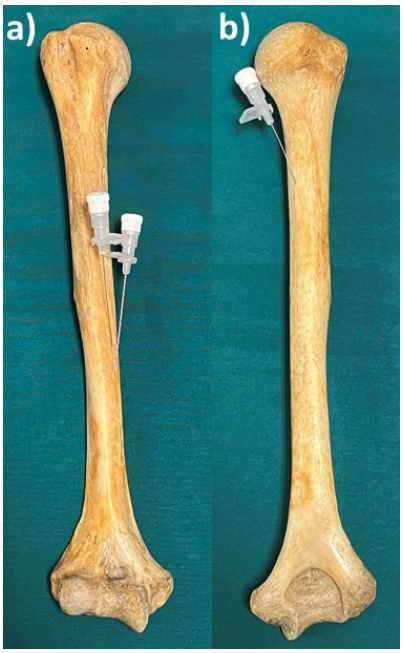

Figure 3. Demonstration of the three nutrient foramina on humerus, a) anterior view of humerus and anteromedial locations of two nutrient foramina, b) posterior view of humerus and posterior location of nutrient foramen. Hypodermic needles show the entrance points of nutrient foramina.

RESULTS

Number of NF: In total, 123 NFs were observed on 113 humeri. Only one NF was observed on 41 of 58 (70.69%) right humeri and 45 of 55 (81.82%) left humeri. Overall, 86 of 113 (76.11%) humeri were found to have one NF each. Besides these, humeri that had 2 NFs were observed on 10 of 58 (17.24%) right humeri, and 7 of 55 (12.73%) left humeri. Overall, 17 of 113 (15.05%) humeri had 2 NFs (Figure 2.). On one of the right sided humerus, 3 NFs were observed (Figure 3.). There was no NF on 6 of 58 (10.35%) right humeri, 3 of 55 (5.45%) left humeri for a total of 9 of 113 (7.96%) humeri.

Direction of NF: While 122 (99.19%) of all NFs were directed distally, one (0.81%) NF was directed horizontally. All of the NFs on the right side, were directed distally, while 58 of 59 (98.3%) of the NFs on the left side were directed distally, and only one of these 59 (1.7%) NFs was directed horizontally.

Localization of the NF on the humeral surfaces or borders: Eighty-six (69.92%) NFs were located on the anteromedial surface, while 18 (14.63%) NFs were found to be located on the medial border, 12 (9.76%) NFs on the posterior surface,

4 (3.25%) NFs on the anterolateral surface and 3 (2.44%) NFs on the lateral border. On the right side, 43 of 64 (67.19%) NFs were located on the anteromedial surface, while 10 of 64 (15.63%) NFs were found to be located on the posterior surface, 9 of 64 (14.06%) NFs on the medial border, 1 of 64 (1.56%) NF on the anterolateral surface and 1 of 64 (1.56%) NF on the lateral border. On the left side, 43 of 59 (72.88%) NFs were located on the anteromedial surface, while 9 of 59 (15.25%) NFs were found to be located on medial border, 3 of 59 (5.09%) NFs on anterolateral surface, 2 of 59 (3.39%) NFs on posterior surface and 2 of 59 (3.39%) NFs on lateral border (Figure 4.). No NF was observed on the anterior border of the humerus.

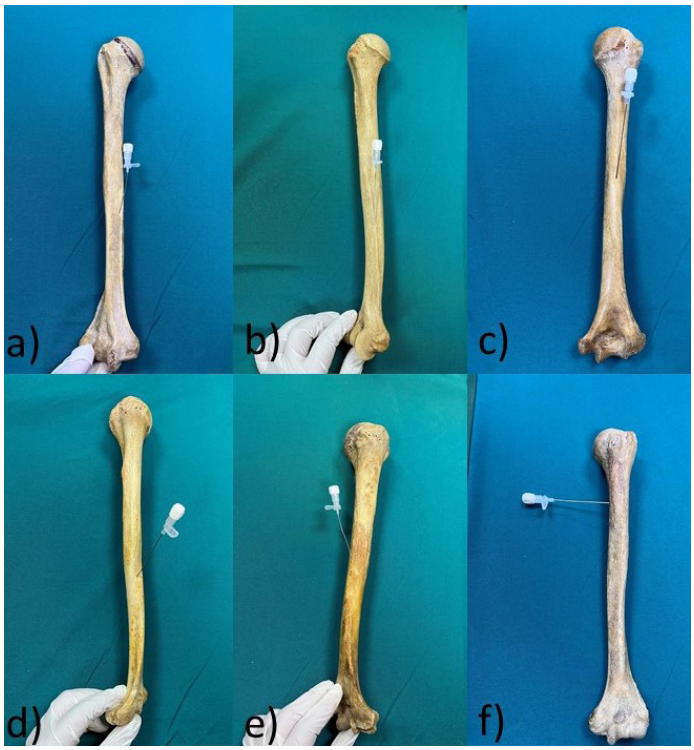

Figure 4. Views of the localizations of the nutrient foramen on humerus. a) on the anteromedial surface, b) on the medial border, c) on the posterior surface, d) anterolateral surface, e) lateral border, f) horizontally directed nutrient foramen

Size of NF: The most common sized NFs were detected as 20G sized, comprising of 28 of 64 (43.75%) NFs on the right side, 22 of 59 (37.29%) NFs on the left side and 50 of 123 (40.65%) NFs in total. 14G and 16G sized NFs were not detected.

Position of NF on the humerus: 5 (4.07%) NFs were located on the proximal 1/3, 113 (91.86%) NFs were located on middle 1/3 and 5 (4.07%) NFs were located on distal 1/3 of the humerus. On the right side, 3 of 64 (4.68%) NFs were located on the proximal 1/3, 58 of 64 (90.64%) NFs on middle 1/3 and 3 of 64 (4.68%) NFs on distal 1/3. On the left side 2 of 59 (3.39%) NFs were located on the proximal 1/3, 55 of 59 (93.22%) NFs on middle 1/3 and 2 of 59 (3.39%) NFs on the distal 1/3.

Total length of humerus: The mean value of the total length of humerus was measured 301.68±**20.61** mm in all humeri, while observed to be 302.69±21.83 mm on the right side, and 300.67±**19.48** mm on the left side.

Distance between NF and proximal end of the humerus: The mean value of distance between the NF and the proximal end of the humerus was measured 166.70±**32.50** mm in all humeri, and 165.86±**36.43 mm** on the right side, and 167.61±**27.91 mm** on the left side.

Foraminal index: The mean value of foraminal index was calculated to be 55.33±**9.48% in all humeri, and** 54.95±**10.63% on the right side and** 55.73±**8.13% on the left side.**

Morphological and morphometric properties of NFs were summarized under Table 1. and Table 2. There were no significant differences between the right and left side regarding the total length of the humerus (p=0.620), distance between NF and proximal end of the humerus (p=0.767) and foraminal index (p=0.649) measurements.

Table 1. Morphometric properties of the nutrient foramen

Parameter	Side	Mean±SD	P value
TL (mm)	Right	302.69±21.83	0.620
	Left	300.67±**19.48**	
	Total	301.68±**20.61**	
DNF (mm)	Right	165.86±**36.43**	0.767
	Left	167.61±**27.91**	
	Total	166.70±**32.50**	
FI (%)	Right	54.95±**10.63**	0.649
	Left	55.73±**8.13**	
	Total	55.33±**9.48**	

TL: total length of humerus, DNF: distance between nutrient foramen and proximal end of the humerus, FI: foraminal index

Table 2. Morphologic properties of the nutrient foramen

Parameter		Side		
		R n(%)	L n(%)	T n(%)
Direction	Proximally n (%)	0	0	0
	Distally n (%)	64 (100)	58 (98.3)	122 (99.19)
	Horizontally n (%)	0	1 (1.7)	1 (0.81)
Localization	Anteromedial surface n (%)	43 (67.19)	43 (72.88)	86 (69.92)
	Anterolateral surface n (%)	1 (1.56)	3 (5.09)	4 (3.25)
	Posterior surface n (%)	10 (15.63)	2 (3.39)	12 (9.76)
	Medial border n (%)	9 (14.06)	9 (15.25)	18 (14.63)
	Lateral border n (%)	1 (1.56)	2 (3.39)	3 (2.44)
	Anterior border n (%)	0 (0)	0 (0)	0 (0)
Size	14G n (%)	0 (0)	0 (0)	0 (0)
	16G n (%)	0 (0)	0 (0)	0 (0)
	18G n (%)	4 (6.25)	7 (11.87)	11 (8.94)
	20G n (%)	28 (43.75)	22 (37.29)	50 (40.65)
	22G n (%)	18 (28.13)	21 (35.59)	39 (31.71)
	24G n (%)	14 (21.87)	9 (15.25)	23 (18.70)
Number of NF	0 n (%)	6 (10.35)	3 (5.45)	9 (7.96)
	1 n (%)	41 (70.69)	45 (81.82)	86 (76.11)
	2 n (%)	10 (17.24)	7 (12.73)	17 (15.05)
	3 n (%)	1 (1.72)	0 (0)	1 (0.88)
Position	Proximal 1/3 n (%)	3 (4.68)	2 (3.39)	5 (4.07)
	Middle 1/3 n (%)	58 (90.64)	55 (93.22)	113 (91.86)
	Distal 1/3 n (%)	3 (4.68)	2 (3.39)	5 (4.07)

R: right, L: left, T: total, n: number, G: gauge, NF: nutrient foramen

DISCUSSION

In the previous studies about the morphology and morphometry of the NFs of the humerus and in our study show that there is usually one NF on the humerus, directed distally, localized on the anteromedial surface and the middle 1/3 of the humerus [1, 10, 11, 13-18]. The knowledge of the morphologic variations and morphometric properties of the NF is important for the orthopaedic surgeons for the purpose of avoiding damage to the nutrient artery while performing open reduction and internal fixation of the fracture [19]. Existence of intact arterial supply is significant in healing of fractured bones [16]. In some fractures, despite appropriate treatment, bone healing is observed to be either slow or not at all [6]. The lack of adequate arterial supply is known as one of the most important reasons for such condition [1].

When we examine the number of the NFs as per previous studies, 1 NF was generally observed in each humerus. This study reported one NF on 86 (76.11%) of the humeri, similar to the findings of Desai and Damor [14] in India 69 (74.11%), Poudel and Satyal [18] in Nepal 40 (80%), Pereira et al. [17] in Brasil 154 (88,5%), and Ehab Kamal Ali [15] in Egypt 210 (84%). The incidence of 1 NF on the humerus was found to be lower in the studies performed by Öztürk et al. [11] in Türkiye 39 (66.1%), Singh et al. [1] in India 38 (61.29%), Mansur et al. [16] in India 154 (60.87%), Arfan et al. [13] in India 52 (60.40%), Cihan and Toma [10] in Türkiye 59 (57.28%) and higher in the study by Kumar et al. [20] in India 73 (91.25%) compared to our study. The present study showed that no NF was detected on 9 (7.96%) of the humeri, which was similar to the findings of Desai and Damor [14] 10 (10.8%), Ehab Kamal Ali [15] 5 (2%), Mansur et al. [16] 5 (1.98%), Cihan and Toma [10] 7 (6.80%) and Kumar et al. [20] 3 (3.75%). Unlike these studies, Singh et al. [1] and Pereira et al. [17] did not detect a humerus devoid of NF. Only a few studies observed triple NFs in the humerus. We found one humerus with triple NFs in compared to the findings of Arfan et al. [13] 5 (5.81%), Mansur et al. [16] 16 (6.32%), Singh et al. [1] 5 (8.06%), Cihan and Toma [10] 8 (7.77%) and Öztürk et al. [11] 1 (1.7%). There are very rare studies that found out more than 3 NFs. Öztürk et al. [11] found 4 foramina on 1 humerus

and 5 foramina on 1 humerus. In the study of Mansur et al. [16], 5 humeri had 4 NFs. Cihan and Toma [10] detected 4 NFs on 3 humeri (2.91%) and 5 NFs on 2 humeri (1.94%). In our study, no humerus with more than 3 foramina was observed (Table 3.).

The NF was generally directed distally in all humeri, similar to in previously conducted studies [1, 10, 13, 14, 16, 18]. Unlike these studies, 2 (2.4%) of the NFs were directed proximally in the study of Öztürk et al. [11], while in our study, 1 (0.81%) of the NFs were found out to be directed horizontally (Table 3.). To our knowledge, there is no study which observed horizontally directed NF.

While the NF is usually located on the anteromedial surface of the humerus in the studies, it can also be observed on other borders and surfaces of the humerus [1, 10, 11, 13, 15, 16, 18]. In the studies conducted by Ehab Kamal Ali [15], Mansur et al. [16], Singh et al. [1], Cihan and Toma [10] and Kumar et al. [20] no NF was detected on the anterior border, as is the case in our study. No NF was observed to be located on the anterolateral surface and lateral border by Öztürk et al. [11], and Ehab Kamal Ali [15] and Mansur et al. [16] have not observed any NF on the medial and lateral border, and no NF was observed on the lateral border according to the study by Arfan et al. [13] (Table 3.).

NFs are usually localized in the middle 1/3 of the humerus, there may also be NFs localized on the proximal 1/3 and distal 1/3 [1, 10, 11, 13, 14, 16]. Unlike other studies, no NF was detected on the proximal 1/3 of the humerus in the studies performed by Poudel and Satyal [18], Kumar et al. [20] and Cihan and Toma [10] (Table 3).

Table 3. Comparison of the morphologic properties of nutrient foramen

Studies	Population	Sample size	Number of NF n(%)	Direction of NF n(%)	Localization of NF n(%)	Position of NF n(%)	Size of NF n(%)
Cihan and Toma (2023) [10]	Türkiye	103 (52 R, 51 L)	0: 7 (6.80) 1: 59 (57.28) 2: 24 (23.30) 3: 8 (7.77) 4: 3 (2.91) 5: 2 (1.94)	PD: - DD: 153 (100) HD: -	AM: 96 (62.7) AL: 9 (5.8) AS: 3 (1.9) PLS: 26 (16.9) PS: 18 (11.7)	P: - M: 42 (89.3) D: 5 (10.6)	
Kumar et al. (2022) [20]	India	80 (40 R, 40 L)	0: 3 (3.75) 1: 73 (91.25) 2: 3 (3.75) 3: 1 (1.25) 4: - 5: -	PD: - DD: 82 (100) HD: -	AM: 73 (89.02) AL: 8 (9.76) PS: 1 (1.22) MB: - LB: - AB: -	P: - M: 71 (86.58) D: 11 (13.42)	
Öztürk et al. (2022) [11]	Türkiye	59 (24 R, 35 L)	0: 1 (1.7) 1: 39 (66.1) 2: 16 (27.1) 3: 1 (1.7) 4: 1 (1.7) 5: 1 (1.7)	PD: 2 (2.4) DD: 81 (97.6) HD: -	AM: 38 (45.79) AL: - PS: 21 (25.30) MB: 16 (19.28) LB: - AB: 1 (1.20) ITS: 7 (8.43)	P: 9 (10.8) M: 64 (77.1) D: 10 (12.1)	
Singh et al. (2018) [1]	India	62 (32 R, 30 L)	0: - 1: 38 (61.29) 2: 19 (30.65) 3: 5 (8.06)	PD: - DD: 91 (100) HD: -	AM: 50 (54.95) AL: 11 (12.08) PS: 11 (12.08) MB: 18 (19.78) LB: 1 (1.09) AB: -	P: 2 (2.19) M: 86 (94.5) D: 3 (3.3)	
Mansur et al. (2016) [16]	Nepal	253 (108 R, 143 L)	0: 5 (1.98) 1: 154 (60.87) 2: 73 (28.85) 3: 16 (6.32) 4: 5 (1.98)	PD: - DD: 368 (100) HD: -	AM: 327 (88.86) AL: 17 (4.62) PS: 24 (6.52) MB: - LB: - AB: -	P: 2 (0.54) M: 349 (94.84) D: 17 (4.62)	

Ehab Kamal Ali (2021) [15]	Egypt	250 (125 R, 125 L)	0: 5 (2) 1: 210 (84) 2: 35 (14)		AM: 171 (61) AL: 28 (10) PS: 81 (29) MB: - LB: - AB: -		
Pereira et al. (2011) [17]	Brasil	174	0: - 1: 154 (88.5) 2: 20 (11.5)				
Arfan et al. (2022) [13]	India	86 (40 R, 46 L)	0: 4 (4.65) 1: 52 (60.40) 2: 25 (29.06) 3: 5 (5.81)	PD: - DD: 117 (100) HD: -	AM: 64 (55.17) AL: 3 (2.58) PS: 20 (17.24) MB: 27 (23.27) LB: - AB: 2 (1.72)	P: 8 (6.89) M: 103 (88.79) D: 5 (4.31)	0.814+-0.213 mm
Poudel and Satyal (2019) [18]	Nepal	50 (29 R, 21 L)	0: 2 (4) 1: 40 (80) 2: 8 (16)	PD: - DD: 56 (100) HD: -	AM: 44 (88) AL: 1 (2) PS: 2 (4) MB: LB: AB:	P: - M: 41 (82) D: 5 (10)	
Desai and Damor (2022) [14]	India	93 (44 R, 49 L)	0: 10 (10.8) 1: 69 (74.1) 2: 14 (15.1)	PD: - DD: 97 (100) HD: -		P: 1 (1.03) M: 92 (94.85) D: 4 (4.12)	
This study	Türkiye	113 (58 R, 55 L)	0: 9 (7.96) 1: 86 (76.11) 2: 17 (15.05) 3: 1 (0.88)	PD: - DD: 122 (99.19) HD: 1 (0.81)	AM: 86 (69.92) AL: 4 (3.25) PS: 12 (9.76) MB: 18 (14.63) LB: 3 (2.44) AB: -	P: 5 (4.07) M: 113 (91.86) D: 5 (4.07)	14G: - 16G: - 18G: 8.94 20G: 40.65 22G: 31.71 24G: 18.70

R: right, L: left, NF: nutrient foramen, n: number, PD: proximally directed, DD: distally directed, HD: horizontally directed, AM: anteromedial, AL: anterolateral, PS: posterior surface, AS: anterior surface, PLS: posterolateral surface, MB: medial border, LB: lateral border, AB: anterior border, ITS: intertubercular sulcus, P: proximal, M: middle, D: distal, G: gauge

Table 4. Comparison of the morphometric properties of nutrient foramen

Studies	Population	Sample size	TL (mm)	DNF (mm)	FI (%)
Cihan and Toma (2023) [10]	Türkiye	103 (52 R, 51 L)	R: 304.39±20.04 L: 303.54±20.22	R: 172.49±23.17 L: 166.68±25.26	55.77
Kumar et al. (2022) [20]	India	80 (40 R, 40 L)	301.1±19.4	166.5±12.2	55.53
Öztürk et al. (2022) [11]	Türkiye	59 (24 R, 35 L)	311.33±30.9	160.92±45.67	52.39±14.84
Mansur et al. (2016) [16]	Nepal	253 (108 R, 143 L)	270.22	149.71	55.20
Arfan et al. (2022) [13]	India	86 (40 R, 46 L)	304.22±21.98		56.83±7.80
Poudel and Satyal (2019) [18]	Nepal	50 (29 R, 21 L)	297.09	142.95	48.12
Desai and Damor (2022) [14]	India	93 (44 R, 49 L)	R: 300.8±17.8 L: 302.3±20.8	R: 185.8±40.5 L: 187.8±48.3	R: 53.27 L: 49.96
This study	Türkiye	113 (58 R, 55 L)	301.68±**20.61**	166.70±**32.50**	55.33±**9.48**

R: right, L: left, TL: total length of humerus, DNF: distance between nutrient foramen and proximal end of the humerus, FI: foraminal index

There are only a few studies which measured the diameter of the NF. Arfan et al. [13] measured the mean diameter of the foramen as 0.814+-0.213 mm. In our study, majority of NFs were sized at 20G (1.1 mm) (Table 3.). The diameter of the NF may provide us necessary information about the diameter of the nutrient artery.

The mean TL was measured as 301.68**±20.61 mm in our study which is similar to the studies of** Cihan and Toma [10] in Türkiye (right: 304.39±20.04 mm, left: 303.54±20.22 mm), Kumar et al. [20] in India (301.1±19.4 mm), Öztürk et al. [11] in Türkiye (311.33±30.9 mm), Arfan et al. [13] in India (304.22±21.98 mm), Desai and Damor [14] in India (right: 300.8±17.8 mm, left: 302.3±20.8 mm) and higher than the studies of Mansur et al. [16] in Nepal (270.22 mm) and Poudel and Satyal [18] in Nepal (297.09 mm). The mean DNF was measured 166.70**±32.50 mm in our study which is in accordance to the studies of Kumar et al.** [20] (166.5±12.2 mm), Öztürk et al. [11] (160.92±45.67 mm), Cihan and Toma [10] (right: 172.49±23.17 mm, left: 166.68±25.26 mm), lower than that of Desai and Damor [14] (right: 185.8±40.5 mm, left: 187.8±48.3 mm) and higher than that of Mansur et al. [16] (149.71 mm) and Poudel and Satyal [18] (142.95 mm). The mean FI was calculated in our study as 55.33**±9.48%, which is in accordance to the studies of** Cihan and Toma [10] (55.77%), Kumar et al. [20] (55.53%), Mansur et al. [16] (55.20%), Arfan et al. [13] (56.83±7.80%) and higher than that of Öztürk et al. [11] (52.39±14.84%), Poudel and Satyal [18] (48.12%), and Desai and Damor [14] (right: 53.27%, left: 49.96%). The foraminal index indicates where the NF is located from the proximal to the distal parts of the humerus. Foraminal index values found in studies show us that the NF is generally localized on the middle 1/3 of the humerus. Comparison of the morphologic and morphometric properties of NFs were summarized in Table 3. and Table 4.

Limitations

This study has certain limitations. The age and sex of the bones were unknown. Due to such absence of information, the age and sex differences for NF could not be evaluated. In addition, the sample size was limited to 113 bones. Further studies may be performed with larger sample size and known age and sex of the bones.

CONCLUSION

In conclusion of our study, it is found out that there is usually one NF on the humerus and that this foramen is directed distally, localized on the anteromedial surface and the middle 1/3 of the humerus and sized at 20G. And also, we observed horizontally directed NF on the humerus. To our knowledge, there is no study which observed horizontally directed NF. There may be differences between populations about the morphology and morphometry of NF. Therefore, being aware for the morphology, morphometry and variations of the NF is important for the orthopaedic surgeons in surgeries such as fracture repairing and vascularized bone graft in order to avoid damaging the nutrient arteries.

