ISSN:2149-7893 E-ISSN: 2536-507X

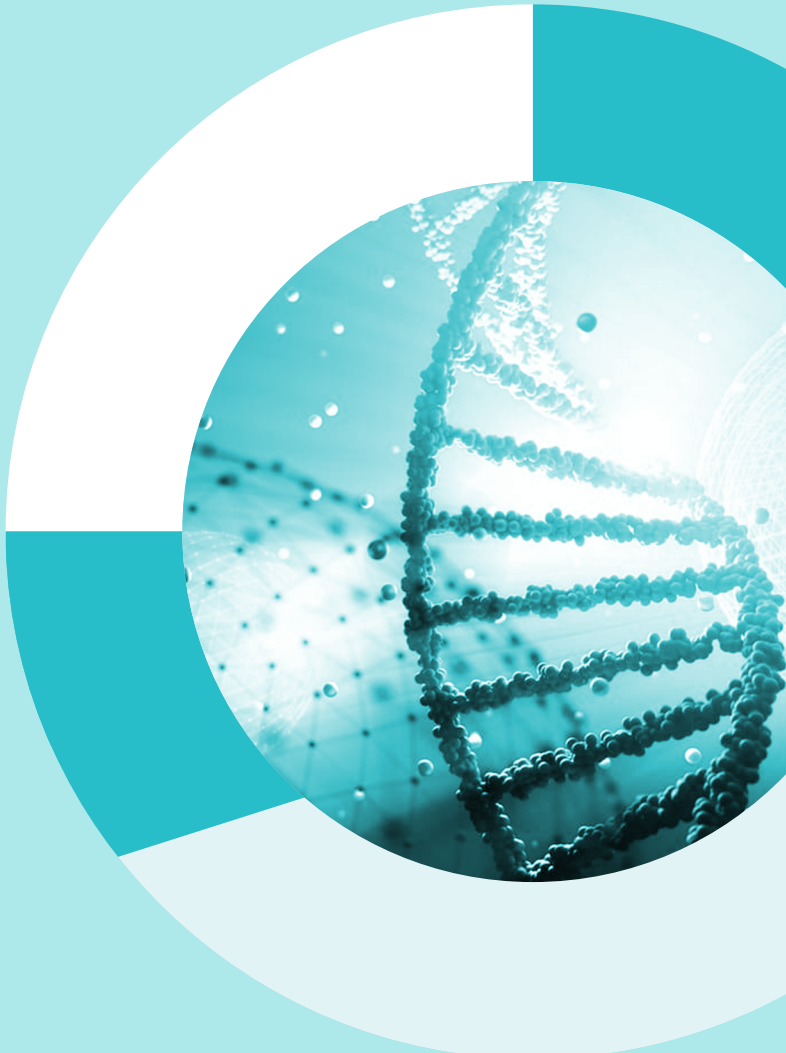
CYPRUS JOURNAL OF MEDICAL SCIENCES

Indexed in the Web of Science

Volume: 8 Issue: 1 February 2023

REVIEW

ORIGINAL ARTICLES



Official Journal of Cyprus Turkish Medical Association

galenos yayınevi

www.cyprusjmedsci.com

CYPRUS
JOURNAL OF MEDICAL SCIENCES
Indexed in Web of Science
Volume: 8 | Issue: 1 | February 2023

EDITORIAL BOARD

Publisher Contact
Address: Molla Gürani Mah. Kaçamak Sk. No: 21/1 34093 İstanbul, Türkiye
Phone: +90 (212) 621 99 25 **Faks/Fax:** +90 (212) 621 99 27
E-mail: info@galenos.com.tr/yayin@galenos.com.tr
Web: www.galenos.com.tr **Yayıncı Sertifika No:** 14521

Publication Date: Aralık 2022/December 2022
E-ISSN: 2536-507X
ISSN: 2149-7893

International scientific journal published bi-annually.

CYPRUS
JOURNAL OF MEDICAL SCIENCES
Indexed in Web of Science
Volume: 8 | Issue: 1 | February 2023

EDITORIAL BOARD

CYPRUS
JOURNAL OF MEDICAL SCIENCES
Indexed in Web of Science
Volume: 8 | Issue: 1 | February 2023

EDITORIAL BOARD

Biostatistical Editors

National Advisory Board

CYPRUS
JOURNAL OF MEDICAL SCIENCES
Indexed in Web of Science
Volume: 8 | Issue: 1 | February 2023

EDITORIAL BOARD

CYPRUS
JOURNAL OF MEDICAL SCIENCES
Indexed in Web of Science
Volume: 8 | Issue: 1 | February 2023

EDITORIAL BOARD

International Advisory Board

CYPRUS
JOURNAL OF MEDICAL SCIENCES
Indexed in Web of Science
Volume: 8 | Issue: 1 | February 2023

EDITORIAL BOARD

CYPRUS
JOURNAL OF MEDICAL SCIENCES
Indexed in Web of Science
Volume: 8 | Issue: 1 | February 2023

EDITORIAL BOARD

CYPRUS
JOURNAL OF MEDICAL SCIENCES
Indexed in Web of Science
Volume: 8 | Issue: 1 | February 2023

EDITORIAL BOARD

CYPRUS
JOURNAL OF MEDICAL SCIENCES
Indexed in Web of Science
Volume: 8 | Issue: 1 | February 2023

AIMS AND SCOPE

Cyprus Journal of Medical Sciences (Cyprus J Med Sci), the official organ of Cyprus Turkish Medical Association.

This journal is an international, open access, scientific, peer-reviewed journal in accordance with independent, unbiased, and double-blinded peer-review principles. As of 2022, the journal has become a bimonthly publication, publishing in February, April, June, and August, October and December. The journal's publication language is English. (E-ISSN:2536-507X)

The aim of the journal is to publish original research papers of the highest scientific and clinical value in all medical fields. Cyprus Journal of Medical Sciences also publishes reviews, rare case report and letters to the editors.

The target audience of the journal includes healthcare professionals physicians, and researchers who are interested or working in in all fields of medicine.

The editorial and publication process of the Cyprus Journal of Medical Sciences 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), and National Information Standards Organization (NISO). The journal is in conformity with the Principles of Transparency and Best Practice in Scholarly Publishing.

Cyprus Journal of Medical Sciences is indexed in Web of Science-Emerging Sources Citation Index, TUBITAK ULAKBIM TR Index, EBSCO, INDEX COPERNICUS, J-GATE and Gale.

All manuscripts must be submitted via the online submission system, which is available at www.cyprusjmedsci.com. The journal guidelines, technical information, and the required forms are available on the journal's web page.

All expenses of the journal are covered by the Cyprus Turkish Medical Association. Potential advertisers should contact the Editorial Office. Advertisement images are published only upon the Editor-in-Chief's approval.

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

All published content is available online, free of charge at www.cyprusjmedsci.com.

Open Access Policy

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

Author(s) and copyright owner(s) grant access to all users for the articles published in the Cyprus Journal of Medical Sciencesas free of charge. Articles may be used provided that they are cited.

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

Creative Commons

The journal's content is licensed under a Creative Commons Attribution-NonCommercial 4.0 International (CC BY-NC 4.0) which permits third parties to share and adapt the content for non-commercial purposes by giving the apropriate credit to the original work.

A Creative Commons license is a public copyright license that provides free distribution of copyrighted works or studies. Authors use the CC license to transfer the right to use, share or modify their work to third parties.

Open access is an approach that supports interdisciplinary development and encourages collaboration between different disciplines. Therefore, Cyprus Journal of Medical Sciences contributes to the scientific publishing literature by providing more access to its articles and a more transparent review process.

Material Disclaimer

Statements or opinions stated in articles published in the journal do not reflect the views of the editors, editorial board and/or publisher; The editors, editorial board and publisher do not accept any responsibility or liability for such materials. All opinions published in the journal belong to the authors.

CYPRUS
JOURNAL OF MEDICAL SCIENCES
Indexed in Web of Science
Volume: 8 | Issue: 1 | February 2023

INSTRUCTIONS TO AUTHORS

Copyright Agreement and Acknowledgement of Authorship Form

ICMJE Form

Cyprus Journal of Medical Sciences (Cyprus J Med Sci) is the scientific, peer-reviewed, open-access international publication organ of Cyprus Turkish Medical Association. The journal is published bimonthly in February, April, June, and August, October and December. The journal's publication language is English.

The journal aims to publish original research papers of the highest scientific and clinical value in all medical fields. Cyprus Journal of Medical Sciences also publishes reviews, rare case reports and letters to the editors.

The target audience of the journal includes healthcare professionals, physicians, and researchers who are interested or working in all fields of medicine.

To read the Article Processing Charge (APC) Policy, please click here.

EDITORIAL AND PUBLICATION PROCESS

The editorial and publication process of the Cyprus Journal of Medical Sciences 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), and National Information Standards Organization (NISO). The journal is in conformity with the Principles of Transparency and Best Practice in Scholarly Publishing.

Originality, high scientific quality, and citation potential are the most significant 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.

PEER REVIEW PROCESS

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

ETHICAL PROCEDURES

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) 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 protect the patients' anonymity carefully.

For photographs that may reveal the identity of the patients, signed consent of the patient or their legal representative should be enclosed, and the publication approval must be provided in the "Materials and Methods" section. However, the identities of the patients should be concealed in the photographs.

PLAGIARISM

Cyprus Journal of Medical Sciences is extremely sensitive about plagiarism. All submissions are screened by a similarity detection software (iThenticate by CrossCheck) at any point during the peer-review and/or production process. Even if you are the author of the phrases or sentences, the text should not have unacceptable similarity with the previously published data.

CYPRUS
JOURNAL OF MEDICAL SCIENCES
Indexed in Web of Science
Volume: 8 | Issue: 1 | February 2023

INSTRUCTIONS TO AUTHORS

When you are discussing others' (or your own) previous work, please make sure that you cite the material correctly in every instance.

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 following COPE guidelines.

PREPRINT

Authors must provide the journal with the preprint server deposition of their article accompanying its DOI during initial submission.

Cyprus Journal of Medical Sciences does not consider preprint publications before publication. In other words, authors are allowed to present and discuss their findings on a non-commercial preprint server before submission to the journal.

If the article is published in the Cyprus Journal of Medical Sciences, it is the responsibility of the authors to update the archived preprint and link it to the published version of the article.

AUTHORSHIP

Each person listed as an author should fulfill the authorship criteria recommended by http://www.icmje.org/recommendations/browse/roles-and-responsibilities/defining-the-role-of-authors-and-contributors.html). The ICMJE recommends that authorship is based on the following four 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. Also, 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 on the title page of the manuscript.

Cyprus Journal of Medical Sciences requires corresponding authors to submit a signed and scanned version of the Copyright Agreement and Acknowledgement of Authorship form (available for download www.cyprusjmedsci.com) during the initial submission process 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.

DISCLOSURE AND CONFLICTS OF INTEREST

All sources of financial support should be disclosed. All authors ought to disclose a meaningful conflict of interest in the process of forming their study. Any financial grants or other support received for a submitted study from individuals or institutions should be disclosed to the Editorial Board of the Cyprus Journal of Medical Sciences. The ICMJE Potential Conflict of Interest Disclosure Form should be filled in and submitted by all contributing authors to disclose a potential conflict of interest. The journal’s Editorial Board determines cases of a potential conflict of interest of the editors, authors, or reviewers 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 claims that cannot be resolved internally. The Editor in Chief is the final authority in the decision-making process for all appeals and complaints.

CYPRUS
JOURNAL OF MEDICAL SCIENCES
Indexed in Web of Science
Volume: 8 | Issue: 1 | February 2023

INSTRUCTIONS TO AUTHORS

COPYRIGHT AND LICENSE

A Creative Commons license is a public copyright license that provides free distribution of copyrighted works or studies. Authors use the CC license to transfer the right to use, share or modify their work to third parties.

Open access is an approach that supports interdisciplinary development and encourages collaboration between different disciplines. Therefore, Cyprus Journal of Medical Sciences contributes to the scientific publishing literature by providing more access to its articles and a more transparent review process.

The journal's content is licensed under a Creative Commons Attribution-NonCommercial 4.0 International (CC BY-NC 4.0) which permits third parties to share and adapt the content for non-commercial purposes by giving the appropriate credit to the original work.

DISCLAIMER

Statements or opinions expressed in the manuscripts published in Cyprus Journal of Medical Sciences reflect the views of the author(s) and not the opinions of the editors, the editorial board, or the publisher; the editors, the editorial board, and the publisher disclaim any responsibility or liability for such materials. The final responsibility regarding the published content rests with the authors.

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 December 2019). 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.cyprusjmedsci.com. Manuscripts submitted via any other medium and submissions by anyone other than one of the authors will not be evaluated.

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

Authors are required to submit the following:

- Copyright Agreement and Acknowledgement of Authorship Form, and
- ICMJE Potential Conflict of Interest Disclosure Form (should be filled in by all contributing authors) during the initial submission. These forms are available for download at www.icmje.org.

Preparation of the Manuscript

Title page: A separate title page should be submitted with all submissions and this page should include:

The full title of the manuscript as well as a short title (running head) of no more than 50 characters,

- Name(s), affiliations, highest academic degree(s), and ORCID IDs of the author(s),
- Grant information and detailed information on the other sources of support,
- Name, address, telephone (including the mobile phone number), and e-mail 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 (Background/Aims, Material and Methods, Results and Conclusion). Please check Table 1 below for word count specifications.

Keywords: Each submission must be accompanied by a minimum of three to a maximum of five 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/MBrowser.html).

CYPRUS
JOURNAL OF MEDICAL SCIENCES
Indexed in Web of Science
Volume: 8 | Issue: 1 | February 2023

INSTRUCTIONS TO AUTHORS

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" target the experts and specialists of the field, each item should be written as plain and straightforward as possible.

Manuscript Types

Original Articles: This is the most important type of article since it provides new information based on original research. Acceptance of original papers will be based upon the originality and importance of the investigation. The main text of original articles should be structured with Introduction, Material and Methods, Results, and Discussion subheadings. An original article can be signed by maximum 6 authors unless it is a multi-center study or that it required extensive labour. Please check Table 1 for the limitations for Original Articles.

Clinical Trials

Cyprus Journal of Medical Sciences adopts the ICMJE's clinical trial registration policy, which requires that clinical trials must be registered in a publicly accessible registry that is a primary register of the WHO International Trials Registry Platform (ICTRP) or in ClinicalTrials.gov.

Instructions for the clinical trials are listed below.

Clinical trial registry is only required for the prospective research projects that study the relationship between a health-related intervention and an outcome by assigning people.

- To have their manuscript evaluated in the journal, author should register their research to a public registry at or before the time of first patient enrollment.
- Based on most up to date ICMJE recommendations, Cyprus Journal of Medical Sciences accepts public registries that include minimum acceptable 24-item trial registration dataset.
- Authors are required to state a data sharing plan for the clinical trial registration. Please see details under "Data Sharing" section.
- For further details, please check ICMJE Clinical Trial Policy at

http://www.icmje.org/recommendations/browse/publishing-and-editorial-issues/clinical-trial-registration.html

Data Sharing

As of 1 January 2019, a data sharing statement is required for the registration of clinical trials. Authors are required to provide a data sharing statement for the articles that reports the results of a clinical trial. The data sharing statement should indicate the items below according to the ICMJE data sharing policy:

Whether individual deidentified participant data will be shared

- What data in particular will be shared
- Whether additional, related documents will be available
- When the data will be available and for how long
- By what access criteria will be shared

Authors are recommended to check the ICMJE data sharing examples at

http://www.icmje.org/recommendations/browse/publishing-and-editorial-issues/clinical-trial-registration.html

While submitting a clinical trial to Cyprus Journal of Medical Sciences;

- Authors are required to make registration to a publicly accessible registry according to ICMJE recommendations and the instructions above.
- The name of the registry and the registration number should be provided in the Title Page during the initial submission.
- Data sharing statement should also be stated in the Title Page even the authors do not plan to share it.

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

CYPRUS
JOURNAL OF MEDICAL SCIENCES
Indexed in Web of Science
Volume: 8 | Issue: 1 | February 2023

INSTRUCTIONS TO AUTHORS

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).

Editorial Comments: Invited brief editorial comments on selected articles are published in The Cyprus Journal of Medical Sciences. Editorials should not be longer than 1000 words excluding references. Editorial comments aim to provide a brief critical commentary by reviewers with expertise or with high reputation in the topic of the research article published in the journal. Authors are selected and invited by the journal to provide such comments. Abstract, Keywords, and Tables, Figures, Images, and other media are not included.

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 subheadings of the review articles should be planned by the authors. However, each review article should include an "Introduction" and a "Conclusion" section. Please check Table 1 for the limitations for Review Articles.

Case Reports: There is limited space for case reports in the journal and reports on rare cases or conditions that constitute challenges in diagnosis and treatment, those offering new therapies or revealing knowledge not included in the literature, and interesting and educative case reports are accepted for publication. The text should include Introduction, Case Presentation, and Discussion with an unstructured abstract. Please check Table 1 for the limitations for Case Reports.