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REFERENCES

[1] Singh V, Saxena AK, Aneja PS, Mahato P (2018) A Study on Variations of Nutrient Foramen of Humerus and Its Clinical Significance. Int J Med Res Prof. 4(5):255-258. https://doi.org/10.21276/ijmrp

[2] Bhojaraja VS, Kalthur SG, Dsouza AS (2014) Anatomical study of diaphyseal nutrient foramina in human adult humerus. Arch Med Health Sci. 2(2):165-169. https://doi.org/10.4103/2321-4848.144325

[3] Pasli B, Gündoğdu HY, Abbasoğlu TT, Güneş BE (2023) Morphometric and topographic evaluations of principal nutrient foramina of the ilium. Turk J Med Sci. 53(1):243-252. https://doi.org/10.55730/1300-0144.5579

[4] Ülkir M, Karaçoban L, Günes BE (2023) Morphologic and Morphometric Evaluation of Nutrient Foramina of Tibia. J Anat Soc India. 72(3):239-245. https://doi.org/10.4103/jasi.jasi_87_22

[5] Khandve B, Verma A (2018) A study of nutrient foramina of humerus at chhattisgarh state. Int J Inn Res Med Sci. 3(3):1794-1798. https://doi.org/10.23958/ijirms/vol03-i03/04

[6] Asharani S, Ningaiah A (2016) A study on the nutrient foramen of humerus. Int J Anat Res. 4(3):2706-2709. https://doi.org/10.16965/ijar.2016.312

[7] Azizi J, Danish H, Kabul A (2019) Anatomical study of nutrient foramen in the long bones of upper extremities. Int J Eng Appl Sci Technol. 4(6):7-10.

[8] Yaseen S, Nitya W, Ravinder M (2014) Morphological and topographical study of nutrient foramina in adult humerii. Int J Innov Res Sci Eng Technol. 3(4):7-10.

[9] Mishra AK, Jaiswal S, Sharma P, Tewari V (2018) The Study Of Nutrient Foramen Of Bones Of Arm And Forearm In Adults. EJMR. 5(1):28-31. https://doi.org/10.24041/ejmr2018.59

[10] Cihan ÖF, Toma S (2023) Morphology and Topography of the Nutrient Foramina in the Shoulder Girdle and Long Bones of the Upper Extremity. Eur J Ther. 29(3):359-369. https://doi.org/10.58600/eurjther.20232902-395.y

[11] Öztürk K, Dursun A, Ayazoğlu M, Kastamoni Y (2022) An Investigation of the Nutrient Foramen in the Long Bones of the Upper and Lower Limbs in Turkish Population. Med Records. 4(2):179-186. https://doi.org/10.37990/medr.1049282

[12] Hughes H (1952) The factors determining the direction of the canal for the nutrient artery in the long bones of mammals and birds. Acta Anat (Basel). 15(3):261-280. https://doi.org/10.1159/000140748

[13] Arfan N, Suresh N, Suma M (2022) A morphometric study on variations of nutrient foramen of humerus with its clinical implication. Indian J Clin Anat Physiol. 9(1):29-34. https://doi.org/10.18231/j.ijcap.2022.008

[14] Desai U, Damor B (2022) Morphometric Study of Nutrient Foramina in Typical Adult Human Long Bones of Superior Extremity with its Clinical and Surgical Importance A Cross-sectional Study. Int J Anat Radiol Surg. 11(3):14-17. https://doi.org/10.7860/IJARS/2022/43270.2796

[15] Kamal Ali E (2021) Morphometric and comparative study of nutrient foramina of femur and humerus bones and its clinical importance in Egyptian population. Sys Rev Pharm. 12(7):2387-2392.

[16] Mansur D, Manandhar P, Haque M, Mehta D, Duwal S, Timalsina B (2016) A study on variations of nutrient foramen of humerus with its clinical implications. Kathmandu Univ Med J 14(53):78-83.

[17] Pereira G, Lopes P, Santos A, Silveira F (2011) Nutrient foramina in the upper and lower limb long bones: morphometric study in bones of Southern Brazilian adults. Int J Morphol. 29(2):514-520.

[18] Poudel A, Satyal B (2019) A Study of Variation of Nutrient Foramen of Dry Adult Humerus. JNGMC. 17(1):38-42.

[19] Joshi H, Doshi B, Malukar O (2011) A study of the nutrient foramina of the humeral diaphysis. NJIRM. 2(2):4-17.

[20] Kumar S, Sinha SK, Akhtar MJ, Kumar B, Sinha RR, Kumar A, kumar Sinha S (2022) Morphometric Study of the Nutrient Foramen of the Humerus in the Population of Bihar. Cureus. 14(12):1-6. https://doi.org/10.7759/cureus.32856

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Original Research

Systemic Immune Inflammation Index and Neutrophil-to-Lymphocyte Ratio Correlate with Fasting Glucose Levels Among Type 2 Diabetic Patients

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ABSTRACT

Objective: Type 2 diabetes mellitus (T2DM) pathogenesis involves low-grade chronic inflammation, which can be measured via surrogate markers such as neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), systemic immune inflammation index (SII), and pan-immune inflammation value (PIIV). They were demonstrated to be correlated with T2DM-related outcomes, including mortality, akin to glycemic indices of fasting blood glucose and glycated hemoglobin. However, it is not clear whether a correlation exists between inflammatory markers and glycemic indices.

Methods: A retrospective study was designed. Clinical and medication variables, glycemic control variables, and complete blood count differential variables were acquired via electronic medical records. NLR, PLR, SIIV, and PIIV values were calculated. Correlation analyses between fasting blood glucose, glycated hemoglobin values, and inflammatory indices were conducted.

Results: Sixty-three patients were included in the study. The median fasting blood glucose and glycated hemoglobin levels were 115 mg/dL and 6.2%, respectively. SII and NLR significantly correlated with fasting blood glucose levels ($r = .271$, $p = .032$, and $r = .364$ $p = .003$, respectively). Although PIIV and NLR showed a correlation trend with glycated hemoglobin ($r = .238$ and $r = .236$, respectively), this correlation did not reach statistical significance ($p = .061$ and $p = .062$, respectively).

Conclusion: This study demonstrated that SII and NLR are not only associated with long-term diabetic complications but are also correlated with the cross-sectional glycemic index of fasting blood glucose. Further studies with larger patient groups have the potential to demonstrate significant correlations between inflammatory indices and glycated hemoglobin levels.

Keywords: Type 2 diabetes mellitus, Glucose, Glycated Hemoglobin, Neutrophil, Lymphocyte

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a prevalent metabolic disorder with a significant economic and health burden [1]. T2DM-related morbidity is mainly caused by microvascular complications (i.e., neuropathy, retinopathy, and diabetic kidney disease) and macrovascular complications (i.e., atherosclerotic cardiovascular disorders), and various studies indicated that glycemic control is associated with lower T2DM-related complications [2-4]. There is no gold standard methodology to assess glycemic control in T2DM, and new device-based modalities such as continuous glucose monitoring are promising; however, commonly used and widely available methods include

fasting blood glucose (FBG) and glycated hemoglobin [5]. These two methods were consistently shown to be correlated with T2DM-related outcomes, thus forming the main glycemic control targets of treatment protocols [5].

T2DM pathogenesis involves multiple mechanisms, and insulin resistance is the most widely pronounced [6] ; however, emerging data suggests that low-grade chronic inflammation drives both T2DM pathogenesis as well as T2DM-related complications [7, 8]. Since demonstration of tissue-scale inflammatory processes is not straightforward for routine clinical practice, various surrogate markers were developed to reflect multiple aspects of the innate immune system. The most commonly investigated markers are neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), systemic immune inflammation index (SII), and pan-immune inflammation value (PIIV) [9-12]. While the first two markers include the data that their names denote, SII incorporates neutrophils, platelets, and lymphocytes, whereas PIIV comprises monocyte counts in addition to SII. Previous studies indicate a correlation between these inflammatory markers and diabetes-related outcomes as well as cardiovascular and all-cause mortality among diabetic patients [13-14]. However, it is not well defined whether these inflammatory markers are also associated with glycemic control indices such as fasting blood glucose and glycated hemoglobin.

The aim of this study was to investigate the possible relationships between inflammatory markers and glycemic control indices among T2DM patients who receive diabetes treatment.

MATERIALS AND METHODS

This study was designed as a retrospective electronic medical record (EMR)-based study. Patients who were admitted to the general internal medicine outpatient clinic of the Başkent University Ankara Hospital, a tertiary-level academic hospital, between June 1, 2023, and January 1, 2024 were evaluated for eligibility. Patients over 18 years old with a diagnosis of type 2 diabetes mellitus who had clinical and laboratory data in the EMR that were required for the study were included in the study. Demographic and clinical features that are thought to have possible effects on SII, PIIV, NLR, and PLR were acquired via EMR and were as follows: age, gender, and comorbidities of hypertension, rheumatological diseases, pulmonary diseases, and malignancies. Patients who had an acute infection or rheumatological disease flare (both deduced from the clinical notes or laboratory results) were excluded from the study since both could falsely increase the aforementioned indexes and distort the data. Laboratory data that were acquired were fasting levels of blood glucose, glycated hemoglobin, total cholesterol, high and low-density lipoproteins, triglycerides, and counts of leucocytes, neutrophils, lymphocytes, monocytes, and platelets. Four indexes were calculated as follows:

- Systemic immune inflammation index: (neutrophil x platelet) / lymphocyte
- Pan-immune inflammation value: (neutrophil x platelet x monocyte) / lymphocyte
- Neutrophil-to-lymphocyte ratio: neutrophil / lymphocyte
- Platelet-to-lymphocyte ratio: platelet / lymphocyte

Statistical Analysis

Continuous variables were shown as median (interquartile range) or mean (± standard deviation), according to their distribution patterns. Categorical variables were shown as numbers (percentages). Between-group differences for categorical variables were analyzed via Pearson's Chi-squared test ($\chi 2$ test) or Fisher's exact test, if needed. Between-group differences for continuous variables were analyzed using the Student's t-test or Mann–Whitney U test, according to their distribution patterns. Relationships between continuous variables were analyzed using Spearman's correlation test, and Spearman's rho values were provided. All analyses were conducted using IBM SPSS Software version 23.0 (SPSS Inc., Chicago, IL). Two-sided significance testing was performed, and p values less than 0.05 were considered statistically significant.

Main Points:

Type 2 diabetes mellitus (T2DM) pathogenesis involves low-grade chronic inflammation. Neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), systemic immune inflammation index (SII), and pan-immune inflammation value (PIIV) are inflammatory markers and correlated with T2DM complications but it is not clear whether a correlation exists between inflammatory markers and glycemic indices. This study demonstrated that SII and NLR are correlated with fasting blood glucose in addition to previously demonstrated associations.

RESULTS

Sixty-three patients were found to be eligible to be included in the study, of whom 45 (71.4%) were female, and the median age was 66 (16). All patients except for five were on oral antidiabetic

medications, whereas 18 (28.6%) were on insulin treatment. Statins were used by 25 (39.7%) patients.

Laboratory results demonstrated well-regulated T2DM overall, with a median glycated hemoglobin level of 6.2 (1.4) % and a median glucose level of 115 (36) mg/dL. Regarding complete blood count values, neutrophil, lymphocyte, monocyte, and platelet levels were 4.2 (1.9), 2.4 (0.8), .54 (.17) and 244 (97) x109/L, respectively. The calculated inflammatory index values of NLR, PLR, SII, and PIIV were 404 (310), 231 (184), 1.69 (.83), and 99 (59), respectively. Table 1 demonstrates the demographic, clinical, and laboratory characteristics of patients in detail.

Correlation analysis demonstrated that SII and NLR values correlated with fasting blood glucose levels ($p < .05$). While spearman's rho for the correlation between PIIV and fasting blood glucose was .223, it did not reach statistical significance ($p = .079$), as did PLR. Correlation analysis between glycated hemoglobin and inflammatory indexes was not statistically significant; however, spearman's rho for PIIV and NLR were .238 and .236, respectively, with significance levels of .061 and .062, respectively. Table 2 demonstrates the correlation analysis between fasting blood glucose levels, glycated hemoglobin, and inflammatory indexes. Figure 1 shows the statistically significant correlations with a trendline.

Mann-Whitney-U analysis to analyze whether inflammatory indexes differed with regards to gender, underlying comorbidities, insulin, or statin treatments, but it did not reach statistically significant results (all $p > .05$). However, although the level of significance was .065, NLR levels showed a higher trend in insulin users compared to non-users (1.88 vs. 1.62). Inflammatory indexes did not show a statistically significant correlation with age as well (all $p > .05$).

Table 1. Demographic, clinical and laboratory characteristics of the patients

Feature	Value*
Demographics & Comorbidities	
Age	66 (16)
Gender (Female)	45 (71.4%)
Hypertension	50 (79.4%)
Chronic Kidney Disease	8 (12.7%)
Rheumatological	4 (6.3%)
Pulmonary	6 (8.5%)
Malignancy	4 (6.3%)
Medications	
Oral antidiabetics	58 (92.1%)
Insulin	18 (28.6%)
Statin	25 (39.7%)
Laboratory Values	
Glucose (mg/dL)	115 (36)
Glycated hemoglobin (%)	6.2 (1.4)
Leucocyte ($x10^9$/L)	7.9 (2.5)
Neutrophil ($x10^9$/L)	4.2 (1.9)
Lymphocyte ($x10^9$/L)	2.4 (0.8)
Monocyte ($x10^9$/L)	.54 (.17)
Platelet ($x10^9$/L)	244 (97)
Indexes	
Systemic immune inflammation index	404 (310)
Pan-immune inflammation value	231 (184)
Neutrophil-to-lymphocyte ratio	1.69 (.83)
Platelet-to-lymphocyte ratio	99 (59)

* Numbers in brackets are median levels for continuous variables, and percentages for categorical variables

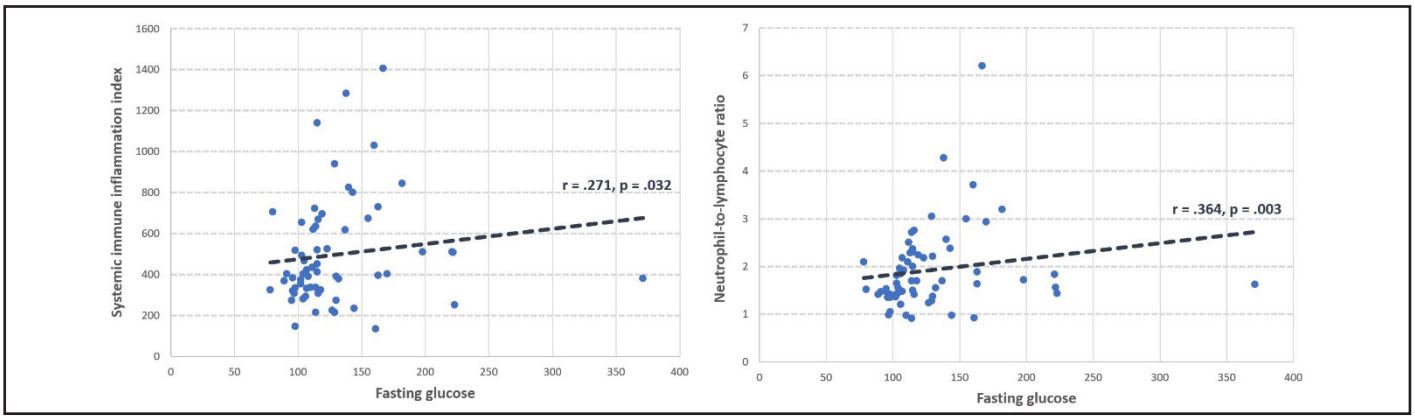

Figure 1. Correlations of systemic immune inflammation index and neutrophil-to-lymphocyte ratio to fasting glucose levels.

Table 2. Correlations between fasting glucose levels and immune indexes, values and ratios

Index	Fasting glucose	Glycated Hemoglobin
Systemic immune inflammation index	**r = .271 , p = .032**	r = .172, p = .177
Pan-immune inflammation value	r = .223, p = .079	r = .238, p = .061
Neutrophil-to-lymphocyte ratio	**r = .364, p = .003**	r = .236, p = .062
Platelet-to-lymphocyte ratio	r = -.019, p = .884	r = -.173, p =.176

Statistically significant correlations are shown in bold

DISCUSSION

This study demonstrated that extensively investigated biomarkers of systemic immune inflammation index and neutrophil-to-lymphocyte ratio values have a positive correlation with fasting blood glucose levels among patients with type 2 diabetes mellitus who receive treatment. This is of particular importance since various studies investigating this relationship have shown associations with long-term outcomes; however, this study described that SII and NLR are correlated with fasting glucose levels even in cross-sectional cohorts.

Various studies have indicated that systemic, low-grade inflammation among T2DM patients is associated with the development of chronic diabetic complications [15]. High-sensitive C-reactive protein (hs-CRP) is an inflammatory marker that is used to define the level of low-grade systemic inflammation. It has been shown that hs-CRP is associated with cardiovascular complications and all-cause mortality [16]. Similarly, the ADVANCE study demonstrated that the pro-inflammatory cytokine interleukin-6 (IL-6) was an independent predictor of macrovascular complications and mortality among diabetic patients [17]. However, both hs-CRP and IL-6 are not widely available, are costly, and are source-consuming; therefore, more feasible approaches are needed to define the level of low-level chronic systemic inflammation. These efforts have led to the emergence of various complete blood count parameter-derived inflammatory markers, of which NLR, PLR, SII, and PIIV are the most extensively studied among both diabetics and non-diabetics. They were first developed for prognosis prediction among malignancies; however, subsequent studies indicated that they are not only associated with outcomes among patients with malignancies but are also associated with outcomes among diabetic patients [13-14].

There are few studies investigating the correlation of inflammatory markers with glycemic indices among diabetic patients. Determination of glycemic control is of paramount importance since treatment intensifications are formed according to fasting blood glucose and glycated hemoglobin levels. Despite their wide use, they have their own limitations; thus, newer methodologies such as continuous blood glucose monitoring systems (CGMS) are being developed to overcome these limitations, but CGMSs have their own constraints as well, such as cost and availability [18]. Considering the limitations of the current glycemic indices and taking into account the disturbances occurring in diabetic patients, pathophysiology-based markers associated with glycemic control are needed. This study has shown that widely available inflammatory markers are associated with fasting blood glucose levels among diabetic patients and have development potential among diabetic patient subgroups, provided that larger patient numbers are involved.

Limitations

This study's limitations are acknowledged. Firstly, this study includes a retrospective cohort, making it prone to biases related to retrospective studies. Secondly, patient number was not high; hence, several correlations could falsely lack significance, such as lack of correlation between PIIV and fasting blood glucose (p = .079), PIIV and glycated hemoglobin (p = .061), as well as NLR and glycated hemoglobin (p = .062). Thirdly, several determinants that could have an effect on the indexes, such as diabetes duration and anti-diabetic medication types, were not evaluated.

CONCLUSIONS

The correlation between fasting blood glucose levels and systemic immune inflammation values, as well as the neutrophil-to-lymphocyte ratio, reflects the low-grade chronic inflammation in type 2 diabetes. Whether these indexes have a role as treatment target markers, such as fasting blood glucose levels or glycated hemoglobin, should be tested in further prospective studies.

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REFERENCES

[1] Sun H, Saeedi P, Karuranga S, Pinkepank M, Ogurtsova K, Duncan BB, Stein C, Basit A, Chan JCN, Mbanya JC, Pavkov ME, Ramachandaran A, Wild SH, James S, Herman WH, Zhang P, Bommer C, Kuo S, Boyko EJ, Magliano DJ (2022) IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. Diabetes Res Clin Pract. 183:109119. https://doi.org/10.1016/j.diabres.2021.109119

[2] American Diabetes Association Professional Practice Committee (2024) 10. Cardiovascular Disease and Risk Management: Standards of Care in Diabetes-2024. Diabetes Care. 47(Suppl 1):S179-S218. https://doi.org/10.2337/dc24-S010

[3] American Diabetes Association Professional Practice Committee (2024) 11. Chronic Kidney Disease and Risk Management: Standards of Care in Diabetes-2024. Diabetes Care. 47(Suppl 1):S219-S230. https://doi.org/10.2337/dc24-S011

[4] American Diabetes Association Professional Practice Committee (2024) 12. Retinopathy, Neuropathy, and Foot Care: Standards of Care in Diabetes-2024. Diabetes Care. 47(Suppl 1):S231-S243. https://doi.org/10.2337/dc24-S012

[5] American Diabetes Association Professional Practice Committee (2024) 6. Glycemic Goals and Hypoglycemia: Standards of Care in Diabetes-2024. Diabetes Care. 47(Suppl 1):S111-S125. https://doi.org/10.2337/dc24-S006

[6] Dimas AS, Lagou V, Barker A, Knowles JW, Mägi R, Hivert MF, Benazzo A, Rybin D, Jackson AU, Stringham HM, Song C, Fischer-Rosinsky A, Boesgaard TW, Grarup N, Abbasi FA, Assimes TL, Hao K, Yang X, Lecoeur C, Barroso I, Bonnycastle LL, Böttcher Y, Bumpstead S, Chines PS, Erdos MR, Graessler J, Kovacs P, Morken MA, Narisu N, Payne F, Stancakova A, Swift AJ, Tönjes A, Bornstein SR, Cauchi S, Froguel P, Meyre D, Schwarz PE, Häring HU, Smith U, Boehnke M, Bergman RN, Collins FS, Mohlke KL, Tuomilehto J, Quertemous T, Lind L, Hansen T, Pedersen O, Walker M, Pfeiffer AF, Spranger J, Stumvoll M, Meigs JB, Wareham NJ, Kuusisto J, Laakso M, Langenberg C, Dupuis J, Watanabe RM, Florez JC, Ingelsson E, McCarthy MI, Prokopenko I, and MAGIC Investigators (2014) Impact of type 2 diabetes susceptibility variants on quantitative glycemic traits reveals mechanistic heterogeneity. Diabetes. 63(6):2158-2171. https://doi.org/10.2337/db13-0949

[7] Donath MY, Shoelson SE (2011) Type 2 diabetes as an inflammatory disease. Nat Rev Immunol. 11(2):98-107. https://doi.org/10.1038/nri2925

[8] Pérez-Morales RE, Del Pino MD, Valdivielso JM, Ortiz A, Mora-Fernández C, Navarro-González JF (2019) Inflammation in Diabetic Kidney Disease. Nephron. 143(1):12-16. https://doi.org/10.1159/000493278

[9] Fucà G, Guarini V, Antoniotti C, Morano F, Moretto R, Corallo S, Marmorino F, Lonardi S, Rimassa L, Sartore-Bianchi A, Borelli B, Tampellini M, Bustreo S, Claravezza M, Boccaccino A, Murialdo R, Zaniboni A, Tomasello G, Loupakis F, Adamo V, Tonini G, Cortesi E, de Braud F, Cremolini C, Pietrantonio F (2020) The Pan-Immune-Inflammation Value is a new prognostic biomarker in metastatic colorectal cancer: results from a pooled-analysis of the Valentino and TRIBE first-line trials. Br J Cancer. 123(3):403-409. https://doi.org/10.1038/s41416-020-0894-7

[10] Hu B, Yang XR, Xu Y, Sun YF, Sun C, Guo W, Zhang X, Wang WM, Qiu SJ, Zhou J, Fan J (2014) Systemic immune-inflammation index predicts prognosis of patients after curative resection for hepatocellular carcinoma. Clin

Cancer Res. 20(23):6212-6222. https://doi.org/10.1158/1078-0432.CCR-14-0442

[11] Balta S, Ozturk C (2015) The platelet-lymphocyte ratio: A simple, inexpensive and rapid prognostic marker for cardiovascular events. Platelets. 26(7):680-681. https://doi.org/10.3109/09537104.2014.979340

[12] Zahorec R (2021) Neutrophil-to-lymphocyte ratio, past, present and future perspectives. Bratisl Lek Listy. 122(7):474-488. https://doi.org/10.4149/BLL_2021_078

[13] Guo W, Song Y, Sun Y, Du H, Cai Y, You Q, Fu H, Shao L (2022) Systemic immune-inflammation index is associated with diabetic kidney disease in Type 2 diabetes mellitus patients: Evidence from NHANES 2011-2018. Front Endocrinol (Lausanne). 13:1071465. https://doi.org/10.3389/fendo.2022.1071465

[14] Dong G, Gan M, Xu S, Xie Y, Zhou M, Wu L (2023) The neutrophil-lymphocyte ratio as a risk factor for all-cause and cardiovascular mortality among individuals with diabetes: evidence from the NHANES 2003-2016. Cardiovasc Diabetol. 22(1):267. https://doi.org/10.1186/s12933-023-01998-y

[15] Yamamoto Y, Yamamoto H (2013) RAGE-Mediated Inflammation, Type 2 Diabetes, and Diabetic Vascular Complication. Front Endocrinol (Lausanne). 4:105. https://doi.org/10.3389/fendo.2013.00105

[16] Lin K, Lan Y, Wang A, Yan Y, Ge J (2023) The association between a novel inflammatory biomarker, systemic inflammatory response index and the risk of diabetic cardiovascular complications. Nutr Metab Cardiovasc Dis. 33(7):1389-1397. https://doi.org/10.1016/j.numecd.2023.03.013

[17] Lowe G, Woodward M, Hillis G, Rumley A, Li Q, Harrap S, Marre M, Hamet P, Patel A, Poulter N, Chalmers J (2014) Circulating inflammatory markers and the risk of vascular complications and mortality in people with type 2 diabetes and cardiovascular disease or risk factors: the ADVANCE study. Diabetes. 63(3):1115-1123. https://doi.org/10.2337/db12-1625

[18] American Diabetes Association Professional Practice Committee (2024) 7. Diabetes Technology: Standards of Care in Diabetes-2024. Diabetes Care. 47(Suppl 1):S126-S144. https://doi.org/10.2337/dc24-S007

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Original Research

Effect of Vitamin K2 on Blood Rheology and Vascular Responses in Diabetic Rats

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ABSTRACT

Objective: Diabetes is manifested by endothelial dysfunction and an imbalance between vasoconstriction and vasodilation. The aim of our study is to examine the effect of vitK2 application on vascular and rheological parameters in a rat diabetes model.