Letters to the Editor: This type of manuscript discusses important parts, overlooked aspects, or lacking parts of a previously published article. Articles on subjects within the scope of the journal that might attract the readers' attention, particularly educative cases, may also be submitted in the form of a "Letter to the Editor." Readers can also present their comments on the published manuscripts in the form of a "Letter to the Editor." Abstract, Keywords, and Tables, Figures, Images, and other media should not be included. The text should be unstructured. The manuscript that is being commented on must be properly cited within this manuscript.

Table 1. Limitations for each manuscript type

Type of manuscript	Word limit	Abstract word limit	Reference limit	Table limit	Figure limit
Original Article	4000	250 (Structured)	35	6	5 or total of 10 images
Review Article	5000	250	50	6	10 or total of 15 images
Case Report	1200	200	15	No tables	4 or total of 8 images
Letter to the Editor	400	No abstract	5	No tables	No media

Tables

Tables should be included in the main document, presented after the reference list, and they should be numbered consecutively in the order they are referred to within the main text. A descriptive title must be placed above the tables. Abbreviations used in the tables should be defined below the tables by footnotes (even if they are defined within the main text). Tables should be created using the "insert table" command of the word processing software and they should be arranged clearly to provide easy reading. Data presented in the tables should not be a repetition of the data presented within the main text but should be supporting the main text.

Figures and Figure Legends

Figures, graphics, and photographs should be submitted as separate files (in TIFF or JPEG format) through the submission system. The files should not be embedded in a Word document or the main document. 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. The minimum resolution of each submitted figure should be 300 DPI. To prevent delays in the evaluation process, all submitted figures should be clear in resolution and large in size (minimum dimensions: 100 × 100 mm). Figure legends should be listed at the end of the main document.

CYPRUS
JOURNAL OF MEDICAL SCIENCES
Indexed in Web of Science
Volume: 8 | Issue: 1 | February 2023

INSTRUCTIONS TO AUTHORS

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.

When a drug, product, hardware, or software program is mentioned within the main text, product information, including the name of the product, the producer of the product, and city and the country of the company (including the state if in USA), should be provided in parentheses in the following format: "Discovery St PET/CT scanner (General Electric, Milwaukee, WI, USA)"

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.

Limitations, drawbacks, and the shortcomings of original articles should be mentioned in the Discussion section before the conclusion paragraph.

References

Both in-text citations and the references must be prepared according to the Vancouver style.

While citing publications, preference should be given to the latest, most up-to-date publications. Authors are responsible for the accuracy of references 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. When there are six or fewer authors, all authors should be listed. If there are seven or more authors, the first six authors should be listed followed by "et al." 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: Yazıcı A. The efficacy of endoscopic ventilation tube insertion in pediatric populations. Cyprus J Med Sci. 2019; 4(2): 73-6.

Book Section: Suh KN, Keystone JS. Malaria and babesiosis. Gorbach SL, Barlett JG, Blacklow NR, editors. Infectious Diseases. Philadelphia: Lippincott Williams; 2004.p.2290-308.

Books with a Single Author: Sweetman SC. Martindale the complete drug reference. 34th ed. London: Pharmaceutical Press; 2005.

Editor(s) as Author: Huizing EH, de Groot JAM, editors. Functional reconstructive nasal surgery. Stuttgart-New York: Thieme; 2003.

Conference Proceedings: Bengisson S. Sothemin BG. Enforcement of data protection, privacy and security in medical informatics. In: Lun KC, Degoulet P, Piemme TE, Rienhoff O, editors. MEDINFO 92.

Proceedings of the 7th World Congress on Medical Informatics; 1992 Sept 6-10; Geneva, Switzerland. Amsterdam: North-Holland; 1992. pp.1561-5.

Scientific or Technical Report: Cusick M, Chew EY, Hoogwerf B, Agrón E, Wu L, Lindley A, et al. Early Treatment Diabetic Retinopathy Study Research Group. Risk factors for renal replacement therapy in the Early Treatment Diabetic Retinopathy Study (ETDRS), Early Treatment Diabetic Retinopathy Study Kidney Int: 2004. Report No: 26.

Thesis: Yılmaz B. Ankara Üniversitesindeki öğrencilerin beslenme durumları, fiziksel aktiviteleri ve beden kitle indeksleri kan lipidleri arasındaki ilişkiler. H.Ü. Sağlık Bilimleri Enstitüsü, Doktora Tezi. 2007.

Manuscripts Accepted for Publication, Not Published Yet: Slots J. The microflora of black stain on human primary teeth. Scand J Dent Res. 1974.

Epub Ahead of Print Articles: Cai L, Yeh BM, Westphalen AC, Roberts JP, Wang ZJ. Adult living donor liver imaging. Diagn Interv Radiol. 2016 Feb 24. doi: 10.5152/dir.2016.15323. [Epub ahead of print].

Manuscripts Published in Electronic Format: Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis (serial online) 1995 Jan-Mar (cited 1996 June 5): 1(1): (24 screens). Available from: URL: http:/ www.cdc.gov/ncidodIElD/cid.htm.

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

CYPRUS
JOURNAL OF MEDICAL SCIENCES
Indexed in Web of Science
Volume: 8 | Issue: 1 | February 2023

INSTRUCTIONS TO AUTHORS

manuscript is not submitted within the allocated time, the revision option may be canceled. If the submitting author(s) believe that additional time is required, they should request this extension before the initial 30-day period is over.

Accepted manuscripts are copy-edited for grammar, punctuation, and format by professional language editors. 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.

CYPRUS
JOURNAL OF MEDICAL SCIENCES
Indexed in Web of Science
Volume: 8 | Issue: 1 | February 2023

CONTENTS

REVIEW

CYPRUS
JOURNAL OF MEDICAL SCIENCES

DOI: 10.4274/cjms.2022.2021-217
Cyprus J Med Sci 2023;8(1):1-7

Synthetic Meshes in Hernia Surgery

Ali Özant, Kalbim Arslan

Department of General Surgery, Near East University Faculty of Medicine, Nicosia, North Cyprus

Abstract

Hernia surgery is among the most common surgical procedures, and more than 80% of them are mesh repairs. Since the introduction of synthetic mesh materials in the 1890s, studies have continued the search for the ideal mesh material with physical, mechanical, structural properties and biocompatibility. It is crucial for surgeons to know the basic structural properties such as the textile fundamentals, porosity, mesh weight, mesh shrinkage and mechanical properties such as mesh bursting strength, elasticity and the anisotropic behavior of the mesh materials in order to make a proper mesh choice. First generation meshes, which are produced from single non-absorbable material are classified as macro-porous, micro-porous or macro-porous with multifilament or microporous components. Second generation meshes are produced from more than one synthetic material. Polypropylene, polyester and polytetrafluoroethylene are the materials used, as in the first generation meshes. As a composite system, other materials such as polyvinylidene fluoride, titanium, poliglecaprone 25 and omega 3 are also combined with these synthetic materials. If the selected mesh is to be used for intra-peritoneal placement, it should be one with a barrier in order to reduce the risk of intestinal adhesions. If the selected mesh material has anisotropic properties, the orientation of the mesh is necessary in the proper direction to match the physiological stretchability, and hence to reduce recurrence.

Keywords: Synthetic surgical meshes, mesh materials, physical properties, mechanical properties

INTRODUCTION

A hernia is described as the protrusion of an organ or tissue through the wall of the physiological cavity. Hernias are classified according to their anatomical locations. The main types of anterior abdominal wall hernias are; inguinal (the most common, accounting for about 75-80% of all types), femoral, umbilical-paraumbilical, epigastric, spigelian, parastomal and incisional hernias.[1] It is well known that the occurrence of hernia is a multifactorial problem incorporating anatomical weakness and many predisposing factors, such as genetic factors and connective tissue disorders.[2] Hernia repair is among the most frequently performed surgical procedures in general surgery. More than 20 million inguinal hernia surgeries are performed annually worldwide, and in the United States, more than 80% of them are mesh repairs.[3] It is obvious that, after the acceptance of mesh implants for hernia repair in both open and laparoscopic repairs, there has been an evident decrease in recurrence rates. Before the introduction of mesh techniques, the expected recurrence rates for primary inguinal hernia repairs and recurrent hernia repairs were 10-30% and 35%, respectively.[4] The recurrence rates after mesh implants were 0-1.7% for tension-free repairs and 0-0.4% for totally extra peritoneal repairs.[5] Although the recurrence rates dropped markedly with the use of prosthetic materials, they still produce some undesired outcomes such as contraction, infection and adhesion between the visceral side and adjacent organs. The prosthetic material used should allow healthy tissue in-growth for incorporation, retaining enough strength to resist abdominal pressure, and sufficient elasticity under physiological pressure and corresponding anisotropy.[6] Since the introduction of prosthetic materials in hernia surgery, many studies have been conducted to find the ideal material in terms of its physical structure and biological responses. The majority of studies have focused on the physical and mechanical properties of mesh materials, such as their weight, pore size and filament type. The aim of this article was to review the main prosthetic materials, structural and mechanical properties of meshes, types of meshes, and currently available surgical synthetic meshes in hernia surgery.

To cite this article: Özant A, Arslan K. Synthetic Meshes in Hernia Surgery. Cyprus J Med Sci 2023;8(1):1-7

ORCID IDs of the authors: A.Ö. 0000-0002-7746-2719; K.A. 0000-0001-5913-0991.

Address for Correspondence: Ali Özant
E-mail: ali.zant@yahoo.com
ORCID ID: orcid.org/0000-0002-7746-2719

Received: 01.10.2021
Accepted: 17.04.2022

History

The first proposal to use a prosthetic material in hernia surgery was put forward by Billroth[7] in 1890. He suggested that finding such material would greatly reduce the recurrence rate after hernia repair.[7] The first prosthetic material used for hernia repair were silver wire coils which were used by Phelps in 1894.[8] Until the 1940s, several surgeons used handmade silver wire filigrees, which became the first prosthetic mesh. Due to various disadvantages such as its inertness in human tissues, fluid accumulation, sinus tract formation, and high infection rates, the use of silver filigrees was abandoned. The second material which was introduced as a prosthetic material in 1940 by Burke was tantalum. It was used until the 1950s with promising study reports and clinical outcomes. However, longer follow-up studies revealed some important disadvantages of this material, where the most undesired outcomes were fragmentation and fracture of the tantalum gauze, which made it unpopular in hernia surgery.[9] Stainless steel was another metallic material which was used in the 1980s and it produced much better outcomes than silver filigrees and tantalum gauze. However, the increasing popularity of other synthetic materials and the use of magnetic resonance imaging reduced its popularity and led to its very limited use in hernia repair. Nylon was the first synthetic prosthetic material used for hernia repair, but due to hydrolytic digestion, it was reported to lose strength and also had to be removed in cases of infection. These undesirable properties made nylon inappropriate for hernia repair.[2] Dr. Francis Usher, started to use other materials such as Teflon, Orlon, Dacron and Propylene in 1955. In 1958, he published a study on hernia repair using polypropylene (PP) mesh, and after 30 years, the Lichtenstein repair, which is now regarded as the "tension-free" mesh technique, became popular for inguinal hernia repair.[10] Although the advantages of meshes were recognized, long-term evidence-based recollection of cases was required to statistically quantify their advantages. In 2002, the European Union Hernia Trialists Collaboration participated in randomized trials of mesh repair, analyzed many randomized controlled trials and concluded that the use of surgical meshes was superior to other techniques.[11]

The Properties of Synthetic Mesh Materials

The ideal mesh should be resistant to infection, chemically inert, non-carcinogenic, inert to the body and its fluids, robust to sterilization, and able to limit hypersensitivity allergic or foreign body reactions.[12] After the implantation of meshes, there are two pathways: Integration or degradation. The expected outcome is tissue incorporation, which is related with the material, pore size, filament type, density, three-dimensional structure, compliance and electric charge.[13] The synthetic materials are mainly classified into two groups: Non-absorbable and absorbable. The non-absorbable synthetic materials are: PP, polytetrafluorethylene (PTFE), expanded polytetrafluoroethylene (ePTFE) and polyester (PET).[2,14] The absorbable materials are: Polylactide, polyglycolic acid, polycaprolactone and polydioxanone. The most common reason for the use an absorbable mesh material is to prevent abdominal compartment syndrome with distended bowels and intra-abdominal infections.[15] By allowing fibrous and connective tissue regeneration, absorbable materials give transient support to the wall of the abdomen and dissolve slowly by hydrolysis.[16]

Currently Used Non-absorbable Materials and Their Properties

Polypropylene: PP is a non-absorbable polymer and it is the most popular mesh material used in hernia surgery with several advantages compared to the other materials. PP is synthesized by the polymerization of propylene.[16] Along with good mechanical stability and strength, it is resistant to biological degradation and it is electrostatically neutral, highly hydrophobic and non-polar.[16] Despite all these advantages, reduced flexibility, shrinkage and local cracks have been observed after the explantation of PP meshes.[17] In spite of these disadvantages, because of its easy fiber manufacturing, durable strength, low cost and ease of handling, PP remains the most commonly used mesh material. Depending on the weight of the mesh, PP meshes may be classified as either light-weight (LW) or heavyweight (HW). The density of a mesh is mainly dependent on its textile structure and pore size along with the molecular weight of the polymer and the diameter of the fiber.[16] The flexibility of LW meshes are generally better than HW meshes and also they mimic human tissues better.[18] PP meshes are manufactured in both coated forms for intra-peritoneal use, and uncoated forms, for extra-peritoneal use.

Polyester: Polyester is composed of polyethylene-terephthalate (PET). It is a soft and compliant material suitable for mesh construction. It is slightly polar, more hydrophilic and hydroscopic than homochain hydrocarbon polymers.[9] The fibers of polyester are very strong, tough and can resist most chemicals.[16] Its textile structure may be mono- or multi-flament. PET surgical meshes were first used for inguinal and abdominal hernias in the 1960s.[19] Meshes now have different configurations for different types of hernia repairs, such as incisional, inguinal and hiatal hernia repair. PET has similarities to PP in biological responses, such as scar formation, complications and side effects.[20] However, degradation after long term is the most undesired complication as shown in some studies. Due to hydrolytic degradation, reduced mechanical strength up to 30% was observed in PET vascular grafts after 10 years, and complete implant failure was observed after 25 to 40 years.[21]

Expanded polytetrafluoroethylene: PTFE is a synthetic polymer consisting of carbon and fluorine with strong bonds between them.[14] Due to its chemical stability, it is inert in nature and resistant to degradation in the body.[11] It has smaller pores sizes than PP, with large pores on one side and smaller pores on the other. Due to this property, it inhibits intestinal adhesion and also does not facilitate tissue in-growth in the abdominal wall, which ultimately results in encapsulation and weaker hernia repair.[22] With the advantages of minimal inflammatory reaction and lower scar density for intraperitoneal use, correct fixation is very important, because ePTFE can be broken easily.[23] The other important feature of this material is its porosity. Due to the microporous structure of its mesh, it is not possible for macrophages to penetrate this material in cases of infection, and the mesh must be removed, particularly if placed during an open ventral hernia repair.[24]

Polyvinylidene fluoride: Polyvinylidene fluoride (PVDF) is manufactured by the polymerization of vinylidene difluoride and is highly inert in nature. It is resistance to degradation and its hydrolysis is better than PET, with high durability and bio-stability.[25] It was demonstrated that the mechanical properties of PVDF are highly stable, as it retains 92.5% of its original strength after 9 years of implantation.[26]

It is crucial for surgeons to understand the basic physical structures and mechanical properties of mesh materials in order to make an informed choice.

The Structural Properties of Mesh

Textile Fundamentals

The manufacturing structure of a mesh may be woven, non-woven or knitted with mono- or multi-filament fibers as its textile structure (Figure 1). Due to the tightness and packed structure of woven meshes, they have a smaller pore size, and hence have the disadvantage of poor fibrous tissue ingrowth.[12] Non-woven mesh structures are produced by the interlocking or bonding of fibers. Due to their micro-porous structure, they allow better fibrous tissue ingrowth and reduced adhesion than woven mesh.[27] Most meshes are warp knitted as textile structures, have larger pore size and greater elasticity. The mechanical characteristics of knitted and woven fabrics are extremely anisotropic.[28]

Pore Size (Porosity)

Pore size is the main factor in the reaction of tissue to the mesh. Porosity simply is the ratio of the volume of voids in the mesh to the total volume of the mesh.[29] Cell proliferation and bacterial growth are highly dependent on porosity. Pores must be larger than 75 µm for the infiltration of blood vessels, fibroblasts, collagen and macrophages. Large pores allow tissue integration without filling with scar tissue, which makes the mesh more flexible since there will be no granuloma bridging (Figure 2). As a part of the foreign body reaction, granulomas normally form around mesh fibers, and bridging is the start of the confluence of individual granulomas with each other. This causes the encapsulation of the entire mesh, which leads to a tough scar plate which reduces flexibility.[30] The encapsulation and bridging of granulomas are more likely to occur in meshes with pores of less than 800 µm. Meshes are classified according to pore size as: Very large pore: >2,000 µm; large pore: 1,000-2,000 µm; medium pore: 600-1,000 µm; small pore: 100-600 µm and microporous (solid): <100 µm.[31]

Mesh Weight and Tensile Strength

The weight of the mesh is directly proportional to the weight and amount of the material used.[32] According to weight, meshes are classified as: heavy-weight (HW), if they are above 80 g/m^2; medium-weight (MW) for 50-80 g/m^2; LW for 35-50 g/m^2; and ultra-lightweight, below 35 g/m^2.[33] HW meshes are known to have a tensile strength up to 100 N/cm^2. It was shown by Klinge at al.[34] that the maximum force on the abdominal wall is about 16 N/cm^2. In an *in vivo* study, Cobb et al.[35] measured bladder pressures on humans while performing different activities, and the maximum tensile strength was determined to be between 11 N/cm^2 and 27 N/cm^2. It is clear that the tensile strength of the HW meshes far exceeds the strength of the abdominal wall. In a porcine model, Cobb at al.[35] tested the burst strength of meshes with different weights, namely HW, MW and lightweight meshes, 5 months after implantation and found that all meshes, regardless of their weight, had significantly greater strength than that of the abdominal wall. Due to less foreign body reaction and inflammatory response, the LW meshes had better tissue integration and improved mesh compliance, which caused less pain and discomfort.