Materials and Methods: A total of 60 male Wistar Albino rats were used to examine vascular responses and hemorheological parameters. A total of 6 groups were: control (C), control+vehicle (Cv), control+vitK2 administered (C + K2), diabetes (D), diabetes+vehicle (Dv), and diabetes+vitK2 (D + K2) group. After the animals were sacrificed, blood and vascular samples were taken and the contraction and relaxation responses of the aorta and erythrocyte deformability and aggregation were examined.

Results: When KCl dose-response curves are evaluated; Increased vasoconstriction response was found in the Dv group compared to the Cv group. The increase in vasoconstriction observed in the Dv group decreased with the application of vit K2. D+vitK2 group thoracic aorta contraction responses returned to Cv group levels. In response to increasing cumulative doses of Phe, a significant increase in vasoconstriction response was observed in the Dv group compared to the Cv group. VitK2 application reduced the Phe-mediated contractile response, which was increased in the Dv group, and returned the contraction response to Cv conditions except for two intermediate Phe doses. In the Dv + K2 group, a significant decrease was observed in the aggregation index, which was tended to increase.

Conclusion: Considering the cardiovascular complications frequently observed in diabetes, it can be suggested that vitK2 therapy may yield positive outcomes in diabetes.

Keywords: Diabetes Mellitus, Vitamin K2, Menaquinone-7, Endothelial dysfunction

INTRODUCTION

Type 2 diabetes mellitus (T2DM) occurs with consequences such as microvascular (retinopathy, nephropathy and neuropathy) and macrovascular (cardiovascular diseases) complications, hyperglycemia and insulin resistance [1]. Diabetes mellitus (DM) is characterized by endothelial dysfunction and decreased nitric oxide (NO) bioavailability [2,3]. Endothelial dysfunction is a critical and initiating factor in the formation of diabetic vascular complications.

Endothelium stimulates the relaxation of vascular smooth muscle by secreting vasodilator substances. The important relaxing factors are; NO, prostacyclin and endothelium-derived hyperpolarizing factor (EDHF). The functional importance of the three substances varies depending on their location. While NO can be effective in large-diameter arteries, EDHF can be a vasodilator in smaller-diameter blood vessels or in some arteries such as coronary arteries when NO release is stopped [4].

The endothelium plays an important role in regulating vascular tone by synthesizing and releasing substances such as prostaglandin I_2 and EDHF [5]. Endothelial dysfunction is an expected finding associated with vascular damage in DM and depends in part on the balance between oxidative stress and NO. Endothelial dysfunction results in decreased NO bioavailability. Changes in NO release are important as they play a major role in the disruption of vascular homeostasis and the development of endothelial dysfunction associated various cardiovascular disorders [6,7].

Vitamin K (vitK) corresponds to 3 separate compounds: vitK1 (phylloquinone), vitK2 (menaquinones) and vitK3 (menadione), which contain methylated naphthoquinone [8]. It functions in the carboxylation of glutamic acid (Gla) residues of proteins that require vitK for their activation. Known as a cofactor for clotting factors such as prothrombin. It also carboxylate some proteins such as osteocalcin [9]. It is stated that osteocalcin concentrations may affect insulin sensitivity and T2DM by regulating the expression of insulin genes and cell proliferation markers. Osteocalcin has been shown to increase insulin secretion and insulin sensitivity and reduce the severity of T2DM in mice [10]. Uncarboxylated osteocalcin increased cell proliferation, insulin secretion, and insulin sensitivity by stimulating adiponectin expression. However, it has been reported in the literature that vitK supplementation (vitK1 and vitK2), which causes a decrease in uncarboxylated osteocalcin levels and an increase in carboxylated osteocalcin, reduces insulin resistance in patients with high risk of T2DM [11].

Main Points:

- Endothelial dysfunction and decreased nitric oxide bioavailability have been observed in rats with type 2 diabetes.
- Vit K2 treatment has reduced increased vasoconstriction induced by phenylephrine and restored the contractile response to normal levels in diabetic rats.
- Vit K2 has reduced increased erythrocyte deformability and aggregation in diabetic rats.
- Vit K2 may be considered a potential therapeutic option in diabetes management.

VitK2 is generally of microbial origin. Important food sources are cheese, curd and natto (a traditional Japanese dish made from fermented soybeans) [12]. It has functions such as protecting the liver and nerves, preventing cardiovascular calcification, relieving menopausal symptoms, bone homeostasis, cognition and energy production. It is effective in reducing the risk of developing T2DM [13]. Many diseases accompanied by inflammation (inflammatory bowel disease, pancreatitis, chronic kidney disease and osteoporosis) are linked to vitK deficiency. It also contributes to endothelial health by reducing inflammation markers [13]. Low dose vitK2 positively affects endothelium-dependent vasodilation and NO release [14]. VitK2 is stored in most tissues; but relatively high in the brain, kidneys and pancreas [15]. Administration of vitK2 increases insulin sensitivity [16]. Its deficiency is responsible for the “calcium paradox”, characterized by low calcium deposition in bone and its deposition in the vascular wall [17]. Based on this information, we aimed to examine the effect of vitK2 application on vascular response and rheological parameters in our study.

MATERIALS AND METHODS

Total of 60 male Wistar Albino rats were used in the experiments examining vascular responses and hemorheological parameters carried out in the laboratories of Akdeniz University Faculty of Medicine, Department of Anatomy and Physiology. Akdeniz University Animal Experiments Local Ethics Committee approval was received (Application Form ID/Protocol no; 1036/2020.02012). The rats were kept in a room at 23 ± 2°C with a 12-hour daylight - 12-hour dark period and were fed with

rat chow without restriction. The animals were left for a one-week adaptation period before being taken to the experimental procedure, and their blood glucose levels were measured during this period to ensure that they had blood glucose values within normal limits. Weekly water and feed consumption and weight measurements of all rats were made until they were sacrified (Table 1 and Table 2).

Control group, (C): Rats in this group were used to determine their normal morphological and physiological characteristics.
Control+Vehicle, (Cv): Rats in this group received an intraperitoneal injection of citrate buffer solution (0.1M, pH 4.5). Since v itK2 is soluble in corn-oil, citrate-buffered corn oil (0.2 ml per 100 g body) was applied via gavage.

Control+VitK2, (C+K2): VitK2 in corn-oil was given at a dose of 35 mg/kg via gavage, 5 days a week, once a day for 8 weeks.
Diabetes group, (D): A single dose of streptozotocin injection (50 mg/kg, dissolved in 10 mmol/L citrate buffer, pH 4.5) was administered intraperitoneally.

Diabetes+Vehicle, (Dv): A single dose of streptozotocin injection (50 mg / kg, dissolved in 10 mmol / L citrate buffer, pH 4.5) was administered intraperitoneally. Since vitK2 is soluble in corn-oil, citrate-buffered corn-oil (0.2 ml per 100 g body) was administered via gavage.

Diabetes+Vit K2, (D+K2): One week after a single dose of intraperitoneal streptozotocin injection, Vit K2 in corn oil was administered at a dose of 35 mg/kg via gavage, 5 days a week, once a day for 8 weeks.

Table 1. Average and standard deviation values of daily feed (g) and water (ml) consumed by the groups. C: control, Cv: control+vehicle, C+K2: control+vit K2, D: diabetes, Dv: diabetes+vehicle, D+K2: diabetes+vit K2.

		Daily feed (g)		Daily water (ml)	
		Mean	Std. deviation	Mean	Std. deviation
Control groups	C	65,59	13,88	58,59	7,74
	Cv	69,06	18,58	53,59	6,64
	C+K2	66,04	14,50	58,44	14,45
Diyabetes groups	D	77,14	22,37	182,26	79,19
	Dv	78,24	21,32	173,93	43,05
	D+K2	90,61	27,79	168,53	36,28

Table 2. Weekly average weight (g) values of the groups. C: control, Cv: control + vehicle, C + K2: control + vitK2, D: diabetes, Dv: diabetes + vehicle, D + K2: diabetes + vitK2.

		0. day	7. day	14. day	21. day	28. day	35. day	42. day	49. day
Control groups	C	226,83	245,00	249,33	260,67	266,00	274,33	280,17	290,33
	Cv	243,83	255,17	263,17	271,17	300,00	302,00	303,17	299,33
	C+K2	232,50	246,83	256,33	263,80	266,00	275,80	282,60	288,80
Diyabetes groups	D	273,42	251,55	247,18	242,64	242,09	239,18	238,09	234,55
	Dv	251,89	248,14	252,86	257,00	259,71	256,00	257,86	259,86
	D+K2	279,88	248,75	243,88	231,50	229,75	225,63	224,75	224,13

Creating Diabetes

Streptozotocin (STZ, 50 mg/kg) dissolved in citrate buffer (0.1M, pH 4.5) (Sigma-Aldrich Cat#S0130) was administered to the animals in the experimental group by intraperitoneal injection under isoflurane anesthesia. Rats with blood glucose levels of 250 mg dl^{-1} or higher were considered diabetic [18].

Application of Vitamin K2

VitK2 applications were administered via gavage at a dose of 35 mg/kg (by preparing a solution in 0.2 ml corn oil per 100 g body weight), once a day, 5 days a week for 8 weeks [19].

After measuring the blood glucose levels of the rats, their thoracic aorta tissues were placed in a cold Krebs solution. The tissue was divided into rings of appropriate length (2-5 mm) and placed in an organ bath containing 10 ml of Krebs solution, oxygenated with a gas mixture containing 95% O_2 - 5% CO_2, temperature 37ºC and pH = 7.4, to be studied.

Endothelium-dependent and independent relaxation responses and contraction responses of the thoracic aorta suspended in the organ bath were examined.

Potassium Chloride (KCl) Mediated Contractile Response: Contraction responses of the vessels to 20, 40 and 80 mM KCl were obtained.

Phenylephrine (Phe) Mediated Contractile Response: Contractile responses were obtained by administering cumulatively increasing doses of Phe (10^{-9} – $3x10^{-5}$ M) to the vessels.

Following contraction with a submaximal dose of Phenylephrine (Phe), cumulative doses of ACh (10^{-9} – $3x10^{-5}$ M) were administered to the vessels and ACh-mediated relaxation responses were obtained.

Following contraction with a submaximal dose of Phenylephrine (Phe), cumulative doses of SNP were administered to the vessels (10^{-10} – $3x10^{-4}$ M), and SNP-mediated relaxation responses were obtained.

Erythrocyte deformability was assessed by laser diffraction analysis at various fluid shear forces using an ektacytometer (LORRCA, RR Mechatronics). Erythrocyte deformability was calculated as elongation index (EI) by the system. EI values were measured between nine shear forces (0.30 – 75.02 Pa), and using these values, the shear force (SS1/2) that caused a deformation of half of the maximum EI was evaluated for each sample. Erythrocyte aggregation was measured in autologous plasma at 37ºC using a photometric aggregometer.

Statistical Analysis

Statistical evaluations were made and the results are shown as ± mean standard deviation. The significance level was accepted as 0.05. Parametric two-way and one-way analyzes of variance (ANOVA) were used for data conforming to normality distribution, and Post-Hoc Bonferroni and Tukey tests were used for pairwise comparisons. Non-parametric Kruskal-Wallis Variance Analysis was used for data that did not comply with normality distribution and Dunn Test was used for pairwise comparisons.

RESULTS

In the vehicle study applied to control (C) and diabetes (D) groups; it was shown that a single injection of citrate buffer solution (0.1M, pH 4.5) into the groups followed by corn-oil (0.2 ml per 100 g body) application for 8 weeks affected conduction type vascular functions (thoracic aorta contraction and relaxation responses). Due to the effects of STZ and Vit K2 solvents, subsequent procedures were continued with Control-vehicle (Cv) and Diabetes-vehicle (Dv) groups instead of C and D groups.

When KCl dose-response curves at 20, 40 and 80 mM concentrations are compared; While vehicle application to group C caused a decrease in the contraction response to KCl ($p<0.05$ and $p<0.01$; Figure 1a), vehicle application to group D caused an increase in the contraction response to the highest dose of KCl ($p<0.01$; Figure 1b).

When comparing Phe dose-response curves with increasing doses; Vehicle application to group C caused a decrease in the contraction response to high doses of Phe ($p<0.05$; Figure 1c). Application of vehicle to group D caused an increase in the contractile response to all Phe doses except the lowest doses ($p<0.001$; Figure 1d).

When comparing ACh dose-response curves with increasing doses; No difference in relaxation response was observed after vehicle application in group C. ($p>0,05$; Figure 2a). Application of vehicle to group D resulted in decreased relaxation responses to all ACh doses except the highest doses. ($p<0,05$ and $p<0,001$; Figure 2b).

When SNP dose-response curves are compared with increasing doses; Vehicle application to groups C and D improved the relaxation responses to low doses of SNP. (* p<0,001; Figure 2c) (#p<0,05, ##p<0,01 and ###p<0,001; Figure 2d).

Corn-oil, which is the solvent of vitK2, was applied to the Cv and Dv groups by gavage for 8 weeks, while vitK2 (0.2 ml/100 g body weight) was dissolved in corn-oil and administered by gavage to the C + K2 and D + K2 groups.

Since vehicle application caused various changes in vascular responses in both C and D groups, statistical evaluation on the effects of vitK2 on vascular responses was performed compared to vehicle groups.

When KCl dose-response curves at 20, 40 and 80 mM concentrations are evaluated; An increase in vasoconstriction response was found in the Dv group compared to the Cv group (p<0,05, p<0,01, p<0,001). The increase in vasoconstriction observed in the Dv group decreased with the application of vitK2 (p<0,01 and p<0,001). D + K2 group thoracic aorta contraction responses returned to Cv group levels (p>0,05). Administration of vitK2 to the Cv group resulted in an increase in contractile response compared to the Cv group (p<0,001) (Figure 3a).

When the dose-response curves obtained in response to increasing cumulative doses of Phe are examined; A significant increase in vasoconstriction response was observed in the Dv group compared to the Cv group (p<0,01 and p<0,001). VitK2 administration reduced the Phe-mediated contractile response, which was increased in the Dv group (p<0,01 and p<0,001). Restored the contractile response to Cv values except for two intermediate doses of Phe (p<0,05). In the C + K2 group, compared to the Cv group, there was a significant decrease in the contractile response with low doses of Phe. (p<0,001). A significant increase in contractile response was observed with high doses of Phe (p<0,01 and p<0,001) (Figure 3b).

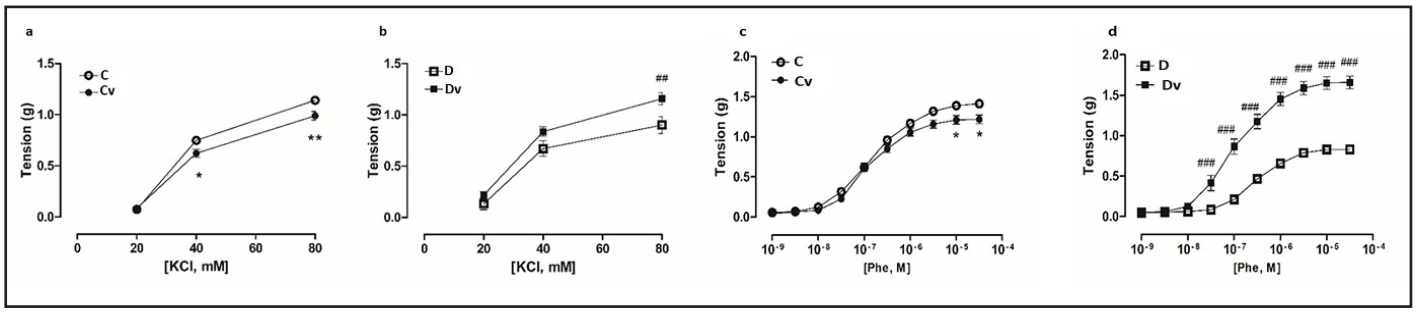

Figure 1. Contraction responses of the thoracic aorta. a) C and Cv, b) D and Dv groups KCl-mediated contraction dose-response curve, c) C and Cv d) D and Dv groups Phe-mediated contraction dose-response curve. *p<0,05, different from K; ###p<0,001, different from D.

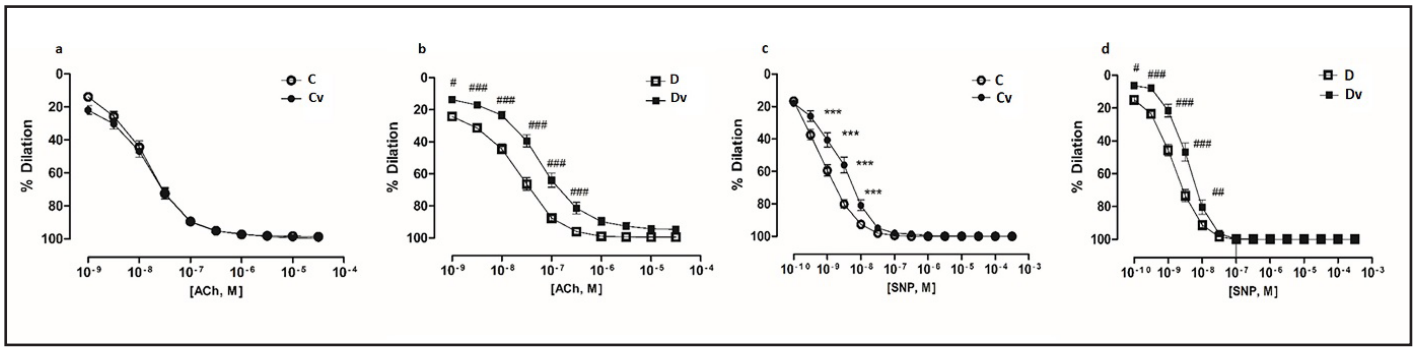

Figure 2. Relaxation responses of the thoracic aorta. a) C and Cv b) D and Dv groups ACh-mediated relaxation dose-response curve. #p<0,05 and ###p<0,001, different from D, c) C and Cv d) D and Dv groups SNP-mediated relaxation dose-response curve. ***p<0,001, different from C; #p<0,05, ##p<0,01 and ###p<0,001, different from D.

When the dose-response curves obtained in response to increasing cumulative doses of ACh are examined; A statistically significant decrease in the vasodilatation response, thus an impairment in the endothelium-mediated relaxation response, was observed in the Dv group compared to the Cv group (p<0,001). VitK2 application increased the diminished vasodilatation response in Dv group vessels (p<0,01 and p<0,001) and returned to the responses observed in the Cv group (p>0,05). Administration of vitK2 to the Cv group resulted in a decrease in relaxation responses compared to the Cv group (p<0,05, p<0,01 and p<0,001) (Figure 4a).

When the dose-response curves in response to increasing cumulative doses of SNP are compared; it was observed that there was a decrease in the relaxation response of the Dv group compared to the Cv group (p<0,05, p<0,01 and p<0,001). Application of vitK2; it was found that it corrected the decrease in the vasodilatation response observed in the Dv group and caused an increase in the relaxation response (p<0,001). The relaxation response observed in the D + K2 group was greater than in the Cv group (p<0,01 and p<0,001). Administration of Vit K2 to the Cv group increased the relaxation response to SNP at intermediate doses (p<0,001) (Figure 4b).

In the diabetes group compared to the control group; an increase in vascular contraction responses and a decrease in relaxation responses were observed. VitK2 partially or completely corrected these changes in contraction and relaxation responses. Considering the cardiovascular complications that frequently occur in diabetes, it can be said that vitK2 treatment will provide positive results.