Mesh Shrinkage

Shrinkage is the name given to the contraction in dimensions in the length and/or width of the mesh material post implantation. It is caused by the contraction of the scar tissue formed at the site of the mesh. Due to its smaller pores, HW mesh shrinks more due to greater scar plate formation. Different types of mesh materials show significant variations

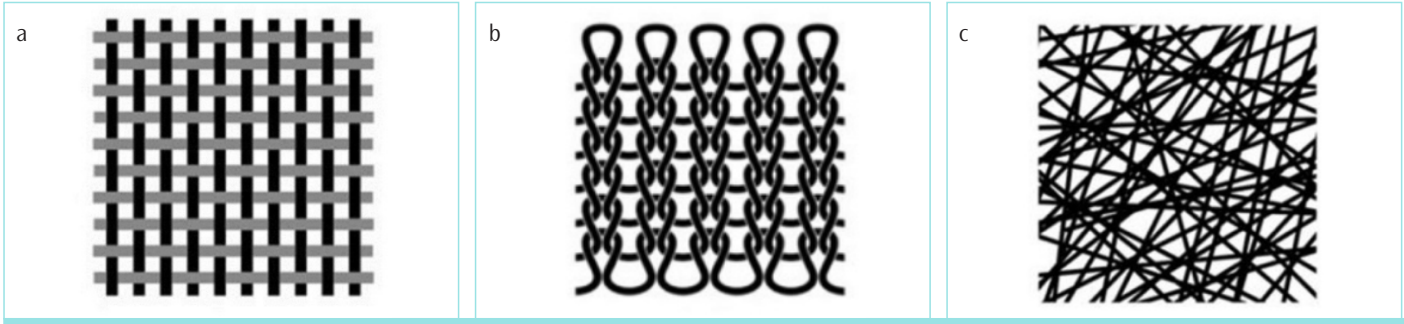


Figure 1. (a-c) Schematic view of the manufactured structure of a mesh. Woven (a) warp knitted (b) and non-woven (c).

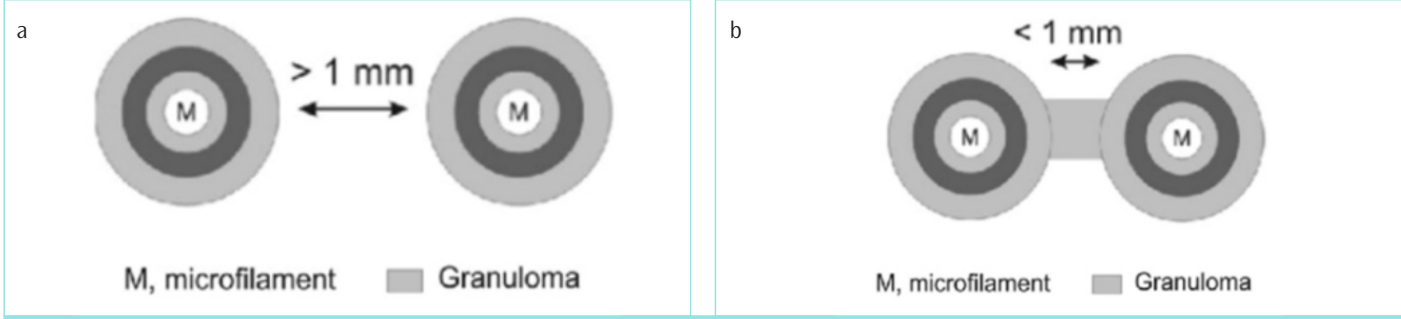


Figure 2. (a,b) Granulomas forming around individual mesh fibers (a) and bridging where individual granulomas become confluent with each other and encapsulate the entire mesh (b).

in their degrees of shrinkage. Synthetic meshes show a minimum and maximum degree of shrinkage as follows: polyester (PET): 6.1% to 33.6%, PTFE: 4% to 51%, and PP: 3.6% to 25.4% (Figure 3).[36]

Mechanical Properties of Mesh

Mesh Bursting Strength

Bursting strength defines the maximum point of exerted uniform pressure, under standardized testing conditions, at the correct angle in relation to the plane of the mesh material at which it ruptures.[37] This strength is related with the mechanical properties, shape, structure density and weight of the mesh. After hernia surgery, the accepted maximum applied forces to the abdominal wall are as follows: 22 N/cm^2 in the craniocaudal and 32 N/cm^2 in the lateral directions.[38] The accepted groin load maximum is 16 N/cm^2.[39] In a study by Deeken et al.[40-43], seven composite meshes were tested by ball bursting (C-QUR, Bard Supramesh, PROCEED, Parietex, BardComposix E/X, Bard Composix L/P, and Dual Mesh) revealing that they had a burst strength higher than 32 N/cm^2. Different studies have shown that the human abdominal wall has a lower burst strength than all knitted synthetic meshes.[41]

Mesh Elasticity and Strength

A materials property in how it changes its dimensions and form when exposed to opposite acting forces, however regains its original dimensions and form when said forces diminish is called elasticity.[42] The abdominal wall has a natural elasticity of 38% at 32 N/cm^2 and it shows a lower resilience in the longitudinal direction than in the horizontal direction. Mesh materials with knitted structures are more elasticity than those with woven structures, and they also have the ability to stretch in all directions.[43] The elasticity exhibited by lightweight meshes at 16 N/cm^2 is approximately 20-35%. On the other hand, HW meshes, at 16 N/cm^2, exhibit an elasticity of 4-15%, which might lead to a restriction in abdominal distension[30]. An appropriate mesh tensile strength along with a suitable stretch capability of the material leads to less pain, less prolapse or recurrence and overall more functional and better results. The elongation rate of the native human abdominal wall is approximately 30%. Meshes with a higher elongation rate than 30% may stretch more than the abdominal wall, which may result in bulging and recurrence.[40]

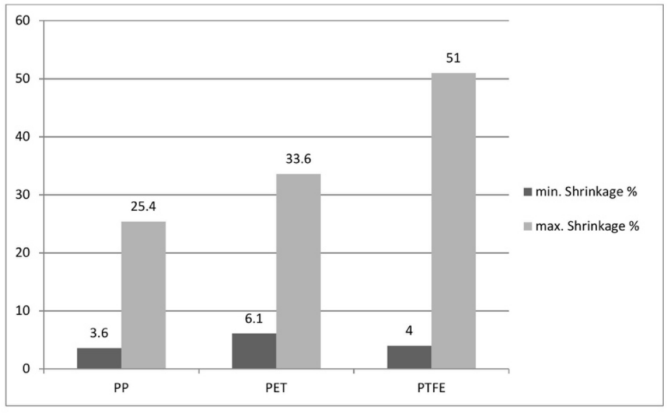


Figure 3. Minimum and maximum shrinkage of synthetic meshes in percentages.

Anisotropic Behavior of Mesh

An anisotropic material is the name given to a material that, if stretched in different directions, responds in different ways. Saberski et al.[6] studied the anisotropic properties of the six most commonly used meshes: PP (Trelex®, Pro-Lite™, Ultrapro™), polyester (Parietex™), and PTFE-based (Dualmesh®, Infinit) meshes, and found that five out of these six meshes presented considerable anisotropic behavior. Ifinit approximately exhibited 20-fold, Ultrapro™ 12-fold, Trelex®, ProLite™, and Parietex™ 2.3-2.4 fold differences between their perpendicular axes. Dualmesh® had the least anisotropic behavior, with an indiscernible difference between the axes.[6] If the selected mesh material has anisotropic properties, the appropriate orientation of the mesh is necessary to comply with the physiological stretchability in order to reduce recurrence.

Classification of Synthetic Surgical Meshes

1. First Generation Meshes

First-generation meshes are classified into three categories according to their structural properties as follows: 1) macro-porous meshes, 2) micro-porous meshes, and 3) macro-porous meshes with multi-filament or micro-porous components. Macro-porous prostheses have a pore size greater than 75 µm and the material of choice is PP. Micro-porous prostheses are commonly made from e-PTFE and have smaller pores, usually less than 10 µm. Macro-porous prostheses containing microporous or multifilament components incorporate plaited multi-filamentary threads in their architecture. They have pore sizes larger than 75 µm, and less than 10 µm of space between their threads.[44]

2. Second Generation Meshes

Due to a number of complications, such as infection, adhesions and recurrence, there is a continuous need to improve surgical meshes. The combination of more than a single synthetic material was used in the development of second generation meshes in order to reduce these complications. Almost the entirety of these meshes consist of PP, e-PTFE or PET combined with one another or different materials such as PVDF, titanium (Ti), omega-3 fatty acids, poliglecaprone 25 (PGC-25), collagen or beta-glucan. Second-generation meshes may be classified as:

2.1. Non-absorbable and synthetic with a barrier: To prevent bowel adhesion when placed intraperitoneally, absorbable or non-absorbable barriers are used in these prostheses. The barrier minimizes the biological response, limiting initial adhesion opportunity to the material. Therefore, it reduces the activation of cells and inflammatory cytokines. Therefore, the onset of an inflammatory cascade is inhibited. Omega-3 fatty acids, ePTFE, collagen, polyurethane, oxidized regenerated cellulose or beta-glucan are possible barriers.[2] The anti-adhesive characteristics of these meshes with non-absorbable or absorbable barriers have been shown in different experimental studies.[45]

2.2. Partially-absorbable and synthetic meshes: Maintaining long-term wound strength and intraoperative handling characteristics by reducing the density of the biomaterial and its subsequent inflammatory reaction is the main goal of constructing a partially absorbable mesh.[2] Recently developed meshes have been improved with the fusion of non-absorbable PP and absorbable materials such as polyglactin 910 and PGC-25.[46] Polyglycolide (L-lactide-co-glycolide) and PP are the most frequently studied materials. These meshes cause less fibrosis, structural changes and chronic inflammation. Some studies have concluded that the use of these prostheses causes less pain and discomfort.[2]

2.3. Combined meshes: The major aim of these meshes is to prevent complications, especially the occurrence of intestinal adhesion, by incorporating the advantages of two different meshes. Polyester and PTFE combined meshes are designed in such a way that the polyester allows for the abdominal wall tissue in-growth and PTFE, which is the surface which comes into contact with the intestines, prevents the occurrence of intestinal adhesion, which is achieved through the different (small) pore size of the mesh. A recent design using a combination of synthetic meshes involves the construction of a mesh which consists of a PET or PP foundation covered with polymers which are absorbable. The adhesion of the intestine with the mesh typically occurs within 7 days of surgery.[47] Therefore, it is thought that only a temporary barrier for adhesion is required for synthetic meshes.

3. Third Generation Meshes (Biological Prosthesis)

The basis of biological mesh materials are scaffolds of collagen acquired from donor sources. Fetal bovine, porcine and human dermis sources are processed by decellularization resulting in highly organized collagen sources exclusively, to remain with the dermal products included in bovine pericardium and porcine small intestine submucosa. The main goal is to provide a matrix of native cells in order to complete healing and synthesize connective tissue which may restore the mechanical and functional integrity of the abdominal wall. Since the aim of this article is to review synthetic meshes, we will not discuss third-generation meshes further.

Discussion

Since the properties of the mesh material play an important role in successful surgical repair, it is important to understand the properties of the mesh material before selecting the most suitable one for the patient. Recent progress has produced a significant number of variations of the mesh materials being used in the repair of hernias. First-generation meshes were mainly based on PP systems. They are classified as macro-porous, micro-porous and macro-porous meshes with multifilament or micro-porous components. Second-generation meshes are composed of a combination multiple synthetic materials. All of them continue to use PP, PET or e-PTFE. They are also systemically combined with different materials such as PVDF Ti, PGC-25 and omega 3.[44] When these composite meshes are applied to intraperitoneal spaces, they form minimal adhesions with the adjacent surfaces provided that each individual side of said mesh is modified to the exact requirements, so they need a specific orientation during implantation. For repairs of hernias where the mesh will contact the bowel, a composite or non-absorbable composite mesh is recommended.[2] To inhibit bowel adhesions, intra-abdominal placement of the prosthesis incorporating a barrier needs to be used. The size of the mesh used is also an important factor.[45-47] Regarding the shrinkage character of the chosen mesh, Elango et al.[2] reported that a minimum of 15x15 cm for the repair of inguinal hernia, and a minimum of 4 cm wider mesh than the defect for umbilical, ventral and incisional repair is required. As we continue to learn more information about the physical and mechanical properties of meshes, mesh selection becomes more important. For inguinal hernia repair, there is little argument about the mesh selection. Although the recurrence rates are similar between HW and LW meshes, for Lichtenstein repairs, it was reported that there were fewer foreign body sensations and less chronic pain reported by those patients who received a LW mesh.[48] However, a longer-term follow-up study with 5-year results revealed a higher recurrence rate for those patients receiving LW meshes in totally extra-peritoneal laparoscopic inguinal repairs, with recurrence rates of 3.8% and 1.1% for patients receiving LW and HW meshes, respectively. A more significant difference with the LW mesh for direct inguinal hernia was seen.[49] Compared with the maximum exerted forces to the abdominal wall, the groin region has a relatively lower force value. Also, the flexibility of the groin region is less than the upper abdominal wall. Therefore, an important mechanical property of the mesh is its elasticity. Based on the knowledge that, at 16 N/cm^2, lightweight meshes have an elasticity range of between 20 to 35%, and they may cause bulging above 30%, the use of such meshes may increase recurrence rates, especially in cases of direct inguinal hernia.[43] Among the mesh materials, PP meshes are the most popular ones for inguinal hernia repair. Decision making in mesh selection for ventral or incisional hernias can often be a confusing problem. There are two important factors in such decisions, namely the patient and the mesh. Factors related with the patient include: The type of abdominal hernia, the size of the defect, the physical capacity and status of the patient, the type of the planned surgery (onlay- inlay placement of the mesh), and the risk of surgical site infection. The factors related with the mesh type are: The material of the mesh, the composition of the mesh, and the physical and mechanical properties of the mesh. For intra-abdominal placements, the mesh must be able to prevent bowel adhesions; this can include an ePTFE surgical mesh or a mesh with an absorbable or non-absorbable barrier. In cases of an increased risk of surgical site infection, a micro-porous (solid) surgical mesh may not be a suitable choice. Due to the micro-porous structure of the mesh, it is not possible for macrophages to penetrate the material in cases of infections, and the mesh must be removed, especially if the placement was carried out in the course of an open ventral hernia repair. In ventral hernia repairs, LW meshes have poorer outcomes compared with MW meshes. In a study of the open retro-muscular placement of meshes for incisional hernia, it was found that use of LW meshes showed a follow-up recurrence of 22.9%, whereas the use of MW meshes showed a follow-up recurrence of 10.6%, where central mesh fracture was the mechanism of recurrence in 46.5% of cases.[50]

Despite all the above mentioned knowledge about meshes and their materials, there are still several points which need clarification. Due to a lack of standardization in test protocols and terminology, material mechanical investigations and clinical study results are mostly confusing. Our knowledge about the mechanical properties of mesh materials may be quite satisfactory. However, considering the structural and mechanical properties of meshes, surgeons still have a lack of knowledge about the exact behaviors of the mesh implanted and the biologic host's responses.

CONCLUSION

This article briefly reviews the properties of mesh materials and discusses which one might be most appropriate. Although there are several studies on this topic, there are currently no standardized test procedures. Although there are more than 70 types of commercially available meshes, it is clear that the ideal mesh has yet to be discovered. The fact that meshes still suffer from infection and/or contraction post implantation is well known. Nevertheless, adhesion amongst the visceral side and neighboring organs still occurs. These mentioned complications might lead to bowel erosion, chronic pain, hernia recurrence or intestinal obstruction. Most of the investigations which were recently published still claim that PP is the "gold standard" mesh. Although the properties of mesh materials are well known, the physical

and mechanical properties of the meshes used need to be clarified and must be listed on the packaging of meshes on the market. This is because without knowing the physical and mechanical characteristics of meshes, such as their elasticity, bursting strength and anisotropic behavior, it is not possible for surgeons to decide which mesh is the right one for their patient. Additionally, the correct orientation of the mesh is necessary according to these characteristics. Regarding the properties of mesh materials, the site of the hernia, the strength of the part of the abdominal hernia site and the physical condition of the patient must also be taken into consideration while selecting the proper mesh. Understanding the properties of the mesh material is an important factor which will help surgeons and will thus reduce complications through the application of the proper mesh material.

MAIN POINTS

- It is crucial for surgeons to know the basic structural and mechanical properties of mesh materials in order to make a proper choice.
- First generation meshes, which are produced from single non-absorbable materials are classified as macro-porous, micro-porous and macro-porous with multifilament or micro-porous components.
- Second generation meshes are produced from more than one synthetic material. PP, Polyester (PET) and ePTFE are the materials used, as in first generation meshes.
- With regards to the properties of the mesh material, the site of hernia, the strength of the part of the abdominal hernia site and the physical condition of the patient must also be taken into consideration while selecting the proper mesh.

Peer-review: Internally peer-reviewed.

Authorship Contributions

Concept: A.Ö., Design: A.Ö., Supervision: K.A., Fundings: K.A., Materials: K.A., Data Collection and/or Processing: A.Ö., Analysis and/or Interpretation: A.Ö., Literature Search: A.Ö., Writing: A.Ö., Critical Review: K.A.

DISCLOSURES

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

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

References

1. Dabbas N, Adams K, Pearson K, Royle GT. Frequency of abdominal wall hernias: Is classical teaching out of date? JRSM Short Rep. 2011; 2(1): 5.
2. Elango S, Perumalsamy S, Ramachandran K, Vadodaria K. Mesh materials and hernia repair. Biomedicine. 2017; 7(3): 16.
3. Wahba M. Evaluation of lightweight polypropylene mesh in Stoppa pre-peritoneal repair of bilateral inguinal hernias. J Am Sci. 2014; 10(5): 116-24.
4. Gilbert AI. Inguinal herniorrhaphy: reduced morbidity, recurrences, and costs. South Med J. 1979; 72(7): 831-4.
5. Bendavid R. Expectations of hernia surgery (inguinal and femoral). In: Principles and Practice of Surgical Laparoscopy, Paterson-Brown S, Garden J, (editors). W.B. Saunders, 1994; 387-414.
6. Saberski ER, Orenstein SB, Novitsky YW. Anisotropic evaluation of synthetic surgical meshes. Hernia. 2011; 15(1): 47-52.
7. Billroth T. The Medical Sciences in the German Universities: A Study in the History of Civilization; Welch WH. Ed. Macmillan: New York, NY, USA; 1924.
8. DeBord JR. The historical development of prosthetics in hernia surgery. Surg Clin North Am. 1998; 78(6): 974.
9. DeBord JR. The historical development of prosthetics in hernia surgery. Surgical Clin North Am. 1998; 78(6): 973-1006.
10. Klinge U, Klosterhalfen B, Birkenhauer V, Junge K, Conze J, Schumpelick VJ. Impact of polymer pore size on the interface scar formation in a rat model. J Surg Res. 2002; 103(2): 208-14.
11. EU Hernia Trialists Collaboration. Repair of groin hernia with synthetic mesh: Meta-analysis of randomized controlled trials. Ann Surg. 2002; 235(3): 322-32.
12. Shankaran V, Weber DJ, Reed RL 2nd, Luchette FA. A review of available prosthetics for ventral hernia repair. Ann Surg. 2011; 253(1): 16-26.
13. Procter L, Falco EE, Fisher JP, Roth JS. Abdominal wall hernias and biomaterials A. Gefen (Ed.), Bioengineering research of chronic wounds (1st ed). Springer Verlag: Berlin Heidelberg; 2009.p.425-47.
14. Cobb WS. A Current Review of Synthetic Meshes in Abdominal Wall Reconstruction. Plast Reconstr Surg. 2018; 142(3 Suppl): 64S-71.
15. Dayton MT, Buchele BA, Shirazi SS, Hunt LB. Use of an absorbable mesh to repair contaminated abdominal-wall defects. Arch Surg. 1986; 121(8): 954-60.
16. Todros S, Pavan PG, Natali AN. Synthetic surgical meshes used in abdominal wall surgery: Part I-materials and structural conformation. J Biomed Mater Res B Appl Biomater. 2017; 105(3): 689-99.
17. Costello CR, Bachman SL, Grant SA, Cleveland DS, Loy TS, Ramshaw BJ. Characterization of heavyweight and ligtweight polypropylene prosthetic mesh explants from a single patient. Surg Innov. 2007; 14(3): 168-76.
18. Hollinsky C, Sandberg S, Koch T, Sedler S. Biomechanical properties of lightweight versus heavyweight meshes for laparoscopic inguinal hernia repair and their impact on recurrence rates. Surg Endosc. 2008; 22(12): 2679-85.
19. Wolstenholme JT. Use of commercial Dacron fabric in the repair of inguinal hernias and abdominal wall defects. Arch Surg. 1956; 73: 1004-8.
20. Leber GE, Garb JL, Alexander AI, Reed WP. Long-term complications associated with prosthetic repair of incisional hernias. Arch Surg. 1998; 133(4): 378-82.
21. Maarek JM, Guidoin R, Aubin M, Prud'homme RE. Molecular weight characterization of virgin and explanted polyester arterial prostheses. J Biomed Mater Res. 1984; 18(8): 881-94.
22. Woloson SK, Greisler HP. Biochemistry, immunology, and tissue response to prosthetic material. In: Bendavid R, Abrahamson J, Arregui ME, Flament JB, Phillips EH, et al., editors Abdominal wall hernias. Principles and management. New York: Springer-Verlag; 2001; 201-7.
23. Koehler RH, Begos D, Berger D, Carey S, LeBlanc K, Park A, et al. Minimal adhesions to ePTFE mesh after laparoscopic ventral incisional hernia repair: reoperative findings in 65 cases. Zentralbl Chir. 2003;128(8): 625-30.
24. Hawn MT, Gray SH, Snyder CW, Graham LA, Finan KR, Vick CC. Predictors of mesh explantation after incisional hernia repair. Am J Surg. 2011; 202(1): 28-33.
25. Klinge U, Klosterhalfen B, Ottinger AP, Junge K, Schumpelick V. PVDF as a new polymer for the construction of surgical meshes. Biomaterials. 2002; 23(16): 3487-93.
26. Laroche G, Marois Y, Schwarz E, Guidoin R, King MW, Pâris E, et al. Polyvinylidene fluoride monofilament sutures: can they be used safely for

long-term anastomoses in the thoracic aorta? Artif Organs. 1995; 19(11): 1190-9.

27. Raptis DA, Vichova B, Breza J, Skipworth J, Barker S. A comparison of woven versus nonwoven polypropylene (PP) and expanded versus condensed polytetrafluoroethylene (PTFE) on their intraperitoneal incorporation and adhesion formation. J Surg Res. 2011; 169(1): 1-6.
28. Zhu LM, Schuster P, Klinge U. Mesh implants: An overview of crucial mesh parameters. World J Gastrointest Surg. 2015; 7(10): 226-36.
29. Karageorgiou V, Kaplan D. Porosity of 3D biomaterial scaffolds and osteogenesis. Biomaterials. 2005; 26(27): 5474-91.
30. Klosterhalfen B, Junge K, Klinge U. The lightweight and large porous mesh concept for hernia repair. Expert Rev Med Devices. 2005; 2(1): 103-117.
31. Earle DB, Mark LA. Prosthetic Material in Inguinal Hernia Repair: how do I choose? Surg Clin North Am. 2008; 88(1): 179-201.
32. Procter L, Falco EE, Fisher JP, Roth JS. Abdominal wall hernias and biomaterials. Gefen A, (editor), Bioengineering research of chronic wounds (1st ed), Springer Verlag: Berlin Heidelberg; 2009.p.425-47.
33. Zogbi L. The Use of Biomaterials to Treat Abdominal Hernias. In: Pignatello R., editor. Biomaterials Applications for Nanomedicine. 1st ed. Volume 18. InTech; Rijeka, Croatia: 2008.p.359-82.
34. Klinge U, Klosterhalfen B, Conze J, Limberg W, Obolenski B, Ottinger AP, et al. Modified mesh for hernia repair that is adapted to the physiology of the abdominal wall. Eur J Surg. 1998; 164(12): 951-60.
35. Cobb WS, Burns JM, Peindl RD, Carbonell AM, Matthews BD, Kercher KW, et al. Textile analysis of heavy weight, mid-weight, and light weight polypropylene mesh in a porcine ventral hernia model. J Surg Res. 2006; 136(1): 1-7.
36. Slater, NJ, Knaapen, L, van Goor, H. Abdominal wall defects: Pathogenesis, prevention and repair. Surgery (Oxford). 2015; 33: 206-13.
37. Zhu, LM, Schuster, P, Klinge, U. Mesh implants: An overview of crucial mesh parameters. World J Gastrointest Surg. 2015; 7(10): 226-36.
38. Pott PP, Schwarz ML, Gundling R, Nowak K, Hohenberger P, Roessner ED. Mechanical properties of mesh materials used for hernia repair and soft tissue augmentation. PLoS One. 2012; 7(10): e46978.
39. Klinge U, Klosterhalfen B, Conze J, Limberg W, Obolenski B, Ottinger AP, et al. Modified mesh for hernia repair that is adapted to the physiology of the abdominal wall. Eur J Surg. 1998; 164(12): 951-60.
40. Deeken CR, Abdo MS, Frisella MM, Matthews BD. Physicomechanical evaluation of polypropylene, polyester, and polytetrafluoroethylene meshes for inguinal hernia repair. J Am Coll Surg. 2011; 212(1): 68-79.
41. Cobb, WS Kercher KW, Heniford BT. The argument for lightweight polypropylene mesh in hernia repair. Surg Innov. 2005; 12(1): 63-9.
42. Hollinsky C, Sandberg S. Measurement of the tensile strength of the ventral abdominal wall in comparison with scar tissue. Clin Biomech (Bristol, Avon). 2007; 22(1): 88-92.
43. Bilsel Y, Abci I. The search for ideal hernia repair; mesh materials and types. Int J Surg. 2012; 10(6): 317-21.
44. Baylón K, Rodríguez-Camarillo P, Elías-Zúñiga A, Díaz-Elizondo JA, Gilkerson R, Lozano K. Past, Present and Future of Surgical Meshes: A Review. Membranes. 2017; 7(3): 47.
45. Bellon JM, Garcia-Honduvilla N, Serrano N, Rodriguez M, Pascual G, Bujan J. Composite prostheses for the repair of abdominal wall defects: effect of the structure of the adhesion barrier component. Hernia. 2005; 9(4): 338-43.
46. Bellon JM, Rodriguez M, Garcia-Honduvilla N, Pascual G, Buján J. Partially absorbable meshes for hernia repair offer advantages over nonabsorbable meshes. Am J Surg. 2007; 194(1): 68-74.
47. Robinson TN, Clarke JH, Schoen J, Walsh MD. Surg Endosc. 2005; 19(12): 1556-60.
48. Uzzaman MM, Ratnasingham K, Ashraf N. Meta-analysis of randomized controlled trials comparing lightweight and heavyweight mesh for Lichtenstein inguinal hernia repair. Hernia. 2012; 16(5): 505-18.
49. Roos MM, Bakker WJ, Schouten N, Voorbrood CEH, Clevers GJ, Verleisdonk EJ, et al. Higher Recurrence Rate After Endoscopic Totally Extraperitoneal (TEP) Inguinal Hernia Repair with Ultrapro Lightweight Mesh: 5-Year Results of a Randomized Controlled Trial (TULP-trial). Ann Surg. 2018; 268(2): 241-6.
50. Cobb WS, Warren JA, Ewing JA, Burnikel A, Merchant M, Carbonell AM. Open retromuscular mesh repair of complex incisional hernia: predictors of wound events and recurrence. J Am Coll Surg. 2015; 220(4): 606-13.

RESEARCH ARTICLE

CYPRUS
JOURNAL OF MEDICAL SCIENCES

DOI: 10.4274/cjms.2022.2021-234
Cyprus J Med Sci 2023;8(1):8-12

Global Analysis of Chronic Osteomyelitis Publications with a Bibliometric Approach

Sabit Numan Kuyubaşı[1], Nihat Demirhan Demirkıran[1], Süleyman Kozlu[1], Süleyman Kaan Öner[1], Sevil Alkan[2]

[1]Department of Orthopedics and Traumatology, Kütahya Health Sciences University Faculty of Medicine, Kütahya, Türkiye
[2]Department of Diseases and Clinical Microbiology, Çanakkale Onsekiz Mart University Faculty of Medicine, Çanakkale, Türkiye

Abstract

BACKGROUND/AIMS: Although there have been innovations in the diagnosis and treatment of chronic osteomyelitis (CO), it is still considered as a challenging situation for both patients and clinicians. In this article, it was aimed to comprehensively examine publications on CO with bibliometric evaluation and to guide researchers and clinicians in reaching articles effectively in their literature searches.

MATERIALS and METHODS: The Scopus bibliometric database was used to identify articles related to CO. Original research articles and reviews made between 1940 and 2021 including the words "chronic" and "osteomyelitis" in their titles and keywords were found. Publications were analyzed according to their research parameters, such as publication year, authors, publication language, institutions, keywords, funding institutions, citations and their field of study.

RESULTS: There were a total of 2,881 publications on CO, and it was observed that the first publication was made in 1890. Among these publications, 2,565 (89.03%) were research articles and 129 (4.47%) were reviews. The United States of America was found to be the most prolific country with 484 (16.79%) articles; it was observed that the China Medical University Hospital was the leading institution in CO. Five hundred sixteen (17.91%) of the articles were published in open access (OA) journals. The largest number of articles on CO were published in the journal Vestnik Khirurgii Imeni II Grekova [n=57 (1.97%)]. The most commonly seen keyword was "osteomyelitis" [n=2,345 (81.39%)]. It was seen that most of the publications were in the field of medicine [n=2,724 (94.55%)] and the most cited article received 4,574 citations. The most pioneering funding sponsor in CO studies was the National Institutes of Health [n=30 (1.04%)]. Pediatricians constituted the majority of the most productive authors in CO studies.

CONCLUSION: In this bibliometric study, the economic size and level of development of countries were important factors in terms of their academic publication efficiency in the field of CO. Developing countries should be encouraged to increase studies on this subject. Considering that the multidisciplinary approach is a significant factor in the follow-up and treatment of CO, we believe that the contributions of surgical branches to this subject should be improved.

Keywords: Bibliometric analysis, xhronic osteomyelitis, Scopus database

INTRODUCTION

The term "osteomyelitis" was first used by the French surgeo Chassaignac[1] in 1852. Injury in adult patients (mostly open fractures) and hematogenous spread after an episode of bacteremia in pediatric patients are among the common etiological factors. It usually results in osteomyelitis, sequestrum and involucrum. This situation represents the presence of chronic osteomyelitis (CO).[2]

Due to the avascular nature of the sequester tissue, osteomyelitis is difficult to treat and can be associated with high morbidity and mortality for the patient. Treatment is aimed at completely eliminating

To cite this article: Kuyubaşı SN, Demirkıran ND, Kozlu S, Öner SK, Alkan S. Global Analysis of Chronic Osteomyelitis Publications with a Bibliometric Approach. Cyprus J Med Sci 2023;8(1):8-12

ORCID IDs of the authors: S.N.K. 0000-0002-3021-0581; N.D.D. 0000-0002-0724-9672; S.K. 0000-0001-5175-0600; S.K.Ö. 0000-0002-4333-0582; S.A. 0000-0003-1944-2477.

Address for Correspondence: Süleyman Kaan Öner
E-mail: skaanoner@gmail.com
ORCID ID: orcid.org/0000-0002-4333-0582

Received: 25.01.2022
Accepted: 17.05.2022

the infection and maximizing the patient's function. Historically, debridement of infected bone with long antibiotic regimens and surgical treatment has been used in the treatment of osteomyelitis.[3] Prior to the clear identification of microorganisms, the significance and principles of infection were not completely understood. Hence, the treatment of osteomyelitis consisted of the amputation of the infected extremity by the surgeon.[2]

Along with the identification of microorganisms in the treatment of CO and variability in age groups, the importance of multidisciplinary approaches has been revealed. Thus, patients were evaluated more effectively with a broad perspective. In conclusion, the multidisciplinary approach has led to great innovations and developments in the medical and surgical fields.[4,5]

With the widespread use of the Internet, many researchers can easily access up-to-date information in the medical literature. However, the increase in the number of scientific publications creates the problem of focusing on the research to be carried out on a subject. It can be seen that there has been an increase in bibliometric studies in parallel with the increasing number of publications, particularly after the 2000s. Bibliometric analysis is a statistical analysis method which allows the examination of scientific publications in terms of many parameters. Researchers can readily and quickly access data on the subjects they wish to study with this method.[6]

Although there have been previous bibliometric studies in the field of orthopedics, there was no up-to-date and comprehensive bibliometric study on CO as of the time of writing.