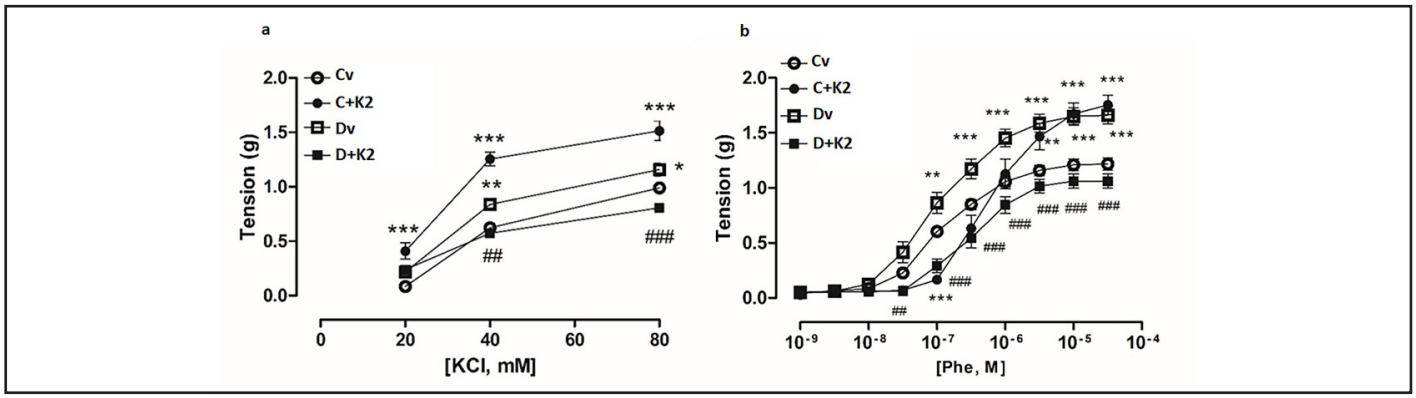

Figure 3. a) KCl-mediated contraction dose-response curve of thoracic aorta in Cv, C + K2, Dv and D + K2 groups. *p<0,05, **p<0,01 and ***p<0,001, different from Cv; ##p<0,01 and ###p<0,001, different from Dv, b) Phe-mediated contraction dose-response curve of thoracic aorta in Cv, C + K2, Dv and D + K2 groups**.** **p<0,01 and ***p<0,001, different from Cv; ##p<0,01 and ###p<0,001, different from Dv.

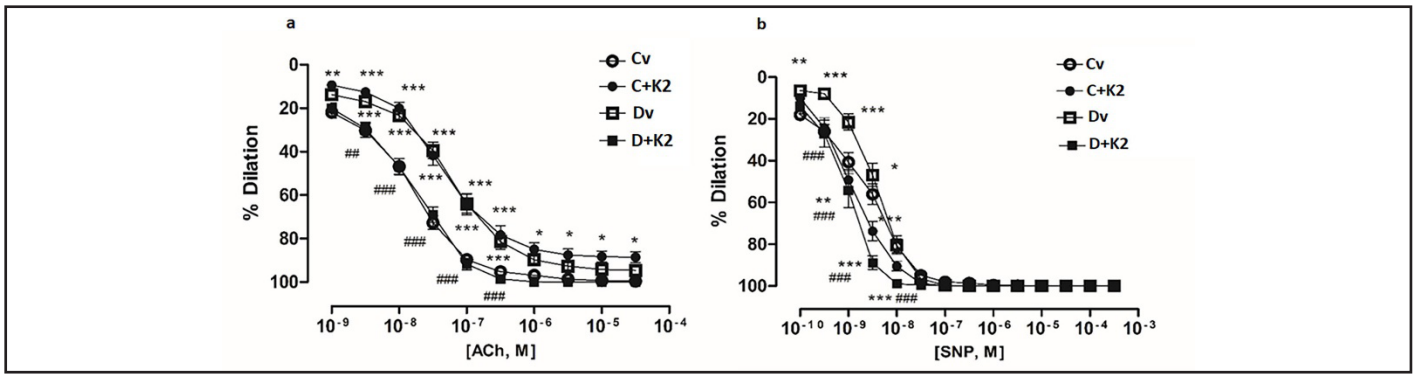

Figure 4. a) ACh-mediated relaxation dose-response curve of thoracic aorta Cv, C + K2, Dv and D + K2 groups. *p<0,05, **p<0,01 and ***p<0,001, different from Kv; ##p<0,01 and ###p<0,001, different from Dv, b) SNP-mediated relaxation dose-response curve of thoracic aorta in Cv, C+K2, Dv and D + K2 groups. *p<0,05, **p<0,01 and ***p<0,001, different from Cv; ###p<0,001, different from Dv.

The results obtained by applying vitK2 to the control group suggest that vitK2 may have different effects on vascular responses under physiological conditions. In line with these results, more studies are needed on the effects of vitK2 under physiological conditions.

In the vehicle study applied to groups C and D; it was shown that the application of corn-oil (0.2 ml per 100 g body) for 8 weeks after a single injection of citrate buffer solution (0.1M, pH 4.5) in the groups did not affect the erythrocyte responses. (Figure 5, Figure 6 and Figure 7). However, since vehicle operation affects the thoracic aorta responses; procedures were continued with Cv and Dv groups instead of C and D groups.

Erythrocyte responses were obtained in Cv and Dv groups after gavage administration of Vit K2 in corn oil (0.2 ml per 100 g body) for 8 weeks. Statistical evaluation of the effects of Vit K2 on vascular responses was performed compared to vehicle groups. Evaluation took place between Cv, C + K2, Dv and D + K2 groups.

When erythrocyte deformabilities were compared, no difference was observed in both EImax and SS 1/2 values between the groups. ($p>0,05$; Figure 8).

When the erythrocyte aggregation indexes of the groups are compared; no significant difference was observed in erythrocyte aggregation in the Dv group compared to the Cv group ($p>0.05$), but the aggregation index of erythrocytes of diabetic rats tended to increase. Application of vitK2 to the Dv group caused a significant decrease in the aggregation index, which tended to increase ($p<0,01$; Figure 9).

The fact that no difference was observed in erythrocyte deformabilities between groups may be due to the fact that erythrocytes are in an intact state. Erythrocyte aggregation increased in the diabetes group; This suggests that blood viscosity increases, resulting in increased resistance to blood flow and deterioration in vascular perfusion in these groups. Application of Vit K2 to the diabetes group had a positive effect.

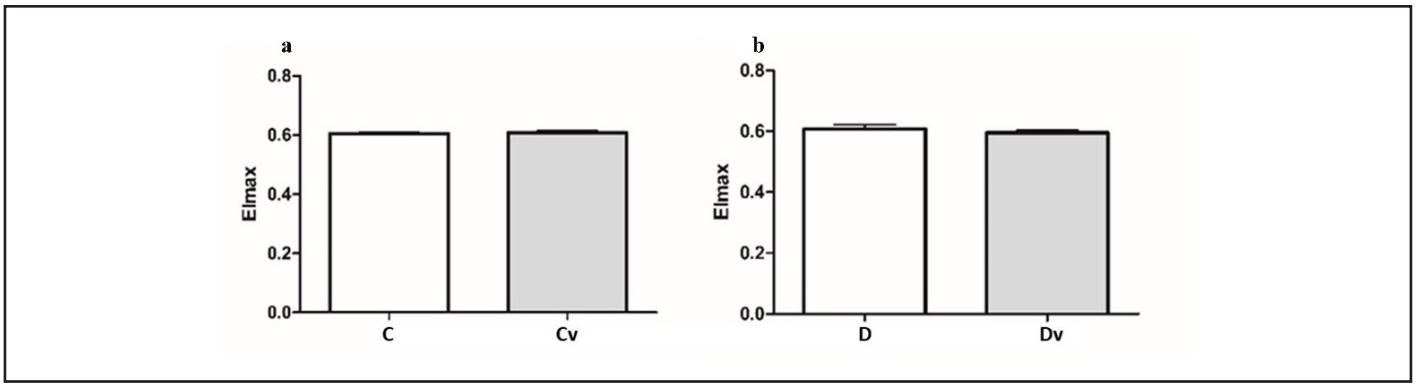

Figure 5. Erythrocyte deformability (EImax) changes a**)** C and Cv groups, b) D and Dv groups.

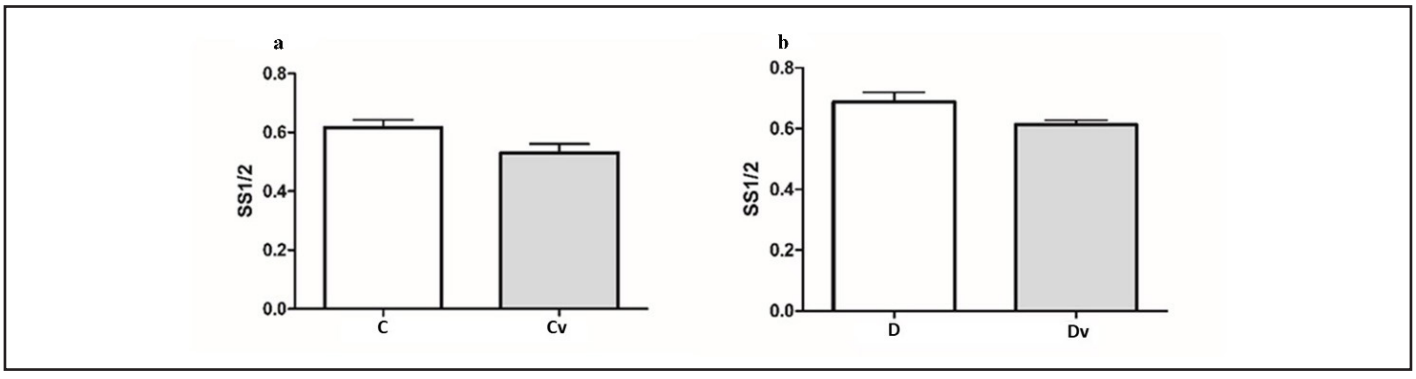

Figure 6. Erythrocyte deformability (SS1/2) changes a) C and Cv groups b) D and Dv groups.

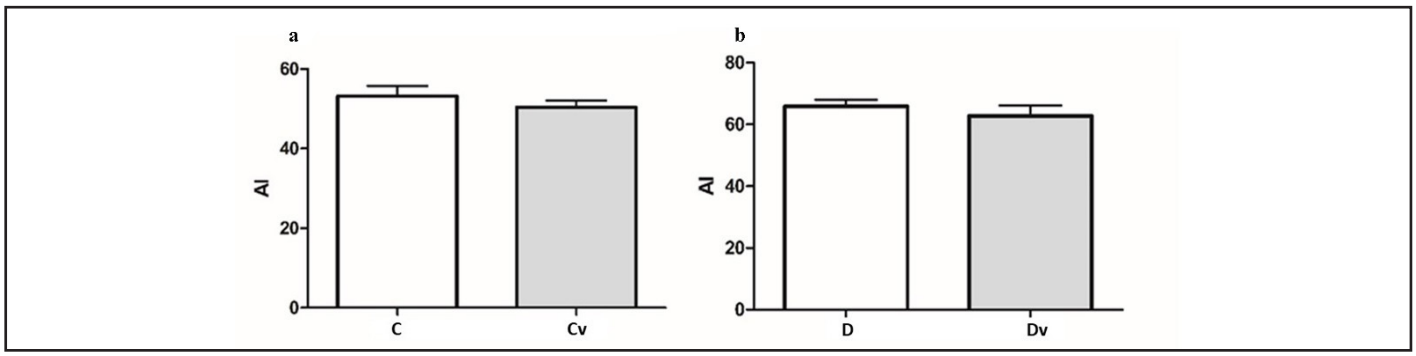

Figure 7. Erythrocyte aggregation changes a) C and Cv groups, b) D and Dv groups.

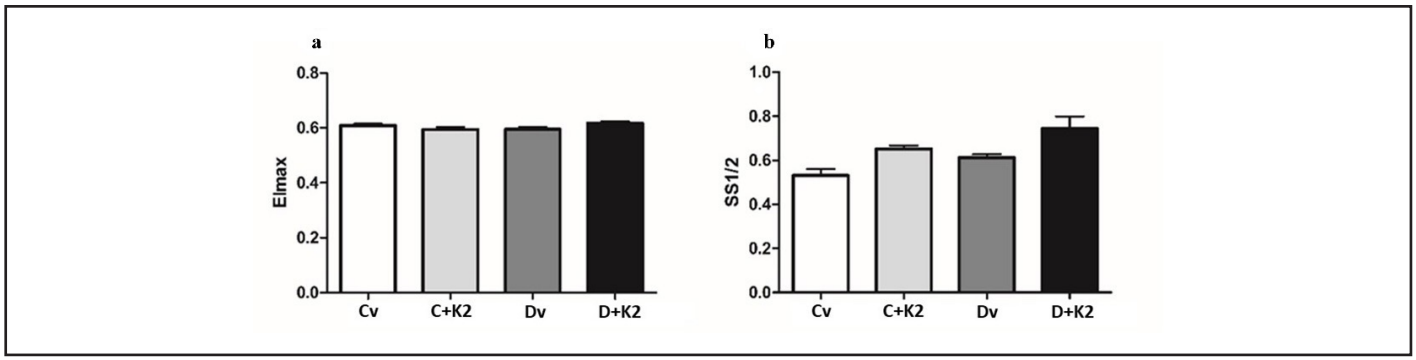

Figure 8. Erythrocyte deformability changes in Cv, C + K2, Dv and D + K2 groups a) EImax, b) SS1/2.

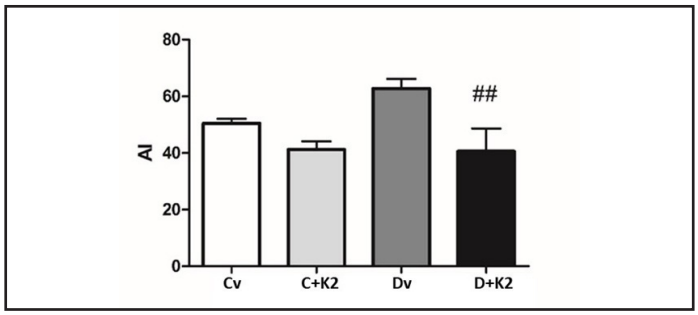

Figure 9. Changes in erythrocyte aggregation in groups Cv, C + K2, Dv and D + K2. ##p<;0,01, different from Dv.

DISCUSSION

The aim of the study was to examine the endothelium-dependent and independent relaxation responses of vitK2 on the aorta throacica, in T2DM rats. In addition, contraction responses were also examined in our study.

The literature, there are studies investigating the effects of vitK2 on intervertebral discs and fibrochondrocytes in diabetic mice, as well as its impact on glycemic status [20,21]. To the best of our knowledge, our study is the first to examine the effect of vitK2 dissolved in corn oil on the vascular endothelium in a rat diabetes model.

Gaertner et al. investigated the effect of corn oil on vascular endothelial responses, they did not find any change in the relaxation response that develops due to low concentrations of ACh in the arteria femoralis. They saw a reduction in SNP-mediated relaxation response at similar doses [22]. In our study, consistent with this study, no change was observed in the vascular relaxation response to increasing doses of ACh in the Cv group given corn oil, while a decrease in vascular relaxation responses was observed in the Dv group, except for the highest doses. Similarly, in our study, the SNP-mediated relaxation response at low doses decreased in both the Cv and Dv groups. These results suggest that the decrease is associated with endothelial dysfunction.

In a placebo-controlled study in individuals with T2DM and cardiovascular disease, the amount of calcification was measured with 18F-NaF PET and CT scans and it was evaluated whether

Vit K2 reduced calcification. In the study, they reported that vascular calcification is an active process and these techniques do not measure it, that the 18F-NaF PET method is a promising technique to detect early changes before calcifications become visible on CT, and that their study also supports that vitK2 supplementation inhibits vascular calcification [23]. Meer et al. in their study, investigated the effect of vitK2 on tendency time (T50) in T2DM individuals, based on the hypothesis that serum calcification T50 may be a new marker of arterial calcification tendency [24]. They reported that further research is needed on the effect of vitK2 supplementation on arterial calcification and the validity of T50 as a marker of arterial calcification [24]. In our study, as expected, an increase in KCl-mediated contractile response was observed in rats in the diabetes group compared to rats in the control group. We found that in vitK2 injected group to diabetic rats, the KCL-mediated endothelial contraction response decreased and returned to the control group level. This showed the positive effect of vitK2 application on vascular endothelial function. An increase in vascular endothelial response was observed in the control group administered vitK2 compared to the control group. It was observed that the vitK2 dose we applied to the diabetes group caused an increase in the vascular endothelial relaxion response. In the ACh-mediated relaxation response, a decrease in the vasodilatation response was observed in the diabetic group compared to the control group. This showed us that there was dysfunction in the endothelium-mediated relaxation response. The decreased vasodilatation response increased in the vitK2 administered group and returned to control levels.

In a study of 16,057 women, Gast et al. showed that individuals with high vitK2 intake had a lower risk of heart disease. They stated that every 10 micrograms of vitK2 consumed per day reduced the risk of heart disease by 9% [25]. A study was conducted to investigate the effects of vitK on calcification and TLR2 (Toll-like receptor 2) and TLR4 (Toll-like receptor 4) expression in aortic tissues and smooth muscle cells in ApoE-/- mice.

They showed that vitK2 could reduce the expression of TLR2 and TLR4 in the aorta and smooth muscle cell, and inhibited high-fat diet-induced intimal calcification and sodium glycerophosphate-induced smooth muscle cell calcification in ApoE-/- mice [26].

When the erythrocyte deformabilities of the groups were compared in our study, no difference was observed in both EImax and SS1/2 values between the groups. When erythrocyte aggregation indexes were compared, although no significant difference was observed in erythrocyte aggregation in the Dv group compared to the Cv group, a tendency to increase in the aggregation index of erythrocytes of diabetic rats was observed. Administration of vitK2 to the Dv group caused a significant decrease in the aggregation index, which tended to increase.

CONCLUSION

As a result, in our study, in the diabetes group compared to the control group; An increase in conduction-type vascular contraction responses and a decrease in relaxation responses were observed. VitK2 application partially or completely corrected these changes in contraction and relaxation responses. Considering the cardiovascular complications that frequently occur in diabetes, it can be said that vitK2 treatment can produce positive results in diabetes. The fact that no difference was observed in erythrocyte deformabilities suggests that this may be due to the fact that erythrocytes are in an intact state. Erythrocyte aggregation increased in the diabetes group; This suggests that blood viscosity increases, increasing resistance to blood flow and causing deterioration in vascular perfusion in these groups. Application of vitK2 to the diabetes group had a positive effect. It was observed that vitK2 administered to the subjects in the control group had a negative effect on vascular responses. The negative effect of this dose, which is curative in diabetes, on the control group showed that dose studies are needed.

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Conflict of Interest Statement

Authors have no conflicts of interest to declare.

Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethical Declaration Statement

Akdeniz University Animal Experiments Local Ethics Committee approval was received (Application Form ID/Protocol no; 1036/2020.02012). All authors should also disclose any conflict of interest that may have influenced either the conduct or the presentation of the research.

REFERENCES

[1] Hu FB (2011) Globalization of diabetes: the role of diet, lifestyle, and genes. Diabetes Care. 34(6):1249-1257. https://doi.org/10.2337/dc11-0442

[2] Avogaro A, Piarulli F, Valerio A et al (1997) Forearm nitric oxide balance, vascular relaxation, and glucose metabolism in NIDDM patients. Diabetes. 46(6):1040-1046. https://doi.org/10.2337/diab.46.6.1040

[3] Hogikyan RV, Galecki AT, Pitt B et al (1998) Specific impairment of endothelium-dependent vasodilation in subjects with type 2 diabetes independent of obesity. J Clin Endocrinol Metab. 83(6):1946-1952. https://doi.org/10.1210/jcem.83.6.4907

[4] Leung SW, Vanhoutte PM (2017) Endothelium-dependent hyperpolarization: age,gender and blood pressure, do they matter? Acta Physiol (Oxf). 219(1):108-123. https://doi.org/10.1111/apha.12628

[5] Shimokawa H (2014) 2014 Williams Harvey Lecture: importance of coronary vasomotion abnormalities-from bench to bedside. Eur Heart J. 35(45):3180-3193. https://doi.org/10.1093/eurheartj/ehu427

[6] Lobato NS, Filgueira FP, Akamine EH et al (2012) Mechanisms of endothelial dysfunction in obesity-associated hypertension. Braz J Med Biol Res. 45(5):392-400. https://doi.org/10.1590/s0100-879x2012007500058

[7] Moncada S, Higgs A (1993) The L-arginine-nitric oxide pathway. N Engl J Med. 329(27):2002-2012. https://doi.org/10.1056/NEJM199312303292706

[8] Yan Q, Zhang T, O'Connor C et al (2023) The biological responses of vitamin K2: A comprehensive review. Food Sci Nutr. 11(4):1634-1656. https://doi.org/10.1002/fsn3.3213

[9] Yamauchi T, Kamon J, Waki H, et al (2003) Globular adiponectin protected ob/ob mice from diabetes and ApoE-deficient mice from atherosclerosis. J Biol Chem. 278(4):2461-2468. https://doi.org/10.1074/jbc.M209033200

[10] Kubota N, Terauchi Y, Yamauchi T et al (2002) Disruption of adiponectin causes insulin resistance and neointimal formation. J Biol Chem. 277(29):25863-25866. https://doi.org/10.1074/jbc.C200251200

[11] Lee NK, Sowa H, Hinoi E et al (2007) Endocrine regulation of energy metabolism by the skeleton. Cell. 130(3):456-469. https://doi.org/10.1016/j.cell.2007.05.047

[12] Beulens JWJ, Booth SL, van den Heuvel EGHM et al (2013) The role of menaquinones (vitamin K) in human health. Br J Nutr. 110(8):1357-1368. https://doi.org/10.1017/S0007114513001013

[13] Mehta D, de Souza A, Jadhav SS (2022) Menaquinone-7: Wide Ranging Physiological Relevance in Muscle and Nerve Health. Vitamin K-Recent Topics on the Biology and Chemistry Eds, 2021, 57-76.

[14] Bar A, Kus K, Manterys A et al (2019) Vitamin K(2)-MK-7 improves nitric oxide-dependent endothelial function in ApoE/LDLR(-/-) mice. Vascul Pharmacol. 122-123:106581. https://doi.org/10.1016/j.vph.2019.106581

[15] Ho HJ, Komai M, Shirakawa H (2020) Beneficial effects of Vitamin K status on glycemic regulation and diabetes mellitus: A Mini-Review. Nutrients. 12(8):2485. https://doi.org/10.3390/nu12082485

[16] Choi HJ, Yu J, Choi H et al (2011) Vitamin K2 supplementation improves insulin sensitivity via osteocalcin metabolism: a placebo-controlled trial. Diabetes Care. 34(9):e147. https://doi.org/10.2337/dc11-0551

[17] Mandatori D, Pelusi L, Schiavone V et al (2021) The dual role of Vitamin K2 in "Bone-Vascular Crosstalk": opposite effects on bone loss and vascular calcification. Nutrients. 13(4):1222. https://doi.org/10.3390/nu13041222

[18] Wu KK, Huan Y (2008) Streptozotocin-induced diabetic models inmice and rats. Curr Protoc Pharmacol. 5(47). https://doi.org/10.1002/0471141755.ph0547s40

[19] Gancheva SM, Zhelyazkova-Savova MD (2016) Vitamin K2 **improves** anxiety and depression but not cognition in rats with metabolic syndrome: a role of blood glucose? Folia Med (Plovdiv). 58(4):264-272. https://doi.org/10.1515/folmed-2016-0032

[20] Mahmoud M, Kokozidou M, Gogele C et al (2023) Does Vitamin K2 **influence** the **interplay** between diabetes mellitus and **intervertebral** disc degeneration in a rat model? Nutrients. 15(13):2872. https://doi.org/10.3390/nu15132872

[21] Hussein AG, Mohamed RH, Shalaby SM et al (2018) Vitamin K(2) alleviates type 2 diabetes in rats by induction of osteocalcin gene expression. Nutrition. 47:33-38. https://doi.org/10.1016/j.nut.2017.09.016

[22] Gaertner S, Auger C, Farooq MA et al (2020) Oral intake of EPA:DHA 6:1 by middle-aged rats for one week **ımproves** age-related endothelial dysfunction in both the femoral artery and vein: role of cyclooxygenases. Int J Mol Sci. 21(3):920. https://doi.org/10.3390/ijms21030920

[23] Zwakenberg SR, de Jong PA, Bartstra JW et al (2019) The effect of menaquinone-7 supplementation on vascular calcification in patients with diabetes: a randomized, double-blind, placebo-controlled trial. Am J Clin Nutr. 110(4):883-890. https://doi.org/10.1093/ajcn/nqz147

[24] Meer R, Romero Prats ML, Vervloet MG et al (2023) The effect of six-month oral vitamin K supplementation on calcification propensity time in individuals with type 2 diabetes mellitus: A post hoc analysis of a randomized, double-blind, placebo-controlled trial. Atherosclerosis. 117307. https://doi.org/10.1016/j.atherosclerosis.2023.117307

[25] Gast GC, de Roos NM, Sluijs I, et al (2009) A high menaquinone intake reduces the incidence of coronary heart disease. Nutr Metab Cardiovasc Dis. 19(7), 504-510. https://doi.org/10.1016/j.numecd.2008.10.004

[26] Wang ZJ, Wang ZQ, Zhu J et al (2018) Vitamin K2 can suppress the expression of Toll-like receptor 2 (TLR2) and TLR4, and inhibit calcification of aortic intima in ApoE mice as well as smooth muscle cells. Vascular. 26(1):18-26. https://doi.org/10.1177/1708538117713395

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Original Research

A Comparison of Cognitive Disengagement Syndrome in Children with Major Depression Versus Attention Deficit and Hyperactivity Disorder

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ABSTRACT

Objective: There is growing evidence that Cognitive Disengagement Syndrome (CDS) is a distinct construct, supported by both empirical research and clinical differentiation. CDS demonstrates a stronger association with the inattentive type of Attention Deficit Hyperactivity Disorder (ADHD-IA), compared to its correlation with hyperactivity/impulsivity symptoms. Several studies have identified a significant positive relationship between CDS and internalizing symptoms such as anxiety and depression. To our knowledge, no study has explored CDS in children with major depression (MD). In the present study, we compared children with MD but without ADHD to children with ADHD but without depression in terms of CDS. We examined whether children with MD would have greater CDS when compared to children with ADHD.

Methods: Thirty-six adolescents with ADHD, and twenty-five adolescents with MD, aged 12 to 18 years old, were administered the Barkley Child Attention Scale (BCAS), Turgay's DSM-IV Based ADHD and Disruptive Behavior Disorders Screening Scale, and the Children's Depression Rating Scale-Revised (CDRS-R).

Results: The majority of the ADHD and MD groups were boys. The MD group had significantly higher BCAS scores than the ADHD group (U=287.0, p=0.016). In the ADHD group, the severity of BCAS scores was inversely correlated with ADHD-Hyperactivity/impulsivity scores (r=-0.681, p<0.001). In the MD group, there was a significant positive relationship between BCAS and CDRS scores (r= 0.493, p=0.012).

Conclusions: The findings of this study highlight the transdiagnostic relevance of CDS by demonstrating its association with depression, independent of ADHD. This underscores the importance of considering CDS in the assessment and treatment of depression across various clinical contexts.

Keywords: Cognitive Disengagement Syndrome; Attention Deficit and Hyperactivity Disorder; Major Depression; Children.

INTRODUCTION

The symptoms of Cognitive Disengagement Syndrome (CDS) (previously called Sluggish Cognitive Tempo, SCT) appear to be a trait that emerges early in childhood [1]. Several studies have defined the cognitive and behavioral dimensions of CDS [2, 3]. CDS is characterized by excessive daydreaming, confusion, drowsiness, absent-mindedness, being lost in one's own thoughts, and a noticeable slowing in behavior and cognitive processes [2]. Until recently, CDS has been studied primarily in the context of Attention Deficit Hyperactivity Disorder (ADHD), specifically as a symptom of the ADHD-inattentive (ADHD-IA) subtype [2, 4]. However, ample evidence suggests that CDS is a distinct entity with both empirical and clinical differentiation [5]. CDS demonstrates a stronger association with ADHD-IA compared to its correlation with hyperactivity/impulsivity symptoms [6]. Some studies specifically suggest that CDS represents a distinct entity with a unique clinical profile, separate from ADHD, implying that the relationship between CDS and ADHD involves the comorbidity of two distinct disorders [4-8]. Studies have identified a significant positive relationship between CDS and internalizing symptoms such as anxiety and depression, as well as social and academic impairment, low self-esteem, and sleep problems, even when controlling for ADHD-IA [3, 6, 9-12]. Bernad et al. [13] conducted a comprehensive 2-year longitudinal study involving a large cohort of school-aged children. The primary objective was to explore the unique predictive value of CDS in relation to various academic and emotional outcomes. The study found that higher CDS were significantly associated with increased levels of teacher-reported depression and academic impairment, independent of ADHD-IA. A recent study found that baseline CDS symptoms predicted future depression and teacher-rated anxiety [2]. Similarly, a 7-year longitudinal study showed that CDS predicted anxious/depressive behaviors, even after controlling for the effects of ADHD [14]. Moreover, a recent study revealed the predictive relationship of CDS with depressive symptoms [15]. Research has also shown that children with severe CDS tend to exhibit increased symptoms of generalized anxiety, social phobia, and obsessions than children with lower levels of CDS [16]. Children with CDS often exhibit significant challenges in various aspects of their daily lives, including social interactions and cognitive functions. Social isolation and loneliness are common among these children due to their tendency to withdraw from social situations. Additionally, they may struggle with reduced efficiency in processing information and face difficulties in managing cognitive tasks and regulating their emotions effectively [8, 17-19]. The emergence of CDS symptoms has been associated with increased activation of the behavioral inhibition system [20, 21], and conflicted shyness [8, 20, 21]. Additionally, some findings suggest that CDS is associated with poor learning strategies, an increased risk of suicide, self-reported rumination and mind wandering (MW), even after controlling for co-occurring conditions such as ADHD, anxiety, and depression [6, 17, 19, 22, 23]. It should be noted that CDS is distinct from both anxiety and depression [8]. A recent study of undergraduate students reported that having more than one other psychiatric disorder was associated with higher levels of CDS symptoms [24].

Main Points

- In this study, we aimed to investigate the associations of CDS with ADHD and depressive symptoms in order to understand unique associations with each disorder.
- In the group of ADHD patients, the severity of CDS scores was inversely correlated with ADHD-Hyperactivity/Impulsivity. In the patients with MD, CDS scores were positively correlated with depression severity.
- The findings of this study highlight the transdiagnostic relevance of CDS by demonstrating its association with depression, independent of ADHD.
- It is important to consider CDS in the assessment and treatment of depression across various clinical contexts.

Adolescents with ADHD are at significant risk of developing depression and related problems compared to their typically developing peers [25]. It was reported that 14% of children and adolescents with ADHD and 1% of youth without ADHD were diagnosed with depression [26]. A longitudinal study showed that children with ADHD exhibited higher depressive symptoms through age 18 compared to children without ADHD, and youth with a history of continued to experience higher depressive symptoms than their age-matched peers from ages 18 to 25 [25]. CDS may contribute to the link between ADHD and depression. It appears to explain much of the covariance between ADHD and depressive symptoms, independently of IA symptoms and externalizing psychopathology [10]. Many studies indicate that the relationship between IA symptoms and depression is no longer significant when CDS is taken into account, but that CDS remains associated with depression when IA is controlled [4, 12,

27]. Therefore, CDS appears to be an important risk factor that should be integrated into research on depression risk in youth with ADHD.

Although a positive relationship between CDS and internalizing symptoms such as anxiety and depression has been recognized, the characteristics of CDS in children with major depression (MD) have not been extensively studied. In this study, we compared children with MD but without ADHD to children with ADHD but without depression in terms of CDS. We examined whether children with MD would have higher CDS compared to children with ADHD. We hypothesized that CDS, as a distinct clinical entity, would exhibit a stronger correlation with depression in children with MD compared to its association with ADHD symptoms in children with ADHD. This is based on the expectation that the emotional characteristics of depression align more closely with CDS symptomatology, whereas the behavioral aspects of ADHD may have a less direct connection. Understanding these differences may lead to more tailored approaches to the diagnosis and treatment of CDS in different clinical conditions.

MATERIALS AND METHODS

Subjects and assessment

Participants consisted of 36 adolescents with ADHD and 25 adolescents with MD, aged 12–18, who applied to the outpatient clinics of Kars Harakani State Hospital between July and November 2023. Data were collected from parents, adolescents, and teachers. All procedures were approved by the institutional review board of Kafkas University (approval date: 04.07.2023, approval number: 274). Parental informed consent and adolescent assent were obtained. Diagnostic assessments for both groups were based on the Diagnostic and Statistical Manual of Mental Disorders, 5th edition [28]. Exclusion criteria for both groups were as follows:

1) Previous diagnoses of neurological disorder, intellectual disability, autism, schizophrenia, bipolar disorder, and substance use disorder;
2) There was no current MD diagnosis in the ADHD group.
3) Participants with MD had no lifetime or current diagnosis of ADHD.

Data Collection Tools

Sociodemographic questionnaire: Sociodemographic characteristics of the sample were obtained through a form prepared by the authors.

Barkley Child Attention Scale [4]: To assess the severity of childhood CDS, all parents completed the Turkish version [29] of the Barkley Child Attention Scale (BCAS) [4]. This scale has been widely used in previous studies involving children and adolescents in Türkiye [30, 31]. The BCAS, for children aged 6 to 12 consists of 12 items divided into two subcategories: sluggishness and daydreaming. The sluggishness dimension includes seven symptoms, such as decreased activity, lethargy, and slowness of behaviors. The daydreaming dimension covers five symptoms, including daydreaming, absentmindedness, and mental confusion. The Cronbach's alpha coefficient was found to be 0.86 in the Turkish reliability study of BCAS

Turgay's DSM-IV Based ADHD and Disruptive Behavior Disorders Screening Scale [32]: This parent-reported scale serves as a screening tool for disruptive behavior disorders based on DSM-IV diagnostic criteria. It has been validated for use in Türkiye by Ercan et al., [33]. The scale consists of a total of 41 items: 9 questions regarding attention deficit, 6 questions regarding hyperactivity, 3 questions regarding impulsivity, 8 questions regarding oppositional defiant disorder and 15 questions regarding conduct disorder. Response options range from zero to three; 0 and 1 indicate normal situations and behaviors, while options 2 and 3 indicate clinically important conditions. A total score of 'six' out of 'nine' criteria rated as 2 or 3 indicates the presence of a serious problem, lasting 'at least six months,' suggestive of ADHD. The Cronbach's alpha coefficients for the sample of the current study was as follows: 0.89 for Inattention, and 0.81 for Hyperactivity.

Children's Depression Rating Scale-Revised: Severity of depression was measured with the clinician-rated Children's Depression Rating Scale-Revised (CDRS-R) [34]. The 16 items on CDRS are measured on 3-, 4-, 5-, and 6-point scales. The CDRS is derived from the Hamilton Rating Scale for Depression (HAM-D); A score of 15 on the CDRS is equivalent to a score of 0 on the HAM-D [35]. Based on family reports and clinical interviews, children demonstrated normal ranges of intelligence, as assessed through adaptive functioning in domains such as communication, self-care, social skills, self-direction, academic skills, work, leisure, health, and/or safety.

Statistical Analysis

Data were analyzed using SPSS 21.0 (IBM Corp., Armonk, NY, USA). Normality assumptions were evaluated through Skewness and Kurtosis values. Descriptive statistics for continuous

variables are presented as mean (M), standard deviation (SD), and standard error (SE). In addition, the number (n) and percentage (%) values of categorical variables were determined for each study group. Sociodemographic and clinical variables were compared between groups using Student's t Test or Mann-Whitney-U Test. Categorical variables were compared between groups using the Chi-Square Test. The relationship between clinical variables in both groups was evaluated by Pearson's or Spearman's correlation analysis.

RESULTS

Comparison of both groups in terms of some sociodemographic and clinical variables is presented in Table 1. The majority of ADHD and MD groups consisted of boys (72.2%, 64.0 %, respectively). There were no significant differences between ADHD (median: 14 (11-17)) and MD (median: 15 (12-18)) groups in terms of age (U=336.5, p=0.092). As expected, Inattention (17.33±3.48 for ADHD; 13.96±1.59 for MD) and Hyperactivity (14.22±7.46 for ADHD; 5.16±1.34 for MD) scores were significantly higher in ADHD group (T=5.096, p<0.001; T=7.123, p<0.001; respectively). Additionally, CDRS scores (median: 27 (18-49) for ADHD; 69 (53-82) for MD) were higher in the MD group (U=0.0, p<0.001). BCAS scores of the MD group (median: 24 (22-29)) were significantly higher than those of the ADHD group (median: 21 (12-31)) (U=287.0, p=0.016).

As seen in Table 2, the severity of BCAS scores in the ADHD group was inversely correlated with ADHD-HI/impulsivity scores (r=-0.681, p<0.001). There is a significant positive relationship between the BCAS and CDRS scores in the MD group, (r= 0.493, p=0.012) (Table 3).

Table 1. Comparison of Sociodemographic and Clinical Characteristics Between ADHD and Depression in Adolescents

	ADHD n=36	**Depression n=25**	**Statistical Analysis**	
	N (%)	**N (%)**	**X^{2*}**	**p**
Gender			0.465*	0.495
Boy	26 (72.2)	16 (64.0)		
Girl	10(28.8)	9(36.0)		
Parents' Marital Status			2.847*	0.092
Married	32 (88.9)	18 (72.0)		
Divorced/separated	4(11.1)	7(28.0)		
Type of Delivery			0.014*	0.905
Normal	25 (69.4)	17 (68.0)		
Caesarean	11(31.6)	8 (32.0)		
Any obstetric complication (yes)	2 (5.6)	0.0 (0.0)	-	0.508
	M±SD Median (min-max)	**M±SD Median (min-max)**	**T**/U*** **	**P**
Age	14.16±1.79 14 (12-18)	15.00±1.82 15 (12-18)	336.5***	0.092
Mothers' Age at Birth	23.55±4.65 24 (16-32)	25.88±5.21 26 (16-33)	-1.825**	0.073
Fathers' Age at Birth	26.55±5.07 27 (18-36)	32.00±5.28 32 (20-42)	-4.053**	**<0.001**
Birth Weight (kg)	3.22±0.63 3.25 (2.00-4.50)	3.32±0.46 3.25 (2.60-4.50)	-0.706**	0.483
Walking onset (month)	13.27±2.36 13 (10-19)	13.20±2.50 13 (10-19)	430.5***	0.766
Speech onset (month)	13.91±2.66 13 (10-24)	13.04±2.22 13 (10-18)	368.0***	0.223

Duration of Breastfeeding (month)	16.69±8.15 13.50 (0-36)	18.40±8.54 19 (0-36)	-0.788**	0.434
BCAS	20.58±5.76 21 (12-31)	24.24±1.78 24 (22-29)	287.0***	**0.016**
Turgay-Inattention	17.33±3.48 17.50 (7-23)	13.96±1.59 14 (11-17)	5.096**	**<0.001**
Turgay-Hyperactivity	14.22±7.46 15 (2-27)	5.16±1.34 5 (3-7)	7.123**	**<0.001**
CDRS	30.00±9.65 27 (18-49)	67.00±8.28 69 (53-82)	0.0***	**<0.001**

**Chi-Square Test **Student's T Test ***Mann-Whitney-U Test*

ADHD: Attention Deficit Hyperactivity Disorder BCAS: Barkley Child Attention Scale

CDRS: Children's Depression Rating Scale

Table 2. Correlation Analysis Between Clinical Variables in the Group of Adolescents Diagnosed with ADHD (n=36)

(R,p)	1	2	3	4	5	6
1. Age	-					
2. Mothers' Age at Birth	.114, .509	-				
3. Father's Age at Birth	.274, .106	**.783, <.001**	-			
4. BCAS	.145, .398	.110, .523	.088, .610	-		
5. Turgay-IN	-.125, .466	-.162, .345	-.236, .166	.097, .574	-	
6. Turgay-HI/I	-.147, .392	-.253, .137	-.282, .095	**-.681, <.001**	.321, .056	-

**Spearman's Correlation Coefficient*

ADHD: Attention Deficit Hyperactivity Disorder BCAS: Barkley Child Attention Scale

Turhay-IN: Inattention Turgay-HI/I: Hyperactivity/Impulsivity

Table 3. Correlation Analysis Between Clinical Variables in the Group of Adolescents Diagnosed with Depression (n=25)

(R,p)	1	2	3	4	5
1. Age	-				
2. Mothers' age at birth	.299, .147	-			
3. Fathers' age at birth	-.184, .380	**.590, .002**	-		
4. BCAS	.052, .807	.069, .743	.153, .466	-	
5. CDRS	.010, .963	.042, .841	.054, .799	**.493, .012**	**-**

**Spearman's Correlation Coefficient*

BCAS: Barkley Child Attention Scale

CDRS: Children's Depression Rating Scale

DISCUSSION

To our knowledge, there are very few studies investigating CDS in children with MD. In this study, it was investigated whether there was a difference in terms of CDS between children with ADHD and children with MD. By excluding cases of clinical depression in the ADHD group, we hypothesized that CDS, as a separate clinical entity would be higher in children with MD than in children with ADHD. Our results demonstrated that there was no significant relationship between CDS and ADHD-IA scores in the ADHD group without depression. In contrast, the severity of CDS was inversely related to ADHD-HA/I scores. Consistently, some previous studies have shown that that CDS is not associated with, or is negatively associated with externalizing behaviours, when controlling for ADHD inattention [8, 12, 36-39]. One reason for the non-significant relationship between CDS and ADHD-IA may be the exclusion of clinical depression in the ADHD group. CDS may contribute to the development of depression in children with ADHD. As previously reported, there is a significant positive association between CDS and internalizing symptoms such as anxiety and depression, even when controlling for ADHD-IA [3, 6, 9-12, 36, 40, 41]. Therefore, the absence of depression in the ADHD group in our study may have prevented the emergence of a relationship between CDS and ADHD-IA.

Another important finding of this study is that depression severity was strongly associated with CDS in the MD group, excluding individuals with ADHD. Although there is an established relationship between CDS and depression, studies have not examined the possible mechanisms of this association. Theoretical models of depression propose domains of competency, self-perceptions, and information processing as key features in the development and maintenance of symptomatology [42]. For example, it is theorized that negative life events and ongoing negative feedback will lead to negative self-perceptions, which in turn will increase negative affect, cognitive biases, and social withdrawal [42, 43]. Depression in adolescents may occur due to high levels of impairment and resulting stress and demoralization secondary to CDS. Among the various clinical features of CDS, social problems occupy an important place. In typically developing children, higher rates of CDS symptoms have been associated with academic problems, emotional and peer relationship problems [44], and more anxiety/depression, emotional reactivity, withdrawal, and somatic complaints [45]. Individuals with CDS exhibit internalizing behaviors such as social withdrawal [8, 18, 46], lower self-esteem, lower teacher-reported social skills [9, 38], sleep problems, being less interactive, and conflicted shyness [47] in school-aged and adolescent samples. Impaired social and academic functioning in individuals with high CDS is associated with increased levels of peer-rejection [8, 40, 41]. Adolescents with elevated CDS may experience more peer bullying and, accordingly, increased depressive symptoms for a variety of reasons. Difficulties in emotional regulation may explain the association between CDS and avoidance of social situations [8]. When specific internalizing comorbidites are considered, CDS appears to be more strongly associated with depression than anxiety [4, 36, 48, 49]. Therefore, we suggest that CDS and depression symptoms exhibit a differential association, indicating distinct interactions or correlation patterns between these two constructs.

Previous studies have investigated CDS in children and adolescents with autism spectrum disorder [50-52], trauma [53], and sleep disorders [6]. These studies indicates that CDS may be a construct of transdiagnostic significance. The association between CDS and depression, independent of ADHD, in our study may underscore the transdiagnostic relevance of CDS, and highlight its potential significance in multiple mental health conditions.

The current study has several limitations. First, our sample size was relatively small, limiting the generalizability of our findings to a broader population. Secondly, the use of a cross-sectional design in this study prevents the establishment of a causal relationship between the variables investigated. Third, CDS in children was assessed using a scale based on parent report. It is noteworthy that children and adolescents tend to report internalizing disorders more accurately than parents [54]. Therefore, relying solely on parental reports to evaluate CDS in this study represents a significant methodological limitation. Using the self-report CDS scale [55] would provide a more appropriate approach.

CONCLUSIONS

In this study, we have found that CDS scores were inversely correlated with hyperactivity/impulsivity, as expected. However, there were no significant relationships between CDS and inattention. Instead, CDS was positively related to depressive symptoms. The strong correlation observed between depression severity and CDS in the MD group highlights the need to assess and address cognitive daydreaming symptoms in patients

with MD. This relationship may provide insight into cognitive patterns associated with depressive disorders. The findings of this study highlight the transdiagnostic relevance of CDS by demonstrating its association with depression independent of ADHD. This underscores the importance of considering CDS in the assessment and treatment of depression in a variety of clinical contexts. It has been known that CDS symptoms have been associated with several academic and social problems which may lead to anxiety or depression. Understanding the differential relationship between CDS and depression symptoms may help clinicians develop more specific interventions. It may suggest that treatment strategies should address these symptoms differently depending on the specific profile of the individual. Further research may explore the underlying mechanisms that contribute to the differential association between CDS and depression symptoms. Studies should investigate whether specific cognitive or emotional factors mediate this relationship. Comparative studies should examine whether similar correlations exist in different populations or across other mental health disorders.