In this study, we aimed to present a summary of the articles published on CO between the dates of 1 January, 1940 and 28 September, 2021 by means of bibliometric analysis.

MATERIALS AND METHODS

A quantitative study was planned using the bibliometric data analysis method to analyze global publications on CO. The Scopus bibliometric database (accessed: September 28, 2021) was searched in English. Original research articles and reviews made between 1 January, 1940 and 28 September, 2021 including the words "chronic" and "osteomyelitis" in their titles and keywords were found. Re-publications were included in the one-off review. Publications were analyzed bibliometrically according to their research parameters, such as their publication year, authors and institutions, keywords, funding institutions, and citations.

Ethics Committee

In this study, the Helsinki Declaration, which was revised in 2013, was complied with. Ethics committee approval was not required as there was no human or animal research.

RESULTS

There were a total of 2,881 publications on CO, and it was observed that the first publication was made in 1890. While the total number of articles was 666 (23.1%) before 1890-1979, it was found to be 857 (29.7%) solely between 2011-2021 (Figure 1). It was observed that there was a significant increase in the number of articles, particularly after the 2000s (Figure 2).

The majority of the articles [n=2,724 (94.55%)] were written in English; Russian [n=304 (10.55%)] and German [n=230 (7.98%)] are among the other most preferred publication languages. Moreover, it was determined that there were publications in 32 different languages. The United States was found to be the most prolific country with 484 (16.79%) articles. Germany, China, the United Kingdom, and India were among the top 5 countries to publish on CO. Our country, Türkiye, on the other hand, was in 10th place in this regard (Figure 3).

Among the publications, 2,565 (89.03%) were research articles and 129 (4.47%) were reviews. Five hundred sixteen (17.91%) of the articles were

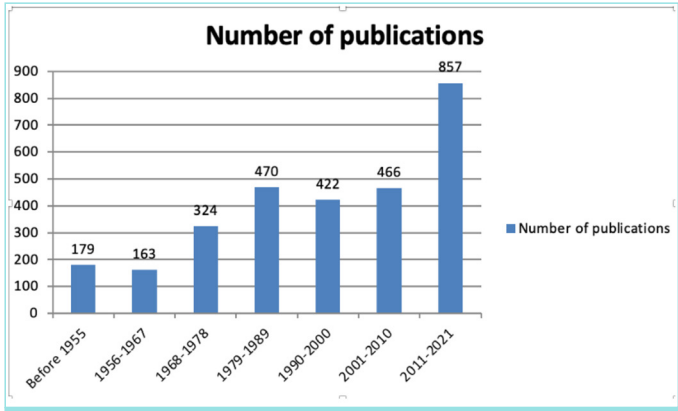


Figure 1. Numerical data of articles by years.

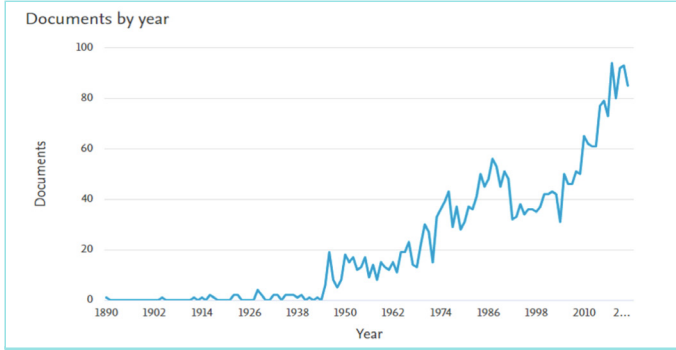


Figure 2. Distribution of articles by years.

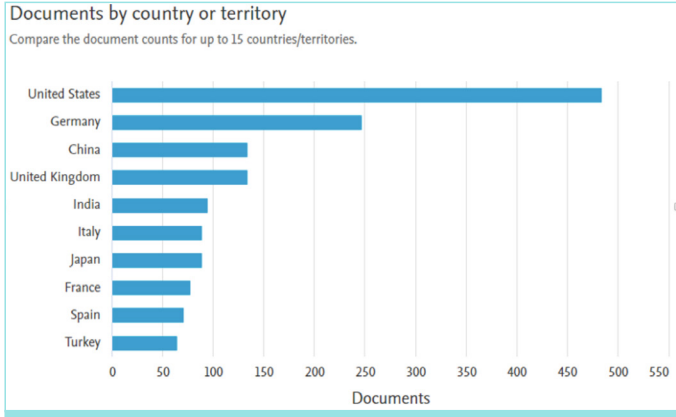


Figure 3. Top 10 countries with the highest number of articles published.

published in open access journals. It was observed that the China Medical University Hospital was the leading institution in publishing articles on CO (Table 1). The largest number of articles on CO were published in the journal Vestnik Khirurgii Imeni II Grekova [n=57 (1.97%)]. The most commonly used keyword was "osteomyelitis" [n=2,345 (81.39%)], followed by human [n=2,176 (75.5%)], article [n=1,854 (64.35%)], chronic disease [n=1,338] (46.44%)] and male [n=1,328 (46.09%)]. Most publications were in the fields of medicine [n=2,724 (94.55%)], dentistry [n =146 (5.06%)], and Immunology and Microbiology [n=129 (4.47%)]. 1,156 (40.12%) of the publications had not been cited at the time of writing. The most cited article received 384 citations (Table 2).

The top three funding sponsors for CO studies were the National Institutes of Health [n=30 (1.04%)], the National Natural Science Foundation of China [n=27 (0.93%)], and the US Department of Health and Human Services [n=21 (0.72%)]. Pediatricians and rheumatologists constituted the majority of the most prolific authors in CO studies. Polly J. Ferguson from the IOWA University Pediatrics Clinic was the clinician with the most publications on CO among the authors, with 22 articles written by her (Table 3).

DISCUSSION

According to our bibliometric analysis results with 22,881 articles, while the number of articles on CO for every 10 years published between 1979-2010 varied between 422 and 470, a significant increase was observed especially between the years 2011-2021. This indicates that researchers value the subject of CO. Based on these data, we anticipate that there may be an increase in the number of articles to be published in the future.

CO causes bone death, soft tissue deterioration, functional impairment, systemic disease, and eventually significant morbidity. For the eradication of infection and the restoration of function, new and reliable techniques, both medical and surgical, have been developed. Surgery continues to be the focus for the treatment of CO. However, multidisciplinary studies are essential to achieve satisfactory results.[7] In our study, Polly J. Ferguson from the IOWA University Pediatrics Clinic was the clinician with the most publications on CO among the authors with 22 articles. In addition to pediatricians, it is noteworthy that the

Table 1. Top 10 institutions with the highest number of articles published

Institution	Number of publications
China Medical University Hospital	21
Klinikum der Universität München	20
Julius-Maximilians-Universität Würzburg	20
China Medical University Hospital	19
National Ilizarov Medical Research Center for Traumatology & Orthopedics	19
Seattle Children's Hospital	19
University of Iowa Carver College of Medicine	18
Technische University Dresden	15
Hospital for Sick Children University of Toronto	15

Table 2. Most cited articles

Document title	Authors, year, country	Source	Citing count	Document type, language
Use of the muscle flap in chronic osteomyelitis: Experimental and clinical correlation	**Mathes Stephen J., 1982, USA**	Plastic and reconstructive surgery	384	Article, English
Subacute and chronic "symmetrical" osteomyelitis	Giedion, A., 1972, Switzerland	Annales de radiologie	338	Article, English
*Homozygous mutations in LPIN2 are responsible for the syndrome of chronic recurrent multifocal osteomyelitis and congenital dyserythropoietic anaemia (Majeed syndrome)	Ferguson, Polly J., History map of the USA in 2005	Journal of medical genetics	282	Article, English
*Osteomyelitis and the role of biofilms in chronic infection	**Brady, Rebecca A., 2008, USA**	FEMS Immunology and Medical Microbiology	266	Short survey, English
*Systemic antibiotics therapy for chronicle osteomyelitis in adults	Spellberg B., 2012, USA	Clinical Infectious Diseases	246	Article, English
The accuracy of diagnostic imaging for the assessment of chronic osteomyelitis: A systematic review and meta-analysis	Termaat MF, 2005, Netherlands	Journal of Bone and Joint Surgery - Series A	222	Review, English
Diagnostic Value of Sinus-tract Cultures in Chronic Osteomyelitis	Mackowiak, PA, 1978, USA	JAMA: The Journal of the American Medical Association	211	Article, English
Chronic recurrent multifocal osteomyelitis: Clinical outcomes after more than five years of follow-up	Huber, A.M., 2002, Canada	Journal of pediatrics	210	Article, English
*Imaging of chronic recurrent Multifocal Osteomyelitis	Khanna, G., 2009, USA	Radiographics	204	Article, English

authors with the most articles were pediatricians, rheumatologists, and neurologists. Although surgery is the main treatment for CO, data demonstrate that orthopedic and plastic surgeons do not show interest in this subject. We believe that surgeons should share their experiences on CO and their innovations in follow-up and treatment methods more frequently in the literature.

The authors, institutions, and countries which have published the largest number of articles each show not only their contribution to the literature on a particular subject, but also their research capabilities and degree of influence on the subject. It is expected that these authors, institutions, and countries will contribute significantly to science on the subject they are interested in going forwards. Furthermore, some studies have reported that the economic size or level of development of countries has a significant effect on their academic publication efficiency.[8,9] In our study, when the distribution of publications of the countries was examined, it was observed that most publications on CO were made by developed countries (USA, Germany, England, Italy, Japan, France, Spain) or by developing countries with large economies (China, India, Türkiye). As a result of our study, it was determined that our country, Türkiye, is in the 10th rank according to the number of publications on CO. We think that the interest of researchers in our country on this subject and the number of effective publications should be increased.

Identifying core journals with high publication and co-citation counts provides significant information for authors to select high-quality journals. Authors may not have the necessary knowledge or experience to select the most appropriate journals for the publication of their research. Therefore, the results from the current bibliographic study provide some guidance to clinical research authors in the field of CO.[10] In our bibliometric study, the research titled "Use of the muscle flap in CO: Experimental and clinical correlation", published by Mathes Stephen et al. was the most cited. The other most cited articles are indicated in Table 2. We consider that it would be beneficial for researchers interested in CO to first read these studies determined by citation. The fact that the rate of uncited articles in our study was 40.2% shows the necessity of improving the level and quality of research in the field of CO.

When the keyword analysis was evaluated, it was seen that the keywords osteomyelitis, human, article, chronic disease, and male are frequently used in articles related to CO. In addition, the use of osteomyelitis with a rate of 81.39% makes us think that this keyword will assist researchers to reach their aims more readily on this subject.

Until 2004, the Web of Science (WoS) database, which was among the publications of Tomson Reuters, was the only database used in bibliometric studies. With the establishment of Scopus and Google Scholar in 2004, the number of bibliometric databases increased.[11,12] In our study, Scopus, which is used for bibliometric analyzes and has the largest database feature, was preferred.

Bibliometrics is the quantitative analysis of research articles using mathematical and statistical methods. It includes a set of criteria to evaluate the impact and trends of research articles published with this analytic method. It can be used to identify models associated with publications in a particular field. Additionally, it is also a useful method for detecting citation data, understanding basic research areas, and predicting future research directions.[13] In the literature, it was seen that bibliographic studies were conducted in the field of orthopedics.[14-16] In our study, we examined the subject of CO, which is still open to debate in the follow-up and treatment of orthopedists, other clinicians, and patients, and had not previously had a bibliometric study.

Study Limitations

One limitation of our study was that we only analyzed research articles and reviews published in the Scopus database. The PubMed and WoS databases were not included. If more than one database is used in bibliometric studies where thousands of articles are analyzed, including the same articles more than once, this may adversely affect the data. Another limitation of bibliometric studies is that they can only be conducted on publications cited and indexed in journals and do not include unpublished studies or publications, theses, and books in non-indexed journals. In our study, there was no analysis of the articles' contents, and only the characteristics of the publications were examined.

CONCLUSION

In this clinical study published on CO, it was found that the number of publications increased rapidly, particularly after the 2000s. It was clearly seen that the most important contribution to the increase in the number of publications was made by those countries with high economic size and development levels. It was remarkable that pediatricians were at the forefront of CO, which is followed up and treated with the contribution of many disciplines.

Table 3. Authors with the most articles in chronic osteomyelitis research

Authors name	Affiliation, country	Number of publications
Polly J. Ferguson	University of Iowa, Department of Pediatrics, Iowa City, United States	22
Hermann Josef Girschick	Vivantes Klinikum im Friedrichshain, Department of Pediatrics and Adolescent Medicine, Berlin, Germany	19
Henner Morbach	Universitätsklinikum Wurzburg, Department of Pediatrics, Wurzburg, Germany	19
Fritz Schilling	Johannes Gutenberg-Universität Mainz, Mainz, Germany	18
Christian Michael Hedrich	University of Liverpool, Department of Women's and Children's Health, Liverpool, United Kingdom Alder Hey Children's NHS Foundation Trust, Department of Pediatric Rheumatology, Liverpool, United Kingdom	17
Chunhuang Tseng	School of Medicine, Department of Neurology, Taichung, Taiwan	17
Yongdong Zhao	University of Washington, Department of Pediatrics, Seattle, United States	16
Ronald M Laxer	Hospital for Sick Children University of Toronto, Division of Rheumatology, Toronto, Canada	12
Chih Hsin Muo	China Medical University, Management Office for Health Data, Taichung, Taiwan	11

MAIN POINT

- Bibliometric studies have become very popular in recent years. It was the first in the literature to conduct a bibliometric study on a subject that should be approached multidisciplinary, such as chronic osteomyelitis
- In this study; It is the examining 2881 articles as a result of scanning the words CO in the Scopus bibliometric database between 1940-2021.

Ethics Committee Approval: Ethics committee approval was not required as there was no human or animal research.

Informed Consent: Informed consent approval was not required as there was no human or animal research.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: S.N.K., N.D.D., S.K., S.K.Ö., S.A., Design: S.N.K., N.D.D., S.K., S.K.Ö., S.A., Data Collection and/or Processing: S.N.K., N.D.D., S.K., S.K.Ö., S.A., Analysis and/or Interpretation: S.N.K., N.D.D., S.K., S.K.Ö., S.A., Literature Search: S.N.K., N.D.D., S.K., S.K.Ö., S.A., Writing: S.N.K., N.D.D., S.K., S.K.Ö., S.A.

DISCLOSURES

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

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

References

1. Chassaignac E. De l'osteo-myelite. Paris: Bull Mem Soc Chir. 1852: 431-6.
2. Parsons B, Strauss E. Surgical management of chronic osteomyelitis. Am J Surg. 2004; 188(1A Suppl): 57-66.
3. Jackson R. Doctors and Diseases in the Roman Empire. University of Oklahoma Press, Oklahoma City. 1988.p.116-7.
4. Patzakis MJ, Abdolahi K, Sherman, Holtom RP, Wilkins J. Treatment of chronic osteomyelitis with muscle flaps. Orthop Clin North Am. 1993; 248(3): 505-9.
5. May JW, Jupiter JB, Gallico GG 3rd, Rothkopf DM, Zingarelli P. Treatment of chronic traumatic bone wounds. Microvascular free tissue transfer: a 13-year experience in 96 patients Ann Surg. 1991; 214(3): 241-50; discussion 250-2.
6. Guler S, Ozmanevra R, Çapkin S. Global Scientific Outputs of Microsurgery Publications: A Bibliometric Approach About Yesterday, Today, and Tomorrow. Cureus. 2020; 12(12): 12205.
7. McNally M, Nagarajah K. Osteomyelitis. Orthop Trauma. 2010; 24(6): 416-29.
8. Doğan G, Karaca O. A bibliometric analysis of the field of anesthesia during 2009-2018. Braz J Anesthesiol. 2020; 70(2): 140-52
9. Kiraz M, Demir E. Bibliometric analysis of publications on spinal cord injury during 1980-2018. World Neurosurg. 2020; 136: 504-13.
10. Wang SQ, Gao YG, Zhang C, Xie YJ, Wang JX, Xu FY. A Bibliometric Analysis Using CiteSpace of Publications from 1999 to 2018 on Patient Rehabilitation After Total Knee Arthroplasty. Med Sci Monit. 2020; 26: e920795.
11. Kawuki J, Yu X, Musa TH. Bibliometric Analysis of Ebola Research Indexed in Web of Science and Scopus (2010-2020). Biomed Res Int. 2020; 2020: 5476567.
12. Karasözen B, Bayram ÖG, Zan BU. Comparison of the WoS and Scopus Databases. Turkish Librarianship. 2011; 25(2): 238-60.
13. Reuters T. Using Bibliometrics: A guide to evaluating research performance with citation data. White paper. 2008. http://ips.clarivate.com/m/pdfs/325133_thomson.pdf
14. Zhou T, Xu Y, Xu W. Emerging research trends and foci of studies on the meniscus: A bibliometric analysis. J Orthop Surg (Hong Kong). 2020; 28(3): 2309499020947286.
15. Zhang Y, Wumaier M, He D, Xiao B, Zhang J. The 100 Top-Cited Articles on Spinal Deformity: A Bibliometric Analysis. Spine. 2020; 45(4): 275-83.
16. He J, He L, Geng B, Xia Y. Bibliometric Analysis of the Top-Cited Articles on Unicompartmental Knee Arthroplasty. J Arthroplasty. 2021; 36(5): 1810-8.