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REFERENCES

[1] Burns GL, Becker SP, Geiser C, Leopold DR, Willcutt EG (2020) Are Sluggish Cognitive Tempo, ADHD, and Oppositional Defiant Disorder Trait- or State-Like Constructs from Prekindergarten to Fourth Grade? J Clin Child Adolesc Psychol 49:460-468. https://doi.org/10.1080/15374416.2019.1567348

[2] Becker SP (2021) Systematic Review: Assessment of Sluggish Cognitive Tempo Over the Past Decade. J Am Acad Child Adolesc Psychiatry 60:690-709. https://doi.org/10.1016/j.jaac.2020.10.016

[3] Burns GL, Montano JJ, Becker SP, Servera M (2023) Cognitive Disengagement Syndrome Symptoms from Early Childhood to Adolescence in a Nationally Representative Spanish Sample. J Clin Child Adolesc Psychol:1-15. https://doi.org/10.1080/15374416.2023.2272944

[4] Barkley RA (2013) Distinguishing sluggish cognitive tempo from ADHD in children and adolescents: executive functioning, impairment, and comorbidity. J Clin Child Adolesc Psychol 42:161-173. https://doi.org/10.1080/15374416.2012.734259

[5] Servera M, Saez B, Burns GL, Becker SP (2018) Clinical differentiation of sluggish cognitive tempo and attention-deficit/hyperactivity disorder in children. J Abnorm Psychol 127:818-829. https://doi.org/10.1037/abn0000375

[6] Becker SP, Leopold DR, Burns GL, Jarrett MA, Langberg JM, Marshall SA, McBurnett K, Waschbusch DA, Willcutt EG (2016) The Internal, External, and Diagnostic Validity of Sluggish Cognitive Tempo: A Meta-Analysis and Critical Review. J Am Acad Child Adolesc Psychiatry 55:163-178. https://doi.org/10.1016/j.jaac.2015.12.006

[7] Becker SP, Vaughn AJ, Zoromski AK, Burns GL, Mikami AY, Fredrick JW, Epstein JN, Peugh JL, Tamm L (2024) A Multi-Method Examination of Peer Functioning in Children with and without Cognitive Disengagement Syndrome. J Clin Child Adolesc Psychol:1-16. https://doi.org/10.1080/15374416.2024.2301771

[8] Willcutt EG, Chhabildas N, Kinnear M, DeFries JC, Olson RK, Leopold DR, Keenan JM, Pennington BF (2014) The internal and external validity of sluggish cognitive tempo and its relation with DSM-IV ADHD. J Abnorm Child

Psychol 42:21-35. https://doi.org/10.1007/s10802-013-9800-6

[9] Bauermeister JJ, Barkley RA, Bauermeister JA, Martinez JV, McBurnett K (2012) Validity of the sluggish cognitive tempo, inattention, and hyperactivity symptom dimensions: neuropsychological and psychosocial correlates. J Abnorm Child Psychol 40:683-697. https://doi.org/10.1007/s10802-011-9602-7

[10] Becker SP, Langberg JM (2013) Sluggish cognitive tempo among young adolescents with ADHD: relations to mental health, academic, and social functioning. J Atten Disord 17:681-689. https://doi.org/10.1177/1087054711435411

[11] Burns GL, Preszler J, Ahnach A, Servera M, Becker SP (2022) Multisource Network and Latent Variable Models of Sluggish Cognitive Tempo, ADHD-Inattentive, and Depressive Symptoms with Spanish Children: Equivalent Findings and Recommendations. Res Child Adolesc Psychopathol 50:881-894. https://doi.org/10.1007/s10802-021-00890-1

[12] Penny AM, Waschbusch DA, Klein RM, Corkum P, Eskes G (2009) Developing a measure of sluggish cognitive tempo for children: content validity, factor structure, and reliability. Psychol Assess 21:380-389. https://doi.org/10.1037/a0016600

[13] Bernad Mdel M, Servera M, Becker SP, Burns GL (2016) Sluggish Cognitive Tempo and ADHD Inattention as Predictors of Externalizing, Internalizing, and Impairment Domains: A 2-Year Longitudinal Study. J Abnorm Child Psychol 44:771-785. https://doi.org/10.1007/s10802-015-0066-z

[14] Vu A, Thompson L, Willcutt E, Petrill S (2019) Sluggish cognitive tempo: longitudinal stability and validity. Atten Defic Hyperact Disord 11:463-471. https://doi.org/10.1007/s12402-019-00287-7

[15] Fredrick JW, Langberg JM, Becker SP (2022) Longitudinal Association of Sluggish Cognitive Tempo with Depression in Adolescents and the Possible Role of Peer Victimization. Res Child Adolesc Psychopathol 50:809-822. https://doi.org/10.1007/s10802-022-00923-3

[16] Harrington KM, Waldman ID (2010) Evaluating the utility of sluggish cognitive tempo in discriminating among DSM-IV ADHD subtypes. J Abnorm Child Psychol 38:173-184. https://doi.org/10.1007/s10802-009-9355-8

[17] Araujo Jimenez EA, Jane Ballabriga MC, Bonillo Martin A, Arrufat FJ, Serra Giacobo R (2015) Executive functioning in children and adolescents with symptoms of sluggish cognitive tempo and ADHD. J Atten Disord 19:507-514. https://doi.org/10.1177/1087054713495442

[18] Becker SP, Garner AA, Tamm L, Antonini TN, Epstein JN (2019) Honing in on the Social Difficulties Associated With Sluggish Cognitive Tempo in Children: Withdrawal, Peer Ignoring, and Low Engagement. J Clin Child Adolesc Psychol 48:228-237. https://doi.org/10.1080/15374416.2017.1286595

[19] Flannery AJ, Luebbe AM, Becker SP (2017) Sluggish Cognitive Tempo is Associated With Poorer Study Skills, More Executive Functioning Deficits, and Greater Impairment in College Students. J Clin Psychol 73:1091-1113. https://doi.org/10.1002/jclp.22406

[20] Becker SP, Burns GL, Garner AA, Jarrett MA, Luebbe AM, Epstein JN, Willcutt EG (2018) Sluggish cognitive tempo in adults: Psychometric validation of the Adult Concentration Inventory. Psychol Assess 30:296-310. https://doi.org/10.1037/pas0000476

[21] Becker SP, McQuade JD (2020) Physiological Correlates of Sluggish Cognitive Tempo in Children: Examining Autonomic Nervous System Reactivity during Social and Cognitive Stressor Tasks. J Abnorm Child Psychol 48:923-933. https://doi.org/10.1007/s10802-020-00651-6

[22] Fredrick JW, Becker SP (2021) Sluggish Cognitive Tempo Symptoms, But Not ADHD or Internalizing Symptoms, Are Uniquely Related to Self-Reported Mind-Wandering in Adolescents With ADHD. J Atten Disord 25:1605-1611. https://doi.org/10.1177/1087054720923091

[23] Shelton CR, Addison WE, Hartung CM (2019) ADHD and SCT Symptomatology in Relation to College Students' Use of Self-Regulated Learning Strategies. J Atten Disord 23:1719-1728. https://doi.org/10.1177/1087054717691134

[24] Nelson JM, Lovett BJ (2023) Sluggish Cognitive Tempo (SCT), Comorbid Psychopathology, and Functional Impairment in College Students: The Clinical Utility of SCT Subfactors. J Atten Disord 27:313-323. https://doi.

org/10.1177/10870547221142458

[25] Meinzer MC, Pettit JW, Viswesvaran C (2014) The co-occurrence of attention-deficit/hyperactivity disorder and unipolar depression in children and adolescents: a meta-analytic review. Clin Psychol Rev 34:595-607. https://doi.org/10.1016/j.cpr.2014.10.002

[26] Larson K, Russ SA, Kahn RS, Halfon N (2011) Patterns of comorbidity, functioning, and service use for US children with ADHD, 2007. Pediatrics 127:462-470. https://doi.org/10.1542/peds.2010-0165

[27] Bernad Mdel M, Servera M, Grases G, Collado S, Burns GL (2014) A cross-sectional and longitudinal investigation of the external correlates of sluggish cognitive tempo and ADHD-inattention symptoms dimensions. J Abnorm Child Psychol 42:1225-1236. https://doi.org/10.1007/s10802-014-9866-9

[28] American Psychiatric Association (2013) The Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition. American Psychiatric Press

[29] Firat S, Unsel Bolat G, Gul H, Baytunca MB, Kardas B, Aysev A, Ercan ES (2018) Barkley Child Attention Scale validity and reliability study. Dusunen Adam: The Journal of Psychiatry and Neurological Sciences 31:284-293. https://doi.org/10.5350/dajpn2018310306

[30] Bolat H, Ercan ES, Unsel-Bolat G, Tahillioglu A, Yazici KU, Bacanli A, Pariltay E, Aygunes Jafari D, Kosova B, Ozgul S, Rohde LA, Akin H (2020) DRD4 genotyping may differentiate symptoms of attention-deficit/hyperactivity disorder and sluggish cognitive tempo. Braz J Psychiatry 42:630-637. https://doi.org/10.1590/1516-4446-2019-0630

[31] Kilicoglu AG, Zadehgan Afshord T, Derin S, Ertas E, Coskun P, Aktas S, Guler EM (2023) Comparison of Possible Changes in Oxidative Stress, DNA Damage, and Inflammatory Markers in Children/Adolescents Diagnosed with Sluggish Cognitive Tempo and Children/Adolescents Diagnosed with Attention-Deficit/Hyperactivity Disorder. J Child Adolesc Psychopharmacol 33:325-331. https://doi.org/10.1089/cap.2022.0081

[32] Turgay A (1994) Disruptive behavior disorders child and adolescent screening and rating scales for children, adolescents, parents and teachers. West Blomfield, MI: Integrative Therapy Institute Publication,

[33] Ercan ES, Amado S, Somer O, Çikoğlu S (2001) Development of a test battery for the assessment of attention deficit hyperactivity disorder [in Turkish]. Cocuk ve Genclik Ruh Sagli{dotless}ği{dotless} Dergisi (Turkish Journal of Child and Adolescent Mental Health) 8:132-144

[34] Poznanski EO, Mokros HB, Western Psychological S (1996) Children's depression rating scale, revised (CDRS-R). Western Psychological Services, Los Angeles, Calif.

[35] Güney S, Baykara H, Emiroğlu N (2018) Psychometric properties of the Turkish adaptation of the Children's Depression Rating Scale: revised in Turkish adolescents. Anatolian Journal of Psychiatry. https://doi.org/10.5455/apd.292666

[36] Becker SP, Marshall SA, McBurnett K (2014) Sluggish cognitive tempo in abnormal child psychology: an historical overview and introduction to the special section. J Abnorm Child Psychol 42:1-6. https://doi.org/10.1007/s10802-013-9825-x

[37] Lee S, Burns GL, Snell J, McBurnett K (2014) Validity of the sluggish cognitive tempo symptom dimension in children: sluggish cognitive tempo and ADHD-inattention as distinct symptom dimensions. J Abnorm Child Psychol 42:7-19. https://doi.org/10.1007/s10802-013-9714-3

[38] McBurnett K, Villodas M, Burns GL, Hinshaw SP, Beaulieu A, Pfiffner LJ (2014) Structure and validity of sluggish cognitive tempo using an expanded item pool in children with attention-deficit/hyperactivity disorder. J Abnorm Child Psychol 42:37-48. https://doi.org/10.1007/s10802-013-9801-5

[39] Sevincok D, Ozbay HC, Ozbek MM, Tunagur MT, Aksu H (2020) ADHD symptoms in relation to internalizing and externalizing symptoms in children: the mediating role of sluggish cognitive tempo. Nord J Psychiatry 74:265-272. https://doi.org/10.1080/08039488.2019.1697746

[40] Burns GL, Servera M, Bernad Mdel M, Carrillo JM, Cardo E (2013) Distinctions between sluggish cognitive tempo, ADHD-IN, and depression symptom dimensions in Spanish first-grade children. J Clin Child Adolesc Psychol 42:796-808. https://doi.org/10.1080/15374416.2013.838771

[41] Carlson CL, Mann M (2002) Sluggish cognitive tempo

predicts a different pattern of impairment in the attention deficit hyperactivity disorder, predominantly inattentive type. J Clin Child Adolesc Psychol 31:123-129. https://doi.org/10.1207/S15374424JCCP3101_14

[42] Cole DA, Dukewich TL, Roeder K, Sinclair KR, McMillan J, Will E, Bilsky SA, Martin NC, Felton JW (2014) Linking peer victimization to the development of depressive self-schemas in children and adolescents. J Abnorm Child Psychol 42:149-160. https://doi.org/10.1007/s10802-013-9769-1

[43] Sinclair SJ, Siefert CJ, Slavin-Mulford JM, Stein MB, Renna M, Blais MA (2012) Psychometric evaluation and normative data for the depression, anxiety, and stress scales-21 (DASS-21) in a nonclinical sample of U.S. adults. Eval Health Prof 35:259-279. https://doi.org/10.1177/0163278711424282

[44] Camprodon-Rosanas E, Ribas-Fito N, Batlle-Vila S, Persavento C, Alvarez-Pedrerol M, Sunyer J, Forns J (2017) Sluggish Cognitive Tempo: Sociodemographic, Behavioral, and Clinical Characteristics in a Population of Catalan School Children. J Atten Disord 21:632-641. https://doi.org/10.1177/1087054716652477

[45] Lee S, Burns GL, Becker SP (2017) Can Sluggish Cognitive Tempo Be Distinguished From ADHD Inattention in Very Young Children? Evidence From a Sample of Korean Preschool Children. J Atten Disord 21:623-631. https://doi.org/10.1177/1087054716680077

[46] Rondon AT, Hilton DC, Jarrett MA, Ollendick TH (2020) Sleep, Internalizing Problems, and Social Withdrawal: Unique Associations in Clinic-Referred Youth With Elevated Sluggish Cognitive Tempo Symptoms. J Atten Disord 24:524-534. https://doi.org/10.1177/1087054718756197

[47] Saez B, Servera M, Becker SP, Burns GL (2019) Optimal Items for Assessing Sluggish Cognitive Tempo in Children Across Mother, Father, and Teacher Ratings. J Clin Child Adolesc Psychol 48:825-839. https://doi.org/10.1080/15374416.2017.1416619

[48] Fenollar Cortes J, Servera M, Becker SP, Burns GL (2017) External Validity of ADHD Inattention and Sluggish Cognitive Tempo Dimensions in Spanish Children With ADHD. J Atten Disord 21:655-666. https://doi.org/10.1177/1087054714548033

[49] Jacobson LA, Murphy-Bowman SC, Pritchard AE, Tart-Zelvin A, Zabel TA, Mahone EM (2012) Factor structure of a sluggish cognitive tempo scale in clinically-referred children. J Abnorm Child Psychol 40:1327-1337. https://doi.org/10.1007/s10802-012-9643-6

[50] Brewe AM, Simmons GL, Capriola-Hall NN, White SW (2020) Sluggish cognitive tempo: An examination of clinical correlates for adults with autism. Autism 24:1373-1383. https://doi.org/10.1177/1362361319900422

[51] Duncan A, Tamm L, Birnschein AM, Becker SP (2019) Clinical correlates of sluggish cognitive tempo in adolescents with autism spectrum disorder. Autism 23:1354-1362. https://doi.org/10.1177/1362361318811329

[52] Mayes SD, Calhoun SL, Waschbusch DA (2021) Relationship between sluggish cognitive tempo and sleep, psychological, somatic, and cognitive problems and impairment in children with autism and children with ADHD. Clin Child Psychol Psychiatry 26:518-530. https://doi.org/10.1177/1359104520978459

[53] Musicaro RM, Ford J, Suvak MK, Sposato A, Andersen S (2020) Sluggish cognitive tempo and exposure to interpersonal trauma in children. Anxiety Stress Coping 33:100-114. https://doi.org/10.1080/10615806.2019.1695124

[54] Silverman WK, Ollendick TH (2005) Evidence-based assessment of anxiety and its disorders in children and adolescents. J Clin Child Adolesc Psychol 34:380-411. https://doi.org/10.1207/s15374424jccp3403_2

[55] Gozpinar N, Cakiroglu S, Gormez V (2023) Sluggish Cognitive Tempo Self Report Scale (SCT-SR): Development and Initial Validation Study. J Atten Disord 27:510-520. https://doi.org/10.1177/10870547231153879

[56] Al. O (2023) Chat GBT. https://chat.openai.com/chat. Accessed Date 25 July 2024

How to Cite;

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Original Research

Benign Fibro-Osseous Lesions of The Jaw: A Retrospective Analysis

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ABSTRACT

Objective: The main goal of this retrospective study was to characterise FOLs in terms of their demographic distribution, prevalence, and clinical and radiological features, and to discuss the treatments for this condition.

Methods: This study included patients with FOLs found in the archives of the Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, University of Harran, Türkiye. The panoramic radiographs and histopathological results of all patients referred to our clinic between 2017 and 2020 were reviewed retrospectively. In total, 18,835 patient records were evaluated. Two oral and maxillofacial surgeons sequentially examined the panoramic radiographs of all patients who presented to our clinic for examination or treatment. In total, 10 patients showed radiological and histopathology results compatible with FOLs.

Results: In total, 18,835 radiographs were evaluated, and 10 (0.00074%) FOLs were seen in 10 patients (8 females and 2 males) ranging in age from 18–64 years. Three of the cases were of FCOD, three were of FaCOD (father and two daughters), one was of of FoCOD, one was of OF, and two were of FD.

Conclusion: FOLs, and in particular FaCFOD, are rarely seen in the clinic. Accurate diagnosis of these diseases is important to avoid inappropriate treatment. In this study, we reported 10 FOLs in 10 patients seen at our institution, and presented a review of the literature.

Keywords: fibroosseoz lesions, familial osseous dysplasia, ossifying fibroma, florid cemental osseous dysplasia

INTRODUCTION

Fibro-osseous lesions (FOLs) are a group of lesions of the jaw and facial bones arising from fibroblast cells. These lesions can be developmental, reactive-dysplastic, or neoplastic. In all FOLs, bone is replaced with fibrous and cement-like tissue, which shows gradual mineralization [1].

Although the classification system for FOLs has undergone several revisions, they are typically classified as fibro-osseouz neoplazms (FOs), osseous dysplasias (ODs), or fibrous dysplasia (FD), based on the system of Waldron,[2] or Ossifiying fibroma (OF), FD, or cemento-osseous dysplasias (CODs), as proposed

by the World Health Organization (WHO, 2017) [3]. The WHO (2017) supports the terminology of 'cemento-osseous dysplasia' because this term more accurately describes the relationship of the lesions to the teeth and their origin from the periodontal tissues. [3].

Radiographic modalities, such as cone-beam computed tomography (CBCT) and orthopantomography, are used to study FOLs in detail, including in terms of their relationship with adjacent structures. Radiographically, FOLs may be radiolucent (early stage), mixed radiolucent-radiopaque (mixed stage) or radiopaque (mature stage) in appearance. It may be well-circumscribed, or it may have an appearance that cannot be clearly separated from the surrounding bone tissue. Resorption and displacement of teeth may occur, in addition to bone expansion [4].

COD is a FOLs subtype classified as periapical cemento-osseous dysplasia (PCOD), focal cemento-osseous dysplasia (FoCOD), florid cemento-osseous dysplasia (FCOD), or familial form of florid cemento-osseous dysplasia (FFCOD) according to the clinical and radiographic findings. The term 'florid' is in reference to the possibility of FCOD being present in multiple quadrants of the jaw, although appearance in all four quadrants is rare [5].

FFCOD is an uncommon hereditary autosomal dominant disorder; only a few cases have been reported in the literature. FaFCOD is characterised by irregular, lobular, intense and opaque masses that spread along the jaws and alveolar processes, and affect multiple members of the same family [6].

Main Points:

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Usually, no treatment is required for FFCOD, such that only regular follow-up examinations are recommended. Accurate diagnosis is crucial because misdiagnosis can lead to the requirement for biopsy, endodontic treatment, or surgical treatment [7].

The main goal of this retrospective study was to characterise FOLs in terms of their demographic distribution, prevalence, and clinical and radiological features, and to discuss the treatments for this condition.

MATERIALS AND METHODS

This study included patients with FOLs found in the archives of the Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, University of Harran, Türkiye. The Ethics Committee of Harran University Faculty of Medicine approved this study, which was conducted in accordance with the Declaration of Helsinki. The panoramic radiographs and histopathological results of all patients referred to our clinic between 2017 and 2020 were reviewed retrospectively. In total, 18,835 patient records were evaluated.

Two oral and maxillofacial surgeons sequentially examined the panoramic radiographs of all patients who presented to our clinic for examination or treatment. In total, 10 patients showed radiological and histopathology results compatible with FOLs, and were thus included in the study (Table 1). They were classified based on the system of WHO [3]. The data analysed included gender, age, symptoms, other medical problems, location of lesions, radiographic technique, histopathological findings, treatment and postoperative complications.

RESULTS

In total, 18,835 radiographs were evaluated, and 10 (0.00074%) FOLs were seen in 10 patients (8 females and 2 males) ranging in age from 18–64 years. Three of the cases were of FCOD, three were of FFCOD (father and two daughters), one was of of FoCOD, one was of COF, and two were of FD (Table 1).

Pathological growth of the jaw bones (resulting from displacement of cortical plates) caused pain and aesthetic problems in three cases, and a decrease in chewing function in five others (three FFCOD, two FD). Thus, contour plasty was performed under general anaesthesia. Postoperatively, there were no complications and patients attended the routine follow-up.

Table 1. Distribution of type of lesions, age/ gender, symptoms, other medical problems, location of lesions, type of radiograhpy, treatment and complication of patients with fibrooseous lesion.

Type of lession	Gender/age	Symptoms	Other Medical Problems	Location of Lesions	Radiograph	Treatment	Post-operative Complications
FOD	F/50	-	-	Right and left mandible	OPG	No treatment	-
FOD	F/37	pain	-	Right and left mandible	OPG	No treatment	-
FOD	F/34	-	-	Right maxillary anterior region, right and left mandible	OPG	No treatment	-
FaOD	M/37	swelling and dull pain	-	involving four quadrants of the maxilla and mandible, left subcodyle region and right mandibular angulus region	OPG	contour plasty	-
FaOD	F/18	swelling and dull pain	-	involving four quadrants of the maxilla and mandible, left mandibular incisura	OPG	contour plasty	-
FaOD	F/15	and dull pain swelling	-	involving four quadrants of the maxilla and mandible, left mandibular angulus	OPG	contour plasty	-
FoOD	F/51	-	-	Anterior mandibular region	OPG	No treatment	-
OF	F/64	Dull pain	-	Right mandibular premolar-molar region	OPG	No treatment	-
FD	M/42	asymmetry	-	Left mandibular molar region	OPG	contour plasty	-
FD	F/22	asymmetry	-	Right maksillar anterior region	OPG	contour plasty	-

DISCUSSION

The aetiology of FOLs is not yet fully understood, but the proximity of the lesions to the periodontal ligament suggests that they originate from the same tissue. FOLs are classified as developmental, reactive-dysplastic or neoplastic. FDs are developmental lesions, fibromas are neoplasms, and CODs are reactive lesions [8].