RESEARCH ARTICLE

CYPRUS
JOURNAL OF MEDICAL SCIENCES

DOI: 10.4274/cjms.2022.2021-176
Cyprus J Med Sci 2023;8(1):13-19

The Value of Strain Elastography in the Distinction Between Benign and Malignant Thyroid Nodules in Patients with Hashimoto Thyroiditis

Evşen Polattaş Solak[1], Halit Nahit Şendur[2], Mahi Nur Cerit[2], Emetullah Cindil[2], Suna Özhan Oktar[2], Cem Yücel[2]

[1]St. Antonius Hospital, Nieuwegein, Netherlands
[2]Department of Radiology, Gazi University Faculty of Medicine, Ankara, Türkiye

Abstract

BACKGROUND/AIMS: To assess the value of strain elastography (SE) in the distinction between benign and malignant thyroid nodules in patients with Hashimoto's thyroiditis (HT).

MATERIALS AND METHODS: The study cohort consisted of 74 thyroid nodules in 69 patients with HT. Fine needle aspiration biopsy (FNAB) or surgical excision were used as the reference standards. Gray-scale properties (internal structure, echogenity, presence or absence of hypoechoic halo sign, microcalcifications, "taller than wide" sign), vascularity scores, elasticity scores and strain ratios of all nodules were noted and compared between benign and malignant nodules. Categorical variables were compared using the Pearson chi-square test. Correlation analyses were performed using the Spearman correlation test. The diagnostic performance of the SE was evaluated using receiver operator characteristic (ROC) curves.

RESULTS: Of the included 74 nodules, 67 (90.5%) and 7 nodules (9.5%) were benign and malignant, respectively. There were significant differences in echogenicity (p=0.017), peripheral halo (p=0.003), microcalcifications (p<0.001), and margin and shape (p=0.009) characteristics between benign and malignant nodules. There was a significant association between elasticity scores and histopathological diagnosis of the nodules (p<0.001). A cut-off point of 6.16 for SR value had sensitivity, specificity, PPV and NPV as 71.4%, 100%, 100% and 97.1%, respectively. Area under the ROC curve for the diagnostic performance of SR values in the differentiation of benign and malignant nodules was 0.797 (p=0.01)

CONCLUSION: Our study revealed that SE is an accurate diagnostic tool to characterize thyroid nodules in patients with HT and this may be useful to avoiding unnecessary invasive procedures.

Keywords: Thyroid nodules, Hashimoto thyroiditis, strain elastography

INTRODUCTION

The thyroid nodule is a discrete lesion that is distinguishable from the surrounding parenchyma in the gland.[1] The prevalence of thyroid nodules varies among regions, and people who live in iodine-deficient areas are more prevalent in this glandular abnormality. The widespread usage of ultrasound led a sizable increase in the detection of thyroid nodules. However, only a few percent (<5%) of these lesions were malignant.[2,3]

Hashimoto's thyroiditis (HT) is an autoimmune disease characterized by lymphocytic infiltration of the thyroid gland and it is the most common cause of hypothyroidism.[4-6] HT most commonly occurs in women, and

To cite this article: Polattaş Solak E, Şendur HN, Cerit MN, Cindil E, Özhan Oktar S, Yücel C. The Value of Strain Elastography in the Distinction Between Benign and Malignant Thyroid Nodules in Patients with Hashimoto Thyroiditis. Cyprus J Med Sci 2023;8(1):13-19

ORCID IDs of the authors: E.P.S. 0000-0003-4818-4016; H.N.Ş. 0000-0003-1690-2538; M.N.C. 0000-0003-2878-6052; E.C. 0000-0002-9345-1577; S.Ö.O. 0000-0003-0112-9992; C.Y. 0000-0002-8014-3874.

Address for Correspondence: Halit Nahit Şendur
E-mail: hsendur@gmail.com
ORCID ID: orcid.org/0000-0003-1690-2538

Received: 24.06.2021
Accepted: 09.04.2022

it demonstrates the peak incidence in the fourth and fifth decades of the life.[7] Clinically, it is presented as diffuse enlargement of the gland with elevated levels of thyroid autoantibodies.[8,9] Ultrasound may aid in the diagnosis of HT, and heterogeneous hypoechogenic parenchyma with increased vascularity in a diffuse enlarged gland is typical for the sonographic appearance of HT.[10,11] However, despite this well-recognized appearance characterization of existing thyroid nodules on gray-scale sonographic evaluation in patients with HT may be challenging and difficult due to these parenchymal alterations. Furthermore, it has been reported that patients with HT have a higher risk of developing thyroid cancer.[12,13] One study reported that the hazard ratio for developing thyroid cancer in HT patients was 11.8 in comparison to the control group.[13] Therefore, in addition to gray-scale evaluation, utilization of relatively newer ultrasound imaging techniques may contribute to more accurate differentiation of benign and malignant thyroid nodules in patients with HT.

Strain elastography (SE) is an imaging technique that estimates the stiffness of the target tissue in response to applied external forces and provides qualitative and semiquantitative assessments.[14,15] Previous studies have reported that SE may improve the diagnostic accuracy of conventional gray-scale ultrasound evaluation in patients with thyroid nodules.[2,3,16-19] However, limited numbers of studies that evaluate the diagnostic performance of SE in thyroid nodules with concurrent HT patients exist in the current literature.[9,20,21] In this regard, this study aimed to assess the value of SE in the distinction between benign and malignant thyroid nodules in patients with HT.

MATERIALS AND METHODS

This prospective study was approved by the Gazi University Institutional Review Board (approval number: 214, date: 05.23.2012) and written informed consent was provided by the patients. Between January and September 2012, all consecutive patients with HT and coexisting thyroid nodules were included in this study. The diagnosis of HT was made based on the symptoms of the patients and clinical and laboratory findings. The patients lacking histopathological assessment, and the patients with cystic or dense-calcified nodules were excluded. Finally, the study cohort consisted of 74 thyroid nodules in 69 patients with HT. Fine needle aspiration biopsy (FNAB) or surgical excision were used as the reference standards. The age and gender of the included patients were noted.

All patients were examined in the supine position using an ultrasound system (Hitachi EUB 7,500) with a 13-8 MHz linear transducer. The tip of the transducer was covered with a generous amount of ultrasound gel. The thyroid glands of the patients were examined in two orthogonal planes. After gray-scale ultrasound assessment, SE was performed for the detected nodules. During elastographic evaluations, the probe was held in the long axis of the thyroid gland to avoid potential mistracking artifacts that may be caused by lateral displacements originating from pulsations of the adjacent carotid artery. To obtain appropriate elastograms, repetitive external forces were applied perpendicular to the gland. The appropriateness of the elastograms was evaluated due to indicators located at the right lower corner of the screen, and the scores that evaluate the quality of manuel compression equal or higher than 3 was determined as appropriate. For all thyroid nodules at least 3 appropriate elastography images were obtained. Gray-scale ultrasound and elastography images of evaluations were recorded on a digital system, and two experienced radiologists assessed all images in a separate reading session with consensus. The patients were categorized into two subgroups (normal or typical for HT) according to parenchymal patterns of the HT disease. The patients whose thyroid gland did not demonstrate any parenchymal abnormality were considered a normal parenchymal pattern. The largest size of the nodules was measured. The composition, echogenicity, shape, and margin characteristics of the nodules and the presence of a peripheral halo and internal microcalcifications were noted. On color Doppler ultrasound vascularity of the nodules was assessed. A vascularity score was given to all nodules based on the vascularity characteristics of the nodules. Score 1 was given if the nodule did not demonstrate peripheral or internal vascularization. Score 2 was given if the nodule demonstrated only peripheral vascularity (no internal vascularity). Score 3 was given if the nodule demonstrated both minimal peripheral and internal vascularity. Score 4 was given if the nodule demonstrated both extensive peripheral and internal vascularity.

Elasticity scores were given for the thyroid parenchymas of the patients according to color codes on the elastograms. Score 1 was given if the parenchyma demonstrated elasticity (green) in all areas. Score 2 was given if the parenchyma predominantly demonstrated elasticity and includes a few areas with low elasticity (blue). Score 3 was given if the parenchyma demonstrated a mixture of green and blue colors. Score 4 was given if the parenchyma demonstrated only low elasticity. Similarly, elasticity scores were given for the thyroid nodules according to the color-coded elasticity characteristics of the nodules on the elastograms. Furthermore, a region of interest (ROI) that covers the entire nodule was placed in all thyroid nodules on elastograms. A second ROI was drawn on the same elastogram in the thyroid parenchyma adjacent to the nodule. The strain ratio (SR) was calculated automatically for each nodule by dividing the elasticity of the parenchyma to the elasticity of the thyroid nodule (Figure 1, 2).

Statistical Analysis

All statistical analyses were performed using SPSS for Windows version 15.0 (IBM Corp, Armonk, NY, USA). Descriptive statistics of the data consisted of mean ± standard deviation or median (minimum-maximum) values. Mann-Whitney U test was used to compare quantitative variables. Categorical variables were compared using the pearson chi-square test. Correlation analyses were performed using the Spearman correlation test. The diagnostic performance of the SE was evaluated using receiver operator characteristic (ROC) curves. A p-value less than 0.05 was used to determine the statistical significance.

Results

The study cohort consisted of 64 (92.8%) women, and 5 (7.2%) men. The mean age of the patients was 49 years (range: 18 to 74 years). The mean size of the nodules was 16.38±8.38 mm (range: 7 to 60 mm). The histopathological diagnosis of the nodules was obtained using FNAB and post-surgical specimen assessment in 60 (81.1%) and 14 (18.9%) nodules, respectively. Of these 74 nodules, 67 (90.5%) nodules were diagnosed as benign, and 7 (9.5%) nodules were diagnosed as malignant. There were no significant differences in the age (p=0.59) and gender (p=0.375) characteristics between the patients with benign and malignant nodules. There were no significant differences in the mean nodule sizes between the benign (15.46 mm) and malignant (25.14 mm) nodules (p=0.168).

The surrounding parenchymal pattern of the nodules were assessed as normal for 7 (9.5%) nodules and typical for Hashimoto thyroiditis

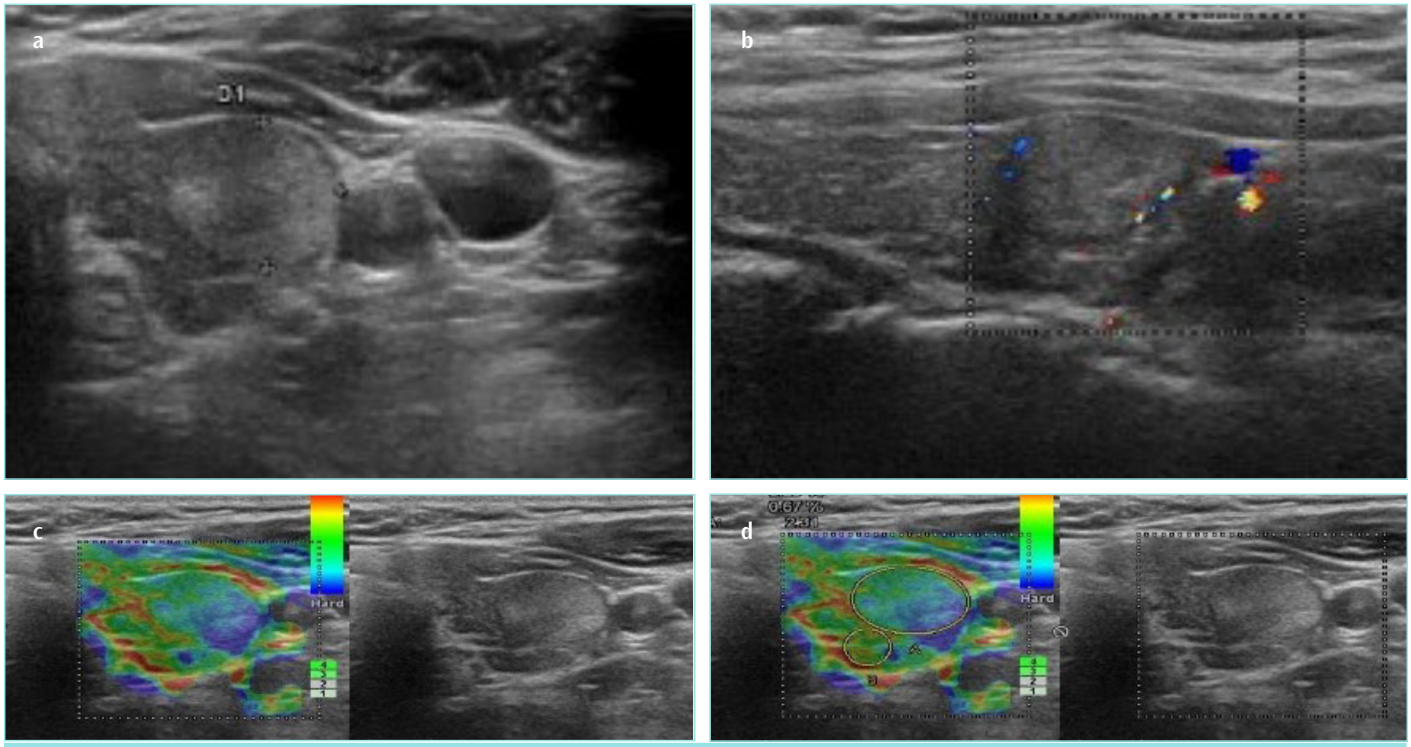


Figure 1. Gray-scale ultrasound (a), color Doppler ultrasound (b), and strain elastography (c, d) images of a 18 year old female patient with Hashimoto thyroiditis demonstrates a round shaped solid nodule with well-defined margin characteristics. The assigned elasticity score was 1, and Strain ratio was calculated as 0.61. Fine needle aspiration biopsy revealed that the nodule was adenomatous nodule.

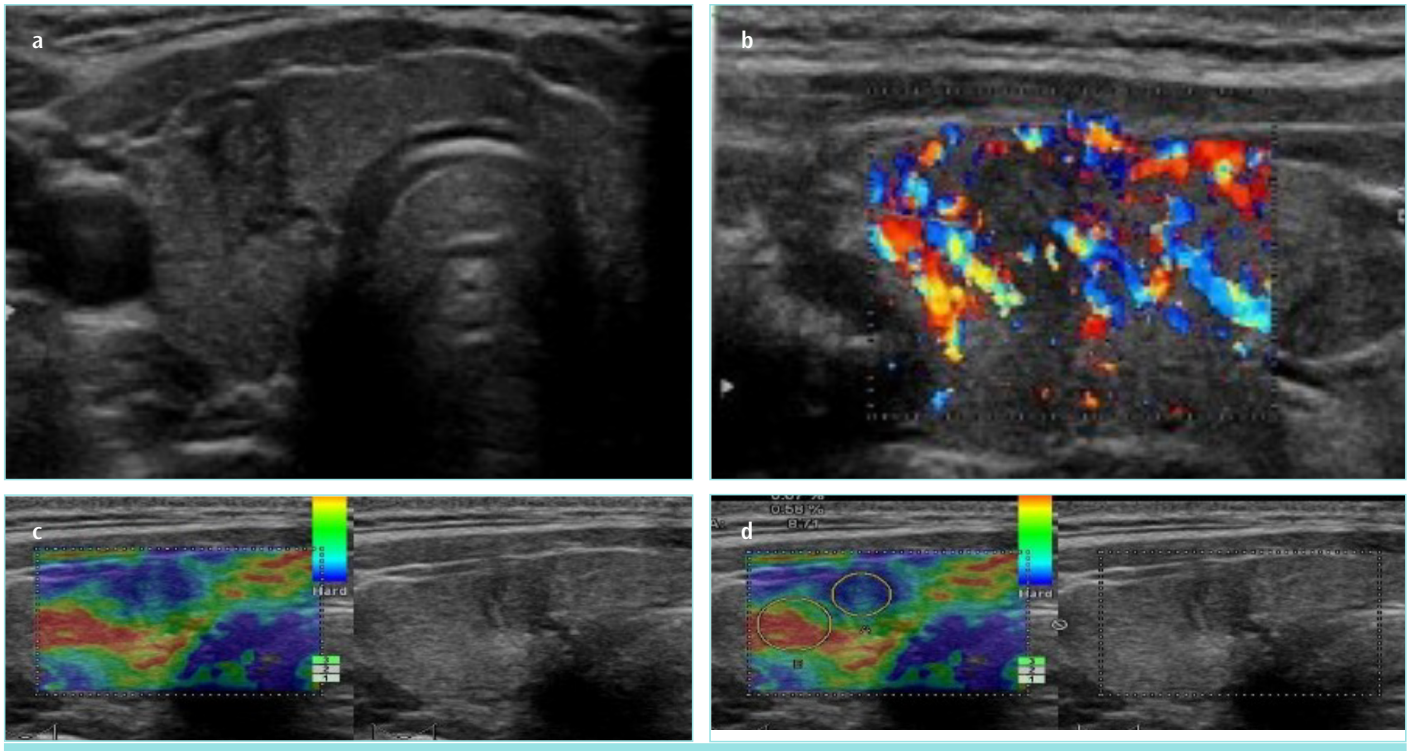


Figure 2. Gray-scale ultrasound (a), color Doppler ultrasound (b), and strain elastography (c, d) images of a 28 year old female patient with Hashimoto thyroiditis demonstrates a hypoechogenic solid nodule with irregular margin characteristics. The assigned elasticity score was 4, and Strain ratio was calculated as 10.5. The histopathological diagnosis was papillary carcinoma.

for 67 (90.5%) nodules. The composition of the nodules were solid and semisolid in 53 (71.6%) and 21 (28.4%) patients, respectively. The echogenicity of the nodules was hypoechogenic in 43 (58.1%) nodules. The margin characteristics were well-defined in 54 (73%) nodules. There were microcalcifications in 10 (13.5%) nodules. There were no statistical significant differences in the sonographic characteristics of the nodules between the parenchymal pattern subgroups (normal and typical for HT) of the patients (p>0.05). There were significant differences in echogenicity (p=0.017), peripheral halo (p=0.003), microcalcifications (p<0.001), and margin and shape (p=0.009) characteristics between benign and malignant nodules. Table 1 represents the distribution of the sonographic characteristics of the benign and malignant nodules. There was a significant difference in vascularity scores between benign and malignant nodules (p<0.001). Table 2 represents the distribution of vascularity characteristics of the benign and malignant nodules.