In the most recent WHO classification, COF was classified as an odontogenic tumor having a mesenchymal origin, distinguishing it from the juvenile types [3]. However, it is still a fibro-osseous lesion and was discussed in detail with the other OFs in the fibro-osseous lesions section of the most recent WHO edition.

OF, which is classified as a benign odontogenic tumour of mesenchymal origin that can occur in any facial bone, is usually seen in the third and fourth decades of life. Among BFOLs, OF is the only group that is neoplastic in origin, and divided into two subgroups as cemento-ossifying fibroma and juvenile ossifying fibroma [9]. Opinions vary regarding gender differences in the prevalence of OF. MacDonald et al. suggested that OF affects both genders equally overall, but women are affected more frequently in the fourth decade of life [10]. OF is frequently seen in the mandibular premolar-molar region, and the maxilla, zygomatic arch and canine fossa are also often affected [9]. OF may present as a well-defined, completely radiolucent mass in the early stages, or with areas of opacity varying according to the degree of calcification. In advanced stages, OF can appear as a mass surrounded by radiopaque and radiolucent bands. Migration and root resorption may be observed in adjacent teeth, and the bone cortex may show expansion [11]. In this study, we found one case of OF. Patient (women aged 64) had chief complaints of expansion of the bone cortex and pain (Figure 1). The lesion was located in the right mandibular premolar-molar region. Radiographically, the lesion appeared as well-defined, dense radiopaque areas surrounded by radiolucent area. The demographic, clinical and radiographic findings of the patient was consistent with previous studies.

The aetiology of FD, a hamartomatous developmental bone disease that arises due to a failure of immature bone to mature, is not fully understood [12]. FD lesions may occur in one bone (monostotic), or in multiple bones (polyostotic). One form of polyostotic FD, namely McCune-Albright syndrome, is accompanied by skin and endocrine anomalies. The most common form of FD is the monostotic type, which accounts for approximately 80–85% of all cases [10]. FDs are frequently seen in the jaw and facial bones. They primarily affect the maxilla, but involvement of the mandible, zygoma, sphenoid, frontal and occipital bones may also be seen [10]. Eversole et al. [11] reported that teeth remain in place when there is bone involvement, but displacement may occasionally occur. Tooth root resorption is rare. Monostotic-type FD is a slow-growing, painless mass generally observed in young adults (second and third decades of life), which affects both sexes equally [13]. The term craniofacial fibrous dysplasia is used to describe monostotic-type FD [11]. On radiographic images, FD may show a radiolucent, sclerotic or mixed radiopaque-radiolucent appearance. Extragnathic FD lesions have better-defined margins compared to gnathic lesions, and the lack of a diffuse structure and borders is an important radiographic feature of FDs of the jaw and facial bones [10]. In this study, we found two cases of FD (female and male aged 22 and 42 years, respectively). In the first patient, the lesion was located in the right maxillary incisor and premolar region, whereas it was located in the left mandibular molar region in the second patient (Figure 2). In both cases, the lesions caused the bone to expand; the expansion was much greater in the second case, enlarging the lingual and buccal cortex and alveolar bone and being in contact with the opposite teeth. Contour plasty was performed in both cases due to the patients' complaints. Radiography revealed sclerosis in the first case, with resorption seen in the roots of the right maxillary first and second incisors. In the second case, the lesion had displaced the teeth and inferior alveolar canal. Expansion of the alveolar bone was also clearly visible. There were no skin or endocrine anomalies in either case. Both cases showed single bone involvement, compatible with monostatic-type FD.

COD is a group of dysplastic bone lesions most commonly seen in the mandible. COD is divided into four subgroups: PCOD, FoCOD, FCOD, and FFCOD [5]. FFCOD is a hereditary form of COD that is much rarer than the other forms. All forms of COD affect the area around the tooth and emerge from the periodontal ligament or cement-like tissue. Although the histopathological features of the different types of CODs are the same, their clinical and radiographic features are different [14].

PCOD is more common in women between the third and fifth decades of life [15]. Radiographically and histopathologically, the lesions show three distinct stages, where progression from the first to the third stage may take months or even years. Early (lytic) stage lesions appear as radiolucent areas in the periapical region, and can be confused with chronic inflammatory lesions [11]. Clinical examination is important for diagnosis. In the second stage, as the lesion matures, radiopaque foci appear within the radiolucent area. In the third (mature) stage, the lesion shows a solid, radiopaque appearance with a surrounding radiolucent band. According to Koenig et al. [16], PCOD lesions are round, typically multifocal, well-defined and smaller than 1 cm. Cortical bone and mucosa are normal. Multifocal lesions can be in different stages within the same patient. In this study, we didin't found any PCOD case.

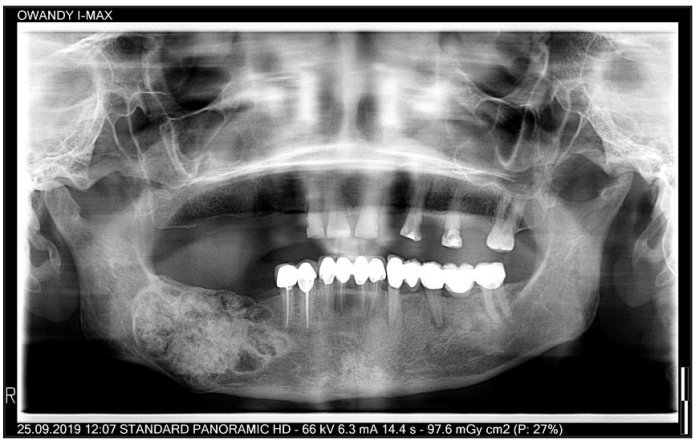

Figure 1. Panoramic radiograph showing large cemento-ossifying fibroma lesion which involve right mandibular premolar- molar region.

Figure 2. Panoramic radiographic appearance of fibrous displasia

FoCOD is the most common type of FOL, and is seen most frequently in African women in the third and fourth decades of life. Although the posterior mandibular region is the most affected area, FoCOD can develop anywhere in the jaw bone. FoCOD usually affects the apical region of the tooth [10]. Radiographically, FoCOD may be well-defined, fully radiolucent, or radiopaque with a partially sclerotic surrounding band. However, these two patterns typically cooccur. FoCOD can also occur in edentulous patients and tooth extraction areas; the lesions are usually 1–2 cm in size and do not tend to show a multifocal distribution. Although the lesion is usually asymptomatic, it can cause pain and swelling if there is secondary infection. FoCOD is generally detected incidentally on routine dental radiographs; the presence of vital teeth in close proximity to the periapical or tooth extraction region is an important diagnostic finding [9]. In this study, we found one FoCOD case (women aged 51 years). The lesions were located in the anterior mandibular region. Radiographically, the lesions were in the mature stage, and appeared as well-defined, dense radiopaque areas.

FCOD is a common form of PCOD [16] White et al. [15] showed that FCOD is most prevalent in women in the fourth and fifth decades of life, typically presenting symmetrically and bilaterally in the mandible or mandibular canal. FCOD can be asymptomatic and is often detected incidentally. Koenig et al. [16] stated that FCOD differs from FoCOD in that it affects more than one quadrant of the jaw and has a larger size. If the lesion becomes very large, it can expand the bone. Radiographic images of FCOD show multiple sclerotic radiopacities surrounded by a peripheral radiolucent rim, accompanied by mixed lesions with ill-defined borders [17]. Extensive lesions can cause cortical expansion and displacement of the mandibular canal [15]. In this study, we found three FCOD cases, (females aged 50, 37, and 34 years). The lesions were well-defined, located bilaterally in the mandible, and showed a multifocal distribution. In the first and second patients (Figure 3), the lesions were well-defined, located bilaterally in the mandible, and showed a multifocal distribution. In the third patient the lesions were well-defined, located bilaterally in the mandible and right maxillary anterior region, and showed a multifocal distribution. Lesions in all three patients show early, mixed and mature stages. The demographic, clinical and radiographic findings of the patients were consistent with previous studies.

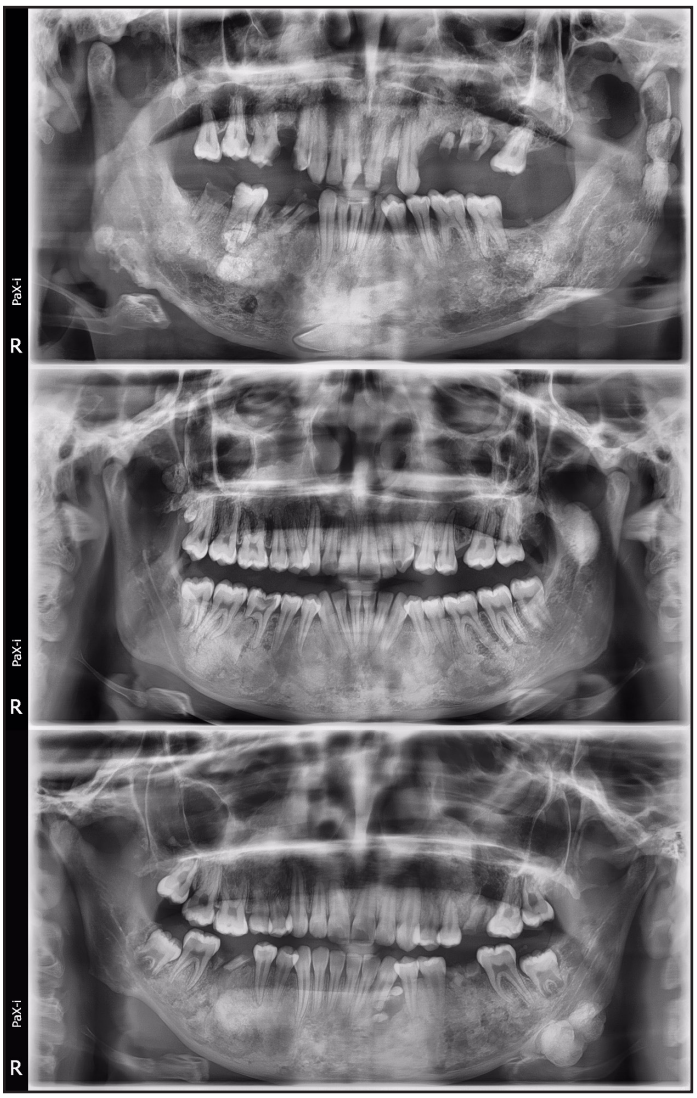

Figure 4. Panoramic radiographs of family members

Figure 3. In the radiographic image, multiple lesions are visible in both quadrants of the mandible. Similar pathologic lesions involvement of mandible and the surrounded the teeth.

Table 2. Review of the literature on familial florid osseous dysplasia (FaOD)

Author	Year	Ethnicity	Involved family members	Reported as
1. Agazzi and Belloni[24]	1953	Italian family	Unspecified	Hard odontomas of the jaws
2. Cannon et al[23]	1980	Unspecified	Mother and son	Familial gigantiform cementoma
3. Sedano et al[21]	1982	White family	10 members	Autosomal dominant cemental dysplasia
4. Young et al[20]	1989	White family	55 members	Familial gigantiform cementoma
5. Musella and Slater[25]	1989	Italian family	Mother and daughter	Familial florid osseous dysplasia
6. Oikarinen et al[22]	1991	Caucasian family	Father and 2 children	Familial gigantiform cementoma
7. Thakkar et al [30]	1993	Caribbean family	Mother, 2 daughters and 1 son	Familial periapical cemental dysplasia
8. Coleman et al[19]	1996	African family	Mother and 2 children	Familial florid osseous dysplasia
9. Toffanin et al[18]	2000	Italian family	Grandmother, son, daughter, grandson, granddaughter	Familial florid osseous dysplasia
10. Hatori et al[26]	2003	Japanese family	Father and daughter	Familial florid osseous dysplasia
11. Srivastava et al[29]	2012	Indian family	Mother and son	Familial florid osseous dysplasia
12. Sim et al[27]	2014	Asian family	Mother and her identical twin daughters	Familial florid osseous dysplasia
13.Thorawat et al [28]	2015	Black family	Mother and daughter	Familial florid osseous dysplasia
14. Kucukkurt et al[17]	2016	Unspecified	Mother, son and mother's brother	Familial florid osseous dysplasia
15. Mingming et al[6]	2019	Chinese family	The family comprises three generations	autosomal dominant Familial florid osseous dysplasia

FFCOD is rarely seen; in our review, only 15 cases were found (Table 2) [6, 17-30]. Küçükkurt et al. [17] noted that although FFCOD is usually asymptomatic, it can cause pain, swelling and facial deformity due to infection. FFCOD tends to occur in younger individuals [6]. Radiographically, FFCOD shows similar features to the other CODs; moreover, maturation and the quantity of mineralised tissue in the lesion affect the radiographic appearance. A completely radiolucent appearance is common in early lesions, while radiopaque foci appear as the lesions mature. In mature lesions, a radiolucent border around the lesion separates it from the adjacent normal bone [15]. We found a case of FFCOD where in a father and two daughters showed similar clinical and histopathological findings. The father was 37 years old and his daughters were aged 15 and 18 years. Orthopantomography revealed multiple radiopaque masses with radiolucent borders in all four quadrants of the jaw, in all three family members (Figure 4). The thin lesions appeared as irregular and lobular radiopacities surrounded by a radiolucent border. All patients exhibited regional swelling. The father had right mandibular angle and left ramus swellings; his older daughter had a left mandibular incisura swelling and the younger daughter showed a left mandibular angle swelling. All three patients complained of extreme swelling and dull pain. Root resorption was present in some teeth in all three cases. In all patients, contour plasty was performed via an extraoral submandibular and intraoral approach. No postoperative complications developed in any of the patients and they were routinely followed-up. Regarding FFCOD cases previously reported, they were more common in white families; only two families were black. Moreover, the mother and children were affected more frequently; in only two cases were the father and children affected, as in our case (Tables 1 and 2).

Limitations: The major limitation of our study is the small number of cases and it was studied single center. The research needs to be supported with more cases and it is thought that multi-center studies will contribute more to the literature.

CONCLUSION

FOLs, and in particular FFCOD, are rarely seen in the clinic. Accurate diagnosis of these diseases is important to avoid

inappropriate treatment. In this study, we reported 10 FOLs in 10 patients seen at our institution, and presented a review of the literature.

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

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Ethical Approval: The study was approved by Harran University local ethics committee (Approval Number: 2020/02/012, Date: 2024-02-19).

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REFERENCES

[1] Köseoğlu S, Günhan Ö, Gülşahı A (2016) Benign fibroosseöz lezyonlar. Acta Odontol Turc 33(2):95-101. https://doi.org/10.17214/aot.25979

[2] Waldron CA (1985) Fibro-osseous lesions of the jaws. J Oral Maxillofac Surg 43:249-62. https://doi.org/10.1016/0278-2391(85)90283-6

[3] Wright JM, Vered M (2017) Update from the 4th Edition of the World Health Organization classification of head and neck tumours: odontogenic and maxillofacial bone tumors. Head Neck Pathol 11:68–77. https://doi.org/10.1007/s12105-017-0794-1

[4] Jk H, Sj S (2013). Misdiagnosis of florid cemento-osseous dysplasia leading to unnecessary root canal treatment: a case report. Restor Dent Endod 38:160–166. https://doi.org/10.5395/rde.2013.38.3.160

[5] Mangala M, Ramesh D, Surekha P, Santosh P (2006). Florid cemento-osseous dysplasia: review and report of two cases. Indian J Dent Res 17:131–134. https://doi.org/10.4103/0970-9290.29875

[6] Mingming Lv, You G, Wang J, Fu Q, Gupta A, Li J, Sun J (2019) Identification of a novel ANO5 missense mutation in a Chinese family with familial florid osseous dysplasia. Journal of Human Genetics 64:599–607. https://doi.org/10.1038/s10038-019-0601-9

[7] Sarmento DJ, Monteiro BV, de Medeiros AM, Silveira EJD (2013) Severe florid cemento-osseous dysplasia: a case report treated conservatively and literature review. Oral Maxillofac Surg 17:43–6. https://doi.org/10.1007/s10006-012-0314-0

[8] Aiuto R, Gucciardino F, Rapetti R, Siervo S, Bianchi AE (2018) Management of symptomatic florid cemento-osseous dysplasia: Literature review and a case report. J Clin Exp Dent 10(3):291-5. https://doi.org/10.4317/jced.54577

[9] Soluk-Tekkesin M, Sinanoglu A, Selvi F, Cakir Karabas H, Aksakalli N (2022) The importance of clinical and radiological findings for the definitive histopathologic diagnosis of benign fibro-osseous lesions of the jaws: Study of 276 cases. Journal of stomatology, oral and maxillofacial surgery 123(3):364–371. https://doi.org/10.1016/j.jormas.2021.04.008

[10] MacDonald-Jankowski DS (2009) Ossifying fibroma: Systematic review. Dentomaxillofac Radiol 38:495. https://doi.org/10.1259/dmfr/70933621

[11] Eversole R, Su L, Elmofty S (2008) Benign fibro-osseous lesions of the craniofacial complex. A review. Head Neck Pathol 2:177. https://doi.org/10.1007/s12105-008-0057-2

[12] Speight PM, Carlos R (2006) Maxillofacial fibro-osseous lesions. Current Diagnostic Pathology 12:1-10. https://doi.org/10.1016/j.cdip.2005.10.002

[13] [Hall G (2012). Fibro-osseous lesions of the head and neck. Diagnostic Histopathol 18:149-58. https://doi.org/10.1016/j.mpdhp.2012.01.005

[14] Abramovitch K, Rice D.D (2016) Benign Fibro-Osseous Lesions of the Jaws. Dent Clin N Am 60 (2016) 167–193. https://doi.org/10.1016/j.cden.2015.08.010

[15] Slootweg PJ (2010) Bone diseases of the jaws. Int J Dent 2010:702314 https://doi.org/10.1155/2010/702314

[16] Koenig, L. J. (2012). Mandible and maxilla. Diagnostic Imaging Oral and Maxillofacial. Koenig LJ, Tamimi D, Petrikowski CG, Harnsberger HR, Ruprecht A, Benson

BW, Van Dis ML, Hatcher D and Perschbacher SE (eds). Amirsys, Milwaukee, WI, pp50-53, pp100-1032.

[17] Kucukkurt S, Rzayev S, Baris E, Atac MS (2016) Familial florid osseous dysplasia: a report with review of the literature. BMJ Case Rep doi:10.1136/bcr-2015-214162. https://doi.org/10.1136/bcr-2015-214162

[18] Toffanin A, Benetti R, Manconi R (2000) Familial florid cemento-osseous dysplasia: a case report. J Oral Maxillofac Surg 58:1440–6. https://doi.org/10.1053/joms.2000.16638

[19] Coleman H, Altini M, Kieser J, M Nissenbaum (1996) Familial florid cemento-osseous dysplasia--a case report and review of the literature. J Dent Assoc S Afr 51:766–70.

[20] Young SK, Markowitz NR, Sullivan S, T W Seale, R Hirschi (1989) Familial gigantiform cementoma: classification and presentation of a large pedigree. Oral Surg Oral Med Oral Pathol 68:740–7. https://doi.org/10.1016/0030-4220(89)90165-5

[21] Sedano HO, Kuba R, Gorlin RJ (1982) Autosomal dominant cemental dysplasia. Oral Surg Oral Med Oral Pathol 54:642–6. https://doi.org/10.1016/0030-4220(82)90078-0

[22] Oikarinen K, Altonen M, Happonen RP (1991) Gigantiform cementoma affecting a Caucasian family. Br J Oral Maxillofac Surg 29:194–7. https://doi.org/10.1016/0266-4356(91)90038-7

[23] Cannon JS, Keller EE, Dahlin DC (1980) Gigantiform cementoma: report of two cases (mother and son). J Oral Surg 38:65–70.

[24] Agazzi C, Belloni L. Hard (1953) Odontomas of the jaws. Clinico-roentgenologic and anatomo-microscopic contribution with special reference to widely extensive forms with familial occurrence. Arch Ital Otol Rinol Laringol 64(16):1–103.

[25] Musella AE, Slater LJ (1989) Familial florid osseous dysplasia: a case report. J Oral Maxillofac Surg 47:636–40. https://doi.org/10.1016/s0278-2391(89)80083-7

[26] Hatori M, Ito I, Tachikawa T, Nagumo M (2003) Familial florid cemento-osseous dysplasia. Asian J Oral Maxillofac Surg Med Pathol 15:135–7. https://doi.org/10.1016/S0915-6992(03)80022-5

[27] Sim YC, Bakhshalian N, Lee J-H, Ahn K-M (2014) Familial florid cemento-osseous dysplasia in mother and her identical twins: a report with review of the literatures. Oral Surgery 7:239–44. https://doi.org/10.1111/ors.12082

[28] Thorawat A, Kalkur C, Naikmasur VG, Tarakji B (2015) Familial florid cemento-osseous dysplasia—case report and review of literature. Clinical Case Reports 2015; 3(12): 1034-1037. https://doi.org/10.1002/ccr3.426

[29] Srivastava A, Agarwal R, Soni R, vesh Sachan, Shivakumar G. C., Chaturvedi T. P (2012) Familial florid cemento-osseous dysplasia: a rare manifestation in an Indian family. Case Rep Dent. 574125. https://doi.org/10.1155/2012/574125

[30] Thakkar NS, Horner K, Sloan P (1993). Familial occurrence of periapical cemental dysplasia. Virchows Arch A Pathol Anat Histopathol 423:233–6. https://doi.org/10.1007/BF01614776

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Letter to Editor

Can Artificial Intelligence be Used Against the Potential Risks of Short Examination Times in Hospitals?