There was no significant difference in the elasticity scores of the nodules between the parenchymal pattern subgroups (p>0.05). There was a weak correlation between the elasticity scores of the nodules and parenchymas (Spearman's rho: 0.308). Table 3 represents the distribution of elasticity scores for the nodules and parenchymas.

Among the nodules within typical for HT surrounding the parenchyma, 91% (61/67) of the nodules were benign, whereas 9% (6/67) of the nodules were malignant. Among the nodules within the normal surrounding parenchyma, 86% (6/7) of the nodules were benign, whereas 14% (1/7) of the nodules were malignant. There were no significant differences in benign and malignant nodules between the parenchymal pattern subgroups (p=0.517).

On elasticity score assessment, 35 of 67 (52.2%) benign nodules showed score 2 characteristics, whereas 5 of 7 (71.4%) malignant nodules showed score 4 characteristics. There was a significant association between elasticity scores and histopathological diagnosis of the nodules (p<0.001). Table 4 represents the distribution of elasticity scores for the benign and malignant nodules. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of elasticity score 4 in the differentiation of malignant thyroid nodules were 71.4%, 95.5%, 62.5%, and 97% respectively. The area under the ROC curve (AUROC) for the diagnostic performance of the elasticity score in the differentiation of benign and malignant nodules was 0.902 (p<0.001) (Figure 3). The means of SR values for benign and malignant nodules were 1.44±0.86 and 7.46±4.82, respectively. There was a significant difference between SR values of benign and malignant nodules (p=0.01). AUROC for the diagnostic performance of SR values in the differentiation of benign and malignant nodules was 0.797 (p=0.01). If a cut-off point was determined as 6.16 for SR value, the sensitivity, specificity, PPV, NPV, and diagnostic accuracy were 71.4%, 100%, 100% 97.1%, and 97.3%, respectively.

DISCUSSION

The combination of superficial location of the thyroid gland and wide usage of sonography in a clinical setting contributes to detection of a large number of thyroid nodules in daily practice. Although malignant transformation of thyroid nodules is rare, it constitutes a diagnostic challenge. This challenge may become more complicated when thyroid nodules occur in patients with HT. The current study revealed that qualitative and semiquantitative assessments depending on SE can

Table 1. The distribution of sonographic characteristics of the benign and malignant nodules

Characteristics of the nodules		% (number of nodules/total)			p
		Benign	Malignant	Total	
Composition of nodule	Solid	68.7 (46/67)	100 (7/7)	71.6 (53/74)	0.181
	Semisolid	31.3 (21/67)	0 (0/7)	28.4 (21/74)	
Echogenicity	Hyperechogenic	19.4 (13/67)	0 (0/7)	17.6 (13/74)	0.017
	Isoechogenic	26.9 (18/67)	0 (0/7)	24.3 (18/74)	
	Hypoechogenic	53.7 (36/67)	100 (7/7)	58.1 (43/74)	
Peripheral halo	Present	59.7 (40/67)	0 (0/7)	54.1 (46/74)	0.003
	Absent	40.3 (27/67)	100 (7/7)	45.9 (34/74)	
Microcalcifications	Present	7.5 (5/67)	71.4 (5/7)	13.5 (10/74)	<0.001
	Absent	92.5 (62/67)	28.6 (2/7)	86.5 (64/74)	
The margin characteristics	Well-defined	79.1 (53/67)	14.3 (1/7)	73 (54/74)	0.001
	Irregular	20.9 (14/67)	85.7 (6/7)	27 (20/74)	
"Taller than wide" sign	Yes	4.5 (3/67)	42.9 (3/7)	8.1 (6/74)	0.009
	No	95.5 (64/67)	57.1 (4/7)	91.9 (68/74)	

Table 2. The distribution of vascularity characteristics of the benign and malignant nodules

Vascularity scores	Benign	Malignant	Total
1	10 (14.9%)	2 (28.6%)	12 (16.2%)
2	25 (37.3%)	0 (0%)	25 (33.8%)
3	29 (43.3%)	0 (0%)	29 (39.2%)
4	3 (4.5%)	5 (71.4%)	8 (10.8%)
Total	67 (100%)	7 (100%)	74 (100%)

successfully differentiate benign and malignant nodules in patients with HT and this may be helpful in reduce unnecessary invasive procedures for thyroid nodules in HT patients.

Anderson et al.[11] analyzed the sonographic appearances of nodular HT patients, and the authors concluded that the sonographic and vascularity features of nodular HT show wide variations. The authors found that the nodules in patients with HT were most commonly hypoechogenic (47%), and showed well-defined (60%) margin characteristics. In their study, 20% of the nodules had calcifications. The current study revealed that 58.1% of the nodules were hypoechogenic; 73% of the nodules had well-defined margin characteristics, and microcalcifications were present in 13.5% of the nodules. These findings may be considered in accordance with the aforementioned study. Conversely, in that study, Anderson et al.[11] found that 45% (29/64) of cases occurred within normal thyroid parenchymal features, whereas 55% (35/64) of cases occurred within the parenchyma that has typical sonographic features of HT. In the current study, only 10% (7/69) patients demonstrated normal thyroid parenchymal features on sonography. Moreover, Anderson et al.[11] also found that 35% of the nodules were hypervascular, while 29% of the nodules were avascular. In our study, we found that 16.2% (12/74) and 50% (37/74, both score 3 and 4) of the nodules were avascular and hypervascular, respectively. The differences in sonographic and vascularity features of the nodules between the studies are more likely due to potential regional variabilities in nodular HT characteristics.

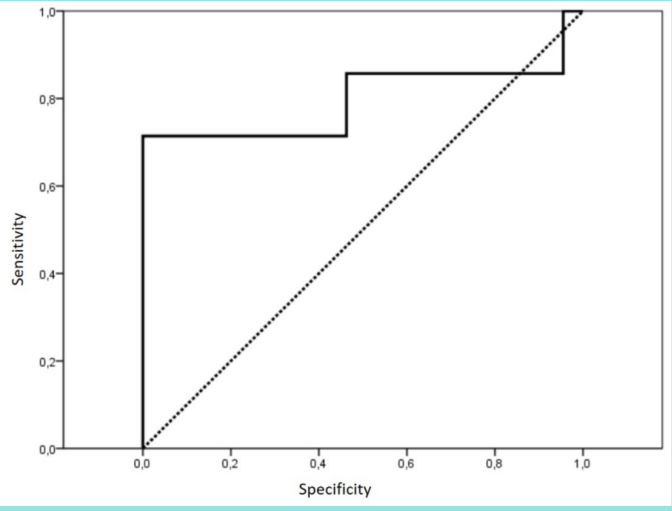


Figure 3. Receiver operating characteristic curve of strain ratio for diagnosis of benign and malignant thyroid nodules.

Cappelli et al.[9] investigated the predictive value of SE in benign thyroid nodules in patiens with co-existing HT. In their study cohort, of the included 242 nodules, 230 (95%) nodules were benign. On elastograms, they found that 79.1% of benign nodules demonstrated elasticity (score 1) in the whole nodule. However, when they categorized the nodules into two subgroups according to parenchymal patterns (mild/moderate versus severe hypoechogenicity) of the gland, they found that the sensitivity of score 1 elasticity on nodules decreased from 75% to 50% in the severe hypoechogenic group. In our study, the distribution of benign (90.5%) and malignant (9.5%) nodules in HT patients was similar to the study cohort of Cappelli et al.[9] We found that 80.6% (54/67) of benign nodules demonstrated score 1 and 2 features on elastograms and this may be considered as in line with the findings of Cappelli et al.[9]

We found a weak correlation between the elasticity scores of the nodules and the gland parenchymas. As lymphocytic infiltration and fibrosis occur in the gland parenchyma of patients with HT, it causes hard tissue characteristics in the gland. This also has the potential to affect the nodule elasticity as the elasticity of the background parenchyma of the nodule may contribute to its elasticity.

Şahin et al.[20] investigated the role of SE in distinguishing benign and malignant thyroid nodules in patients with HT. Their study chort consisted of 267 (92.1%) benign and 23 (7.9%) malignant nodules. The distribution of benign and malignant nodules in their study was quite similar to the current study. They reported that the elasticity score equal or higher than 3 had 82.6% and 50.9% sensitivity and specificity, respectively, for detecting malignant nodules. In the current study, the sensitivity and specificity values for elasticity score 4 in detecting malignant thyroid nodules were 71.4% and 95.5%, respectively. Moreover, Şahin et al.[20] found that a value of 2.45 for the strain index

Table 3. The distribution of elasticity scores for the nodules and parenchymas

Elasticity scores of the nodules	Elasticity scores of parenchymas				
	1	2	3	4	Total
1	13 (68.4%)	2 (10.5%)	4 (21.1%)	0 (0%)	19 (100%)
2	4 (11.1%)	29 (80.6%)	3 (8.3%)	0 (0%)	36 (100%)
3	2 (18.2%)	4 (36.4%)	4 (36.4%)	1 (9,1%)	11 (100%)
4	2 (25%)	5 (62.5%)	1 (12.5%)	0 (0%)	8 (100%)
Total	21 (28.4%)	40 (54.1%)	12 (16.2%)	1 (1,4%)	74 (100%)

Table 4. The distribution of elasticity scores for the benign and malignant nodules

Elasticity scores	Benign	Malignant	Total
1	19 (100%)	0 (0%)	19 (100%)
2	35 (97.2%)	1 (2.8%)	36 (100%)
3	10 (90.9%)	1 (9.1%)	11 (100%)
4	3 (37.5%)	5 (62.5%)	8 (100%)
Total	67 (90.5%)	7 (9.5%)	74 (100%)

had 73.9% and 73% sensitivity and specificity, respectively, for detecting malignant nodules. On the other hand, Wang et al.[21] reported that 5.03 (sensitivity 75%, specificity 92.1%) was the best cut-off point for differentiating benign and malignant thyroid nodules in patients with HT. In our study, a value of 6.16 for SR had 71.4% and 100% sensitivity and specificity, respectively. Differences in the ROI selection criteria and potential reader variabilities may lead to these discordant results between the studies. Moreover, Dietrich et al.[22] reported that for determining benign and malignant thyroid nodules by using SR values, there is no agreement on the best cut-off point.

To maintain the quality of patient care, it is desirable to avoid unnecessary invasive procedures. In a systematic review and meta-analysis,[3] it has been concluded that sonoelastography is an accurate adjunctive technique for evaluating thyroid nodules and it may be helpful to decrease the number of FNAB. Among the 55 nodules that demonstrated elasticity score 1 or 2 features in this study, only 1 (1.8%) nodule had a malignant diagnosis. In this context, in patients with HT, if a nodule has an elasticity score 1 or 2 characteristics on elastograms, to reduce the number of unnecessary biopsies follow-up may be preferrable rather than FNAB.

Study Limitations

The current study has limitations. First, the number of included nodules was small. Second, this study did not evaluate inter- and intraobserver variabilities, which have the potential to lead to wide variations in diagnostic performance of SE. Therefore, future studies with a larger number of nodules that additionally evaluates inter- and intraobserver variabilities will be more impactful.

CONCLUSION

Our study revealed that SE is an accurate diagnostic tool to characterize thyroid nodules in patients with HT and this may be useful to avoid unnecessary invasive procedures.

MAIN POINTS

- SE can be useful in the distinction between benign and malignant thyroid nodules in patients with Hashimoto thyroiditis.
- SE as an adjunctive tool to ultrasound can reduce the number of benign biopsies in patients with Hashimoto thyroiditis.
- Elasticity scores had a significant association with histopathological diagnosis of the thyroid nodules.

ETHICS

Ethics Committee Approval: This prospective study was approved by the Gazi University Institutional Review Board (approval number: 214, date: 05.23.2012).

Informed Consent: Written informed consent was provided by the patients.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: E.P.S., S.Ö.O., C.Y., Design: H.N.Ş., S.Ö.O., Supervision: S.Ö.O., C.Y., Materials: E.P.S., H.N.Ş., M.N.C., Data Collection and/or Processing: E.P.S., H.N.Ş., M.N.C., Analysis and/or Interpretation: E.P.S., H.N.Ş., M.N.C., E.C., Literature Search: E.P.S., Writing: E.P.S., H.N.Ş., Critical Review: E.P.S., H.N.Ş., M.N.C., E.C., S.Ö.O., C.Y.

DISCLOSURES

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

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

REFERENCES

1. Haugen BR, Alexander EK, Bible KC, Doherty GM, Mandel SJ, Nikiforov YE, et al. 2015 American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer: The American Thyroid Association Guidelines Task Force on Thyroid Nodules and Differentiated Thyroid Cancer. Thyroid. 2016; 26(1): 1-133.
2. Rago T, Santini F, Scutari M, Pinchera A, Vitti P. Elastography: new developments in ultrasound for predicting malignancy in thyroid nodules. J Clin Endocrinol Metab. 2007; 92(8): 2917-22.
3. Ghajarzadeh M, Sodagari F, Shakiba M. Diagnostic accuracy of sonoelastography in detecting malignant thyroid nodules: a systematic review and meta-analysis. AJR Am J Roentgenol. 2014; 202(4): W379-89.
4. Vanderpump MP. The epidemiology of thyroid disease. Br Med Bull. 2011; 99(1): 39-51.
5. McLeod DS, Cooper DS. The incidence and prevalence of thyroid autoimmunity. Endocrine. 2012; 42(2): 252-65.
6. Silva de Morais N, Stuart J, Guan H, Wang Z, Cibas ES, Frates MC, et al. The Impact of Hashimoto Thyroiditis on Thyroid Nodule Cytology and Risk of Thyroid Cancer. J Endocr Soc. 2019; 3(4): 791-800.
7. Chiovato L, Magri F, Carlé A. Hypothyroidism in Context: Where We've Been and Where We're Going. Adv Ther. 2019; 36(Suppl 2): 47-58.
8. Dayan CM, Daniels GH, Chronic autoimmune thyroiditis. N Engl J Med. 1996; 335(2): 99-107.
9. Cappelli C, Pirola I, Gandossi E, Formenti A, Agosti B, Castellano M. Elastography Evaluation of Benign Thyroid Nodules in Patients Affected by Hashimoto's Thyroiditis. Int J Endocrinol. 2015; 2015: 367054.
10. Yeh HC, Futterweit W, Gilbert P. Micronodulation: ultrasonographic sign of Hashimoto thyroiditis. J Ultrasound Med. 1996; 15(12): 813-9.
11. Anderson L, Middleton WD, Teefey SA, Reading CC, Langer JE, Desser T, et al. Hashimoto thyroiditis: Part 1, sonographic analysis of the nodular form of Hashimoto thyroiditis. AJR Am J Roentgenol. 2010; 195(1): 208-15.
12. Repplinger D, Bargren A, Zhang YW, Adler JT, Haymart M, Chen H. Is Hashimoto's thyroiditis a risk factor for papillary thyroid cancer? J Surg Res. 2008; 150(1): 49-52.
13. Chen YK, Lin CL, Cheng FT, Sung FC, Kao CH. Cancer risk in patients with Hashimoto's thyroiditis: a nationwide cohort study. Br J Cancer. 2013; 109(9): 2496-501.
14. Cosgrove D, Barr R, Bojunga J, Cantisani V, Chammas MC, Dighe M, et al. WFUMB Guidelines and Recommendations on the Clinical Use of Ultrasound Elastography: Part 4. Thyroid. Ultrasound Med Biol. 2017; 43(1): 4-26.
15. Zhao CK, Xu HX. Ultrasound elastography of the thyroid: Principles and current status. Ultrasonography. 2019; 38(2): 106-24.
16. Asteria C, Giovanardi A, Pizzocaro A, Cozzaglio L, Morabito A, Somalvico F, et al. US-elastography in the differential diagnosis of benign and malignant thyroid nodules. Thyroid. 2008; 18(5): 523-31.