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Dear Editor,

The reason why we wrote this letter is to address the risks that short examination times in our country may pose for patients and doctors and to initiate a discussion on what can be done to find a solution. The recent increase in hospital admissions and the decrease in the number of physicians have created pressure to examine a large number of patients in a short time. Studies show that the general physical examination time of the patient is 20 minutes [1]. This examination period may be longer for some branches. For example, examination time may be longer in cardiology patients due to some procedures, such as effort echocardiography. For psychiatric patients, the first examination can take up to 45 minutes, including the meeting with the patient's relatives. It is reported that as this period shortens, the likelihood of the physician in question facing a malpractice lawsuit in the future increases [2]. Currently, in public hospitals, the system provides an appointment every 10 minutes on average. When we include patients who are taken without an appointment to avoid disruption of their treatment, the examination time per patient sometimes reaches 3-5 minutes. In some hospitals, the number of patients examined by cardiologists per day exceeds 100. The current situation brings with it many problems. First of all, since this period is short, doctors have great difficulty making a diagnosis. There is not enough time for the physician to make a differential diagnosis. Some diseases may be overlooked. If something happens to the patient, the doctor may regret it for life. Additionally, many legal cases can be filed. In short, psychological problems may arise in the physician as a result of many material and moral losses. Short examination times are not good not only for the doctor but also for the patient. Due to the short examination period, the diseases of patients who do not receive adequate and effective treatment may increase. Patients may be dissatisfied because less time is allocated to them. Or he may go from doctor to doctor in search of healing. This situation also leads to a waste of public resources.

In other countries, the examination time allocated per patient is longer than in Turkey. A study conducted in the USA reported that doctors spend an average of 20 minutes per patient and see 11–20 patients a day [3]. On the other hand, the use of artificial intelligence in health is inevitable today, and it is known that it is taught as a course in some medical faculties [4,5]. Artificial intelligence and machine learning can be used to prevent these problems. Studies

have shown that artificial intelligence and machine learning can be useful in the diagnosis of diseases, differential diagnosis, treatment selection, and identification of risky patients [6,7].

As a result, it is extremely important for the relevant authorities to review the inspection periods in consultation with professional organizations due to possible risks. In addition, since we cannot reduce the number of people applying to the hospital and increase the number of doctors in the short term, it would be appropriate to carry out the necessary studies to make artificial intelligence and machine learning a part of the examination for some branches.

Best Regards,

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REFERENCES

[1] Yardım MS, Eser E (2017) How many minutes should be allocated per patient for outpatient diagnosis and treatment applications? Türk J Public Health. 15(1), 58. https://doi.org/10.20518/tjph.326827

[2] Hickson GB, Clayton EW, Entman SS, Miller CS, Githens PB, Whetten-Goldstein K, Sloan FA (1994) Obstetricians' prior malpractice experience and patients' satisfaction with care. 272(20):1583–1587. https://doi.org/10.1001/jama.1994.03520200039032

[3] Number of patients U.S. physicians saw per day 2018 | Statista. https://www.statista.com/statistics/613959/us-physicans-patients-seen-per-day/#statisticContainer. Accessed Date 25 June 2024

[4] Naqvi WM, Sundus H, Mishra G, Muthukrishnan R, Kandakurti PK (2024) AI in Medical Education Curriculum: The Future of Healthcare Learning. Eur J Ther 30:e23-e25. https://doi.org/10.58600/eurjther1995

[5] İmre O (2024) Positive and Negative Aspects of Artificial Intelligence in Psychiatry: Psychiatry and Artificial Intelligence. IJCMBS. 4(2) 27-8 https://doi.org/10.5281/zenodo.10889523

[6] Hayıroğlu Mİ, Altay S (2023) The Role of Artificial Intelligence in Coronary Artery Disease and Atrial Fibrillation. Balkan Med J. 40(3):151-152. https://doi.org/10.4274/balkanmedj.galenos.2023.06042023

[7] Yilmaz R (2023) Artificial Intelligence Evaluation of the Utility of HALP Score and Hematological Indicators in Estimating No-Reflow After Primary Percutaneous Coronary Intervention in Patients With ST-Segment Elevation Myocardial Infarction: Artificial Intelligence and HALP Score No-Reflow After PCI. IJCMBS. 3,147-55. https://doi.org/10.5281/zenodo.10020501

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Letter to Editor

The Influence of Referees on Romeo and Juliet

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Dear Editors,

It is a common situation, especially for young researchers, to receive decision letters from a journal office saying: "Major revision required - No guarantee of acceptance" or "We regret to reject." It is said that experiencing repeated major revisions and even rejections make a researcher more mature, but what is a young researcher to do when even Dr Hwang is badly affected by this situation? [1] Then I read Kundakçı's letter [2] and became even more pessimistic. I worried what if the referees turned into artificial intelligence and even more difficult times awaited the writers. So, I decided to write this letter, thinking that it might be interesting to discuss ideas on the subject with the metaphor of Romeo and Juliet. We all know William Shakespeare's magnificent work Romeo and Juliet [3]. In this work, Romeo and Juliet, who fell in love with each other, failed to be patient in their plans and could not come together until they died. And a great debate has been going on for centuries: What makes their love interesting and unforgettable for all humanity is the greatness of their love or the fact that they could never unite?

In fact, there is a metaphorical relationship between referees and "Romeo and Juliet". Could it be that what determines our love for science and our desire to write scientific articles and makes it unique is that we cannot achieve success as a result of the comments of the referees? Or is it the magnitude of our love for writing that drives us to our enthusiasm for writing?

Generally, it is not taught how to do peer review for scientific journals. If people have not made a special effort to learn, they learn the role of the referee from their own experiences or from studies previously published in the journal. This is actually a situation that may cause some problems in the standardization of the work done. Since the editors will reach a decision based on the recommendations of the referees, the qualifications of the referees and their scientific article evaluation behavior patterns become decisive in this regard.

Since referees are also writers and are not free from all the prejudices of the age, they must act independently of all positive or negative influences when evaluating the manuscripts [4, 5]. The publishing policies of journals (double blind or single blind, etc.) supporting this independence

is the way to prevent this great love from disappearing and not growing further with new separations.

In the story of Romeo and Juliette we see Count Paris who tries to separate them and wants to marry Juliette, and Friar Laurence who is the wise adviser and tries to reunite them. As writers, editors or referees, we scientists perhaps have two options: to hinder this love of science, like Count Paris, or to advance the love of science, like Friar Laurence. Or in other words "*to be or not to be*" a scientist, that is the question.

Kind Regards

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REFERENCES

[1] Hwang K (2021) You Will Be a Researcher, My Student! J Craniofac Surg 32:457. https://doi.org/10.1097/SCS.0000000000005319

[2] Kundakcı YE (2023) ChatGPT's Capabilities for Use in Anatomy Education and Anatomy Research. Eur J Ther 30:200-202. https://doi.org/10.58600/eurjther1842

[3] Shakespeare W (2019) Romeo and juliet. One-Hour Shakespeare. Routledge, pp 304-368.

[4] Fox CW, Meyer J, Aimé E (2023) Double-blind peer review affects reviewer ratings and editor decisions at an ecology journal. Functional Ecology 37:1144-1157. https://doi.org/10.1111/1365-2435.14259

[5] Tennant JP, Ross-Hellauer T (2020) The limitations to our understanding of peer review. Res Integr Peer Rev 5:6. https://doi.org/10.1186/s41073-020-00092-1

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Letter to Editor

A Rare Syndrome in Adolescents: Capgras Syndrome and Its Psychopharmacological Treatment

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ABSTRACT

In this letter to editor, while explaining the process leading to Capgras syndrome (CS), it will be tried to evaluate the psychodynamic, cognitive, neurological, and systemic factors as a result of the anamnesis and detailed examinations. Clinical aspects of the syndrome, psychopharmacologic agents to be used in the treatment process, and these agents' mechanisms of acts will be discussed. Y.B., a 16-year-old female patient, was admitted to our clinic by her family with complaints of restlessness, introversion, and voices in her ears. A psychiatric examination revealed that the patient had persecutory, referential, and somatic delusions and that her family was replaced with other families in her thought content. In the initial treatment of the patient, risperidone was preferred, and sertraline was added to her treatment after the regression in her symptoms was not at the desired level. CS is the most common type of misidentification syndrome. The patient believes that their parents, friends, or themselves have been replaced by people similar to them and may show strong hostility and distrust toward their environment. Although there is no single cause, biological and psychological factors are thought to play a role in its etiopathogenesis. When the literature is examined, it is seen that there are reports of CS in adults, and the number of case reports in adolescence is lower. It should be kept in mind that although rare, CS can be seen in the pediatric and young population and the addition of selective serotonin reuptake inhibitors may be beneficial in cases that do not respond to antipsychotic treatment.

Keywords: Capgras syndrome; adolescent psychiatry; psychotic disorders

Dear Editor,

Delusional misidentification syndromes (DMSs) are considered rare psychopathological phenomena encountered in psychiatric and neurological conditions. It is defined as a delusion in which the person believes that the people, places, objects, and events around him or her have changed or that the same ones have multiplied.[1] Capgras syndrome (CS) is the most common type of misidentification syndrome [1]. The patient believes that their parents, friends, or themselves have been replaced by people similar to them [2]. It is a rare psychiatric disorder characterized by a delusional belief that real and familiar people

have been replaced by mysterious, sometimes malicious, or morally reprehensible impostors [2]. In addition, it is considered a delusional disorder as patients are not expected to improve with explanation and decrease in symptoms over time [2]. Affected individuals have a clear consciousness, often with intact cognitive functions [3]. They may typically show strong hostility and distrust toward their environment [2,3]. Findings on prevalence are generally made from case series including psychotic disorders, and when the literature is examined, it is reported that the diagnosis of CS is higher, especially in male patients diagnosed with paranoid schizophrenia [4]. In a study conducted in the United States in 1983, all 4200 patients admitted to psychiatric emergencies were reviewed, and six cases were found to have a Capgras delusion (0.14% overall and 0.17% of psychotic disorder cases) [5]. There are studies reporting that the prevalence is 0.17/100,000 in adolescents and 0.0032/100,000 in children aged 2–12 years [6]. When the literature is examined, it is seen that there are reports of CS in adults, and the number of case reports in adolescence is lower. In this case, a 16-year-old girl with CS accompanied by somatic delusions, clinical aspects of the syndrome, and psychopharmacologic agents to be used in the treatment process will be discussed.

Patient Information

Y.B., a 16-year-old female patient, was brought to our outpatient clinic by her mother with a complaint of restlessness. According to the history taken from the patient and her family, her complaints started 1.5 years ago, she talked to her middle school mathematics teacher, and she said that her exam paper had been changed in the high school entrance exams, so she could not be placed in the high school she wanted. She repeated the same sentences, and she was not satisfied with the high school she attended. When she told her family that she wanted to investigate this issue and claim her rights, she could not get enough support from them. As a result, she became introverted, especially in the last 1 year; her introversion worsened; she tried to talk to her former middle school mathematics teacher for the last 6 months without her family's knowledge; and she had a sleep disorder in the last few months in the form of not being able to sleep at night and spending the day sleeping. It was learned that she had been saying "You are not my real parents" for the last 3 weeks because her family did not help her in the process of trying to contact her math teacher, and that this situation, which her family initially thought she said in anger, worsened over time, and for the last 10 days she's been saying "My parents live in England; you are not my real parents; you are copies of them." In her psychiatric evaluation, it was determined that she had persecutory delusions that her family and friends were constantly trying to prevent her from being placed in the high school she wanted; grandiose delusions that she was smarter than her other friends and family members; referential delusions that her math teacher's social media posts gave messages about the plans they had prepared to prevent her from being placed in the high school she wanted; somatic delusions that her skin and eye color were changed by her fake family while her skin was normally white and her eyes were blue because she was British; and she had peripheral speech in the thought process. It was learned that the patient heard voices commenting on her delusions during the day. A preliminary diagnosis of "Schizophrenia" was made for the patient whose mood was euthymic and whose affective state was inappropriate, and an electroencephalogram examination, a radiological examination, extensive biochemical examinations, and hormonal examinations were additionally ordered for a differential diagnosis in terms of organicity. All the tests were determined to be within normal limits, and no findings were found on the radiologic examination of the patient. There were no additional findings on the physical and neurologic examinations. In her medical history, it was learned that she was born with a normal birth in the hospital with the help of a midwife, there was no delay in language development, her parents separated during her early childhood and her grandmother took care of her during this period, she had no academic difficulties at the beginning of primary school, and she generally had above-average success at school. No psychotic disorder was reported in the psychiatric family history, and there was no family member with a psychiatric admission. At the time of the patient's first admission, the Clinical Global Impressions Scale severity score was 6 and the Positive and Negative Syndrome Scale (PANSS) score was 116. Organicity was ruled out, and risperidone was gradually started at 2 mg/day. After 1 week, the patient was seen at the follow-up visit and was reported to have anxious and aggressive behaviors. Lorazepam (1 mg/day) was added to her treatment. The patient's symptoms started to regress in the second week of treatment. In the 3rd week of follow-up, it was learned that her delusions and discourses toward her family continued significantly; risperidone treatment was increased to 3 mg/day; the lorazepam dose was decreased; and sertraline 50 mg was added. In the 4th week of follow-up, the patient's persecutory and referential delusions decreased, and somatic delusions improved in the 5th week of follow-up. In the 6th week of follow-up, the patient's delusions improved significantly with 3 mg risperidone and 50 mg sertraline, and according to the information obtained from

her family, there was a decrease in her previous discourse. At the end of the second month, the patient's delusions significantly decreased, no hallucinations were observed, and the PANSS score was 82. The patient's follow-up with the current treatment continues.

DISCUSSION

In this case, we have described CS in a 16-year-old patient with psychosis and somatic delusions. The literature review revealed that there are limited numbers of case reports of adolescents compared to adult case reports, and therefore our case report will contribute to the existing literature. CS, which is one of the DMSs and was first described in an article written by Joseph Capgras, a French psychiatrist, and named after him, was defined as a syndrome that progresses with delusions that the close environment, especially family members, have been changed and replaced by other people [1,7]. In Cotard's Syndrome, one of the DMSs that has common characteristics with CS, such as misidentifying objects and events, the patient has nihilistic delusions in the form of denial of his/her entire being or a part of his/her body, while in Fregoli's Syndrome, the patient has delusions that people he/she knows have become strangers [8-10]. CS, which is the most common DMS, can be secondary to some psychiatric disorders, such as psychotic and mood disorders, as well as some neurological disorders, including traumatic brain injury, epilepsy, stroke, and dementia, or systemic disorders such as pseudohypoparathyroidism, copper poisoning, and hypothyroidism [11]. In addition, there are studies reporting delirium, dementia, or mental retardation in patients with an organic cause [11]. However, the imaging and extensive biochemical examinations performed to screen organicity did not reveal any findings in our case.

Psychodynamic methods have been used to explain Capgras syndrome. In the first psychodynamic explanation, it was thought that it might be a defense against aggressive desires that could be attributed to oedipal conflicts in women and a manifestation of latent homosexuality in men [7], but in the following period, it was thought that it was based on anxiety and a cognitive and emotional regression that develops due to this anxiety [12]. When the detailed anamnesis obtained after ruling out organic causes in our patient was examined, it was observed that the first symptoms of persecutory delusions and psychotic processes started when she failed to achieve the desired success in high school entrance exams. The patient, who could not receive the support she expected from her family, had a one-year separation period in her early childhood, and she lived with her grandmother during this period. It is known that the mother's inability to act as a "shield of protection" will lead to the child receiving a high amount of impulse-based stimuli from the inner world and perceptually overwhelming input from the outside world as well [13]. It was thought that the patient, who could not receive the support she expected from her family after the high school transition exam, was cognitively and emotionally regressed, and that the patient had feelings of alienation and unacceptable bad feelings and images. When the literature is examined, it is reported that the patients stated that the replacement person almost perfectly resembles the original, but they had a significant deficit in forming bonds that would allow them to establish intimacy with the misidentified person [14]. Similar to the literature, based on the fact that her real family would not treat her in such a way, through compartmentalization mechanisms, our patient thought that the people who aroused such thoughts in her could not be the people she previously cared for and loved; they could only be imposters who replaced them, and her real family would support her. Even if a psychodynamic explanation can be made for CS developing in the background of psychosis, as in our case, detailed neuropsychiatric evaluation and psychopharmacologic treatment should be initiated. Because of the low number of cases, especially in children and adolescents, a specific treatment study could not be performed. However, in addition to this, it was observed that antipsychotic drugs were more preferred as the first choice in case reports in the literature, and therefore risperidone treatment was initiated in our patient [15]. When the literature is reviewed, there are case reports of improvement with the addition of antidepressants such as selective serotonin reuptake inhibitors (SSRI) and mirtazapine in cases of delayed response to antipsychotics [16]. In our case, similarly, risperidone treatment was started, and sertraline was added to the treatment because of the lack of expected decrease in symptoms during the follow-up period. After the addition of sertraline treatment, her symptoms decreased. The effects of atypical antipsychotics are due to their blockade of dopamine (DA) 2 receptors, which improves positive symptoms but has little effect on negative symptoms, cognitive impairment, and depressive symptoms [17]. The addition of sertraline in the absence of response to atypical antipsychotics is related to the effects of sertraline on the serotonin (5-HT) system. The combination of sertraline may act on negative symptoms associated with insufficient activation of DA and 5-HT neurons in the prefrontal cortex by means of selective inhibition of serotonin reuptake by sertraline. In particular, sertraline also increases dopaminergic activity by blocking DA transporters

and reuptake [18,19]. These mechanisms may explain how sertraline improves negative and cognitive symptoms. Although our patient did not have these symptoms, we added sertraline to the treatment. Positive symptoms in patients with psychotic disorders are closely associated with central DA hyperfunction [20]. Increased central 5-HT levels may further alleviate patients' positive symptoms by inhibiting DA activity [21]. This latest mechanism may also explain that sertraline enhanced the recovery of positive symptoms, although our patient did not have negative symptoms, depression or cognitive impairment. During treatment, caution should be exercised, especially in terms of metabolic side effects and extrapyramidal symptoms. However, no metabolic side effects were observed in our patient at the end of the third month.

CONCLUSION

In this case, while trying to explain the process leading to CS, we tried to evaluate psychodynamic, cognitive, neurological, and systemic factors as a result of the anamnesis and detailed examinations. We think that our case will contribute to the literature since the development of the condition at a young age is rare. As in our case, patients who are thought to have DMS should be examined in detail in terms of their organic etiology. A holistic evaluation seems to be more valuable in conceptualizing cases than establishing a single model. It is known that a holistic perspective is more valuable in the evaluation of cases. In the treatment of our patient, it was observed that the SSRI group of drugs added to antipsychotic drug treatment accelerated symptomatic improvement, which is consistent with the literature. It was thought that the combination of antipsychotic treatment with SSRIs should be considered in these patients. In addition, it should be kept in mind that CS may also be seen in the pediatric and young populations, although it is rarer. There is a need for more case reports on the clinical presentation and treatment of CS in children and adolescents.

Regards,

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REFERENCES

[1] Cipriani G, Vedovello M, Ulivi M, Lucetti C, Di Fiorino A, Nuti A (2013) Delusional misidentification syndromes and dementia: a border zone between neurology and psychiatry. Am J Alzheimers Dis Other Demen 28(7):671-678. https://doi.org/10.1177/1533317513506103

[2] Berson RJ (1983) Capgras' Syndrome. Am J Psychiatry 140:969–978. https://doi.org/10.1176/ajp.140.8.969

[3] Ellis HD, Luauté JP, Retterstøl N (1994) Delusional misidentification syndromes. Psychopathology 27:117–120. https://doi.org/10.1159/000284856

[4] Ahn BH, Kim JH, Oh S, Choi SS, Ahn SH, Kim SB (2012) Clinical features of parricide in patients with schizophrenia. Aust N Z J Psychiatry 46:621–629. https://doi.org/10.1177/0004867412442499

[5] Fishbain DA (1987) The frequency of Capgras delusions in a psychiatric emergency service. Psychopathology 20:42–47. https://doi.org/10.1159/000284478

[6] Förstl H, Almeida OP, Owen AM, Burns A, Howard R (1991) Psychiatric, neurological and medical aspects of misidentification syndromes: a review of 260 cases. Psychol Med 21:905–910. https://doi.org/10.1017/s0033291700029895

[7] Capgras J, Carrette P (1924) Illusions des sosies et complex d'oedipe. Ann Medico-psychol; 82:48-68.

[8] Young AW, Leafhead KM, Szulecka TK (1994) Capgras and Cotard delusions. Psychopathology 27:226–231. https://doi.org/10.1159/000284874

[9] Berrios GE, Luque R (1995) Cotard Syndrome: clinical analysis of 100 cases. Acta Psychiatr Scand 91:185–188. https://doi.org/10.1111/j.1600-0447.1995.tb09764.x

10] Berrios GE, Luque R. (1999) Cotard's "On hypochondriacal delusions in a severe form of anxious melancholia". Hist Psychiatry 10:269–278. https://doi.org/10.1177/0957154X9901003806

[11] Salvatore P, Bhuvaneswar C, Tohen M, Khalsa HM, Maggini C, Baldesserani RJ, (2014) Capgras' syndrome in first-episode psychotic disorders. Psychopathology 47:261–269. https://doi.org/10.1159/000357813

[12] Bourget D, Whitehurst L (2004) Capgras Syndrome: a review of the neurophysiological correlates and presenting clinical features in cases involving physical violence. *Can J Psychiatry*; 49:719-725. https://doi.org/10.1177/070674370404901102

[13] Akhtar S (2009) Damaged core, origins, dynamics, indicators and treatment. İzmir, Odağ Psychoanalysis and Psychotherapy Education Services.

[14] Fuchs T (2005). Delusional mood and delusional perception, phenomenological analysis. Psychopathology 38:133–139. https://doi.org/10.1159/000085843

[15] Wright S, Young AW, Hellawell DJ (1993) Sequential Cotard and Capgras delusions. Br J Clin Psychol 32:345–349. https://doi.org/10.1111/j.2044-8260.1993.tb01065.x

[16] Khouzam HR (2002) Capgras syndrome responding to the antidepressant mirtazapine. Compr Ther;28(3):238-240. https://doi.org/10.1007/s12019-002-0033-9

[17] Horacek J, Bubenikova-Valesova V, Kopecek M, Palenicek T, Dockery C, Mohr P (2006) Mechanism of action of atypical antipsychotic drugs and the neurobiology of schizophrenia. CNS Drugs 20(5):389-409. https://doi.org/10.2165/00023210-200620050-00004

[18] Popli AP, Fuller MA, Jaskiw GE (1997) Sertraline and psychotic symptoms: a case series. Ann Clin Psychiatry 9(1):15–7. 31. https://doi.org/10.1023/a:1026274123689

[19] Stahl SM. (2013) Essential Psychopharmacology: Neuroscientific Basis and Practical Applications Stahl's, 4th ed. Antidepressants In: Stahl SM ed. Cambridge university press

[20] Kegeles LS, Abi-Dargham A, Frankle WG, Gil R, Cooper TB, Slifstein M, Hwang DR et al. (2010) Increased synaptic dopamine function in associative regions of the striatum in schizophrenia. Arch Gen Psychiatry 67(3):231–9. https://doi.org/10.1001/archgenpsychiatry.2010.10

[21] Thakore JH, Berti C, Dinan TG (1996). An open trial of adjunctive sertraline in the treatment of chronic schizophrenia. Acta Psychiatr Scand 94(3):194–7. https://doi.org/10.1111/j.1600-0447.1996.tb09848.x

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