17. Xing P, Wu L, Zhang C, Li S, Liu C, Wu C. Differentiation of benign from malignant thyroid lesions: calculation of the strain ratio on thyroid sonoelastography. J Ultrasound Med. 2011; 30(5): 663-9.

18. Ning CP, Jiang SQ, Zhang T, Sun LT, Liu YJ, Tian JW. The value of strain ratio in differential diagnosis of thyroid solid nodules. Eur J Radiol. 2012; 81(2): 286-91.

19. Huang Y, Zhou H, Zhang C, Hong Y, Ye Q, Huang P. Diagnostic Performance of Ultrasound Strain Elastography in Transverse and Longitudinal Views in Predicting Malignant Thyroid Nodules. Ultrasound Med Biol. 2019; 45(9): 2289-97.

20. Şahin M, Çakal E, Özbek M, Güngünes A, Arslan MS, Akkaymak ET, et al. Elastography in the differential diagnosis of thyroid nodules in Hashimoto thyroiditis. Med Oncol. 2014; 31(8): 97.

21. Wang J, Li P, Sun L, Sun Y, Fang S, Liu X. Diagnostic value of strain ratio measurement in differential diagnosis of thyroid nodules coexisted with Hashimoto thyroiditis. Int J Clin Exp Med. 2015; 8(4): 6420-6.

22. Dietrich CF, Barr RG, Farrokh A, Dighe M, Hocke M, Jenssen C, et al. Strain Elastography - How To Do It? Ultrasound Int Open. 2017; 3(4): E137-49.

RESEARCH ARTICLE

CYPRUS
JOURNAL OF MEDICAL SCIENCES

DOI: 10.4274/cjms.2022.2022-29
Cyprus J Med Sci 2023;8(1):20-26

A New Gene Therapy Approach by Tenascin-C Genome Editing Induces Apoptosis and Cell Cycle Arrest in Triple-Negative Breast Cancer Cells

Halin Bareke[1], Emine Salva[2], Suna Özbaş[1]

[1]Department of Pharmaceutical Biotechnology, Marmara University Faculty of Pharmacy, Institute of Health Sciences, İstanbul, Türkiye
[2]Department of Pharmaceutical Biotechnology, İnönü University Faculty of Pharmacy, Malatya, Türkiye

Abstract

BACKGROUND/AIMS: There is a pressing need for new therapies for the most aggressive subtype of breast cancer, triple-negative breast cancer (TNBC). *Tenascin-C (TN-C)* codes for a tumor microenvironment-specific protein, which promotes apoptosis evasion and cell proliferation. The aim of this study was to knock down TN-C by using the clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 system to induce cancer cell apoptosis and stunt cell proliferation, laying the grounds for a new gene therapy approach in TNBC.

MATERIALS and METHODS: The human TNBC cell line, MDA-MB-231 cells were transfected by TN-C-specific CRISPR/Cas9 plasmids. TN-C messenger RNA levels were assessed by real-time polymerase chain reaction to determine the knock-down efficiency. Two days after the transfection, the percentage of apoptotic cells and the proportion of cells in cell cycle phases were compared between the treatment and the control groups using flow cytometry. The resultant change in cell proliferation due to the knock-down was determined by MTT assay.

RESULTS: Transfection with the TN-C CRISPR/Cas9 plasmid reduced TN-C levels in the cells by approximately 49% relative to the scrambled-control CRISPR/Cas9 transfected cells. This TN-C downregulation increased the percentage of cells in apoptosis and induced G1-phase arrest. The combined effect of apoptosis and cell cycle arrest led to a significant decrease in the number of cancer cells in the treatment group.

CONCLUSION: Our successful preliminary study of a potential *TNBC* gene therapy based on TN-C genome editing by the CRISPR/Cas9 system led to significant decrease in TNBC cell numbers and it justifies the testing of this system in more advanced preclinical studies.

Keywords: Tenascin-C, CRISPR-cas systems, triple negative breast neoplasms, gene editing, apoptosis

INTRODUCTION

Cancer incidence is increasing, and it is predicted to result in 13 million deaths in 2030.[1] Breast cancer is the most common cancer affecting women worldwide, and its incidence is also projected to increase due to multifactorial variables, including lifestyle changes and increased average lifespan.[2] Apoptosis evasion is one of the hallmarks of cancer, including breast cancer, which enables the tumor cells to thrive despite the presence of anti-apoptotic signals.[3] Apoptosis, or programmed cell death, is induced by cell-intrinsic and extrinsic signals and is vital in tissue development, homeostasis, and the removal of dysfunctional cells, such as tumor cells, from the body.[4] This process is mediated by the serial activation of the caspase enzymes, which culminates in DNA fragmentation, the formation of apoptotic bodies, and the clearance of the apoptotic cell by phagocytic cells.

Cancer cells achieve apoptosis evasion by downregulating pro-apoptotic proteins such as Bcl-2-associated X (Bax) protein and upregulating anti-apoptotic proteins, such as B-cell lymphoma 2 (Bcl-2) protein.[5] Since

To cite this article: Bareke H, Salva E, Özbaş S. A New Gene Therapy Approach by Tenascin-C Genome Editing Induces Apoptosis and Cell Cycle Arrest in Triple-Negative Breast Cancer Cells. Cyprus J Med Sci 2023;8(1):20-26

ORCID IDs of the authors: H.B. 0000-0003-4577-3325; E.S. 0000-0002-1159-5850; S.Ö. 0000-0002-1721-7543.

Address for Correspondence: Halin Bareke
E-mail: halin.bareke@gmail.com
ORCID ID: orcid.org/0000-0003-4577-3325

Received: 22.06.2022
Accepted: 03.08.2022

the administration of most chemotherapeutics leads to apoptosis induction, apoptosis resistance also promotes therapy resistance.[5] Therefore, anti-apoptotic mechanisms in a tumor enable its survival, its resistance to therapy, and its recurrence. Targeting molecules to reverse apoptosis evasion in tumors can restrict tumor growth and overcome therapy resistance. However, since apoptosis is a normal physiological process, the choice of target molecule must be tumor-specific to avoid deleterious side effects.

In solid tumors, the tumor microenvironment (TME) can promote tumor survival, invasiveness, and metastasis.[6] The TME can be defined as non-cancerous cells and structural components which surround the tumor cells. Although the structure of TME might vary slightly among tumor types, it generally consists of stroma cells (e.g., fibroblasts), leukocytes, blood vessels, and extracellular matrix (ECM).[6] The TME can support tumor progression in many ways, including by acting as a growth factor reservoir, modifying cell adhesion, providing nutrients through angiogenesis, and preventing immune cells from reaching the tumor.[7] Some components of the tumor TME, such as growth factors and matricellular proteins, have also been shown to be involved in apoptosis evasion of solid tumors.[8] Tenascin-C (TN-C) is one of the multimeric matricellular proteins in the TME which can promote tumor survival and spread by inducing apoptosis resistance, epithelial-mesenchymal transition, immune evasion, cell proliferation, and ECM degradation.[9,10] Normally, TN-C is found in the embryo near migrating cells, at the interaction sites of epithelial and mesenchymal cells, and in developing connective tissue.[11] TN-C expression after birth is highly restricted, with only minimal expression in tissues which withstand tensile stress, such as tendons and ligaments and in lymphoid organs (thymus, spleen, bone marrow etc.), where there is high cell turnover.[12] Despite its restricted expression in normal healthy tissues, it is consistently upregulated in solid tumors with prognostic significance.[13] TN-C has emerged as an ideal target for gene silencing oncotherapy approaches since its expression is highly restricted to tumors in adults. It is an especially optimum candidate for clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9-based therapies. CRISPR/Cas9 technology is the latest and the most influential genome-editing technology which can create permanent changes (i.e., knock-in, knock-out, and correction of genes) in the genome.[14] Since the created changes in the genome are irreversible, the target gene should be, like TN-C, highly specific only to the tumors.

TN-C is highly expressed and associated with a poorer prognosis in breast cancer.[13] It is expressed both by the stromal cells in the TME and breast cancer cells.[11] TN-C expression in breast cancer is correlated with lymph node and lung metastasis, tumor size, increased angiogenesis, and treatment resistance and rapid invasiveness.[15-17] TN-C can mediate these effects by decreasing cell adhesion, promoting cell survival and angiogenesis, and suppressing immune responses.[18] It has been shown that TN-C levels are especially high in the most aggressive breast cancer subtype, triple-negative breast cancer (TNBC), due to the autophagy deficiency of these cells.[19] This is important because TNBC is negative for estrogen receptors, progesterone receptors, and human epidermal growth factor receptor 2 expression, meaning that treatment targets such as endocrine receptors normally exploited in other subtypes are absent in TNBC.[20] Therefore, TN-C is an important therapeutic target for TNBC, for which such targets are scarce. The TME is shown to be involved in immunomodulation and tumor progression in TNBC, and so, when using a TN-C-based gene therapy, the interaction between the TME and TNBC can potentially be reprogrammed.[21]

As a specific target for oncotherapies, TN-C has been shown to be involved in apoptosis resistance in multiple cancer types, including TNBC. One previous study showed that targeting TN-C with RNA interference induces apoptosis in human TNBC cell lines.[22] Moreover, in breast cancer cell cultures, TN-C reverses the cell cycle arrest induced by doxorubicin by inhibiting p21.[23] In a study of pancreatic cancer cells, it was shown that TN-C induced anti-apoptotic proteins Bcl-Extra-Large protein and Bcl-2 and it decreased caspase activity by activating the ERK1/2/NF-κB/p65 cascade, delineating a mechanistic link between TN-C expression and apoptosis evasion.[24]

To the best of our knowledge, no study has thus far used CRISPR/Cas9 to decrease TN-C expression in TNBC. Thus, the aim of this study was to knock down the *TN-C* gene using the effective genome-editing tool CRISPR/Cas9 in order to inhibit the growth of TNBC cells via apoptosis and hence, provide a foundation for a new gene therapy approach in TNBC.

MATERIALS AND METHODS

Materials

TN-C positive, human TNBC cell line MDA-MB-231 [American Type Cell Collection (ATCC® HTB-26™)] was purchased from ATCC (Manassas, VA, USA). The cells were maintained in a cell culture medium composed of high glucose DMEM medium (Sigma-Aldrich, St. Louis, MO, USA), 10% heat-inactivated fetal bovine serum (Capricorn Scientific, Ebsdorfergrund, Germany), 100 I.U/mL penicillin and 100 µg/mL streptomycin (both from Capricorn Scientific, Ebsdorfergrund, Germany), and 2 mM L-glutamine (Sigma-Aldrich, St.Louis, MO, USA). The cells were passaged twice a week using trypsin/EDTA (Wisent BioProducts Quebec, Canada) for the chemical detachment of the cells from the culture flasks, and Ca^{+2}-and Mg^{+2}-free 1X PBS (Wisent BioProducts Quebec, Canada) for cell washes.

A plasmid coding for two TN-C-specific single guide RNAs (sgRNA) (CTGTTTCGAAGGCTACGCCG and CGGGAGAGGCGGGTGACAGT) and Cas9 enzyme on a single plasmid was synthesized from VectorBuilder (Chicago, IL, USA). A control plasmid containing scrambled sgRNA (sequence: GCACTACCAGAGCTAACTCA) and Cas9 enzyme was purchased from Origene (Rockville, MD, USA).

The cell transfections were carried out using a nanoparticle-based transfection agent, Lipofectamine 3000 (Invitrogen-Thermo Fisher Scientific, Waltham, MA, USA).

Cell proliferation studies were performed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma-Aldrich, St. Louis, MO, USA) and dimethylsulfoxide (DMSO) (Merck, Kenilworth, New Jersey, USA).

In vitro Transfection

Transfection of MDA-MB-231 cells by the CRISPR/Cas9 plasmids was accomplished by the reverse-transfection method, according to the manufacturer's protocol. The plasmids used in transfections were scrambled sgRNA CRISPR/Cas9 plasmid (1 µg) as a control and 1 µg of the TN-C CRISPR/Cas9 plasmid. A plasmid (µg): p3000 (µL): Lipofectamine (µL) ratio of 1:3:2 was used to complex the plasmids with the transfection reagents. The total volume was increased to 50 µL with a reduced serum medium, Opti-MEM (Thermo Fisher Scientific, Waltham, MA, USA). The complexed plasmid was mixed with 150,000 cells in 450 µL of complete medium and plated in triplicate on 24-well culture plates.

For the proliferation studies, the same plasmid-to-transfection agent ratio was adjusted for a total volume of 100 µl (200 ng of each plasmid) and the cells were plated on 96-well plates.

Real-Time PCR

The knock-down effect of the TN-C CRISPR/Cas9 plasmids on TN-C mRNA levels was assessed by real-time PCR (RT-PCR). First, RNA was isolated from the cells 3 days after the transfection according to the manufacturer's protocol (RNeasy Mini Kit, Qiagen, Hilden, Germany). After the spectrophotometric confirmation of RNA quality (A260/A280=2), cDNA was synthesized using 0.5 µg of RNA. cDNA synthesis was performed by reverse transcription according to the manufacturer's protocol (ImProm-II Reverse Transcription System, Promega, Madison, WI, USA).

Primers specific to the human TN-C mRNA were used for the RT-PCR reaction (forward: AGAGAACCAGCCAGTGGTGT, reverse: GCCTGCTCCTGCAGTACATT). The TN-C mRNA levels were normalized using GAPDH mRNA levels as an internal control and the primers with sequences provided in a previous study were used.[25] The RT-PCR reactions were carried out in triplicate for each group, using the SYBR Green PCR Master Mix (Thermo Fisher Scientific, Waltham, MA, USA) in QuantStudio™3 (Thermo Fisher Scientific, Waltham, MA, USA). TN-C mRNA levels were relatively quantified using the ΔΔCt calculation.

Apoptosis

The effect of reducing the expression of the *TN-C* gene on cell apoptosis was investigated using flow cytometry by staining with the ImmunoStep FITC Annexin-V Apoptosis Detection Kit with propidium iodide (PI) (Salamanca, Spain). The cells were detached from the plates 48 hours after transfection and washed 3 times with 1X PBS. The cells were stained according to the manufacturer's protocol. To correct for fluorescence spillover, the cells were also stained with Annexin-V only and PI only. The stained cells were visualized using the BD FACSAria cytometer (BD Biosciences, Franklin Lanes, NJ, USA) and analyzed using Infinicyt 2.0 software (Cytognos, Salamanca, Spain). Unstained untransfected cells were used to gate the quadrants in the dot plots.

Cell Cycle Analysis

A flow cytometry-based cell cycle kit containing RNAse, PI, and detergent was used to determine the effect of TN-C knockdown on the cell cycle (Cytognos, Salamanca, Spain) on day 2 post-transfection. PI enters through the cell membrane as it is permeabilized by a detergent, and then PI binds only to DNA since RNAse removes all RNA from the cell. Therefore, the amount of DNA in the cell could be determined, providing an indirect indicator of cell cycle phases. Similar to the apoptosis assay, the cells were trypsinized and washed 3 times with 1X PBS. Then, 200 µL of the ready solution was added, and after a 10-minute incubation at room temperature in the dark, the cells were immediately visualized using the BD FACSAria cytometer and analyzed using Infinicyt 2.0 software.

Cell Proliferation

The effect of transfection with TN-C CRISPR/Cas9 on cell proliferation was assessed by the formation of formazan salt by the living cells after the addition of MTT. The change in cell proliferation and hence cell number was measured as the difference in the amount of formazan formed. Three days after the transfection, the cell media was removed, and 0.5 mg/mL MTT in complete medium was added. The cells were incubated for 4 hours in a humidified incubator at 5% CO_2 and 37 °C to allow MTT to be converted into formazan. The insoluble formazan salt was subsequently dissolved by DMSO. The colorimetric reading was measured at 570 nm and 690 nm by a microplate reader (SmartSpec 300, Bio-Rad, Hercules, CA, USA). The absorbance at 690 nm was subtracted from that at 570 nm to obtain a corrected reading.

Statistical Analysis

Statistical analysis was conducted using GraphPad Prism Software version 7.00 for Windows (GraphPad Software, San Diego, California, USA). Student's t-test was used to test for statistical significance. Values of p-smaller than 0.05 were considered statistically significant ($^{*}p<0.05$; $^{**}p<0.01$).

RESULTS

CRISPR/Cas9 Plasmids Successfully Downregulate TN-C Expression

Transfection success was investigated by observing green fluorescent protein (GFP) fluorescence under a fluorescent microscope. There were GFP-positive cells 24 hours after transfection in TN-C CRISPR/Cas9 and scrambled CRISPR/Cas9 plasmid-transfected cells, confirming a successful transfection (Figure 1).

The TN-C relative expression study using RT-PCR showed that 3 days after the transfection, TN-C mRNA levels were significantly lowered ($p=0.0007$) to half of the level in the cells transfected with the scrambled control (Figure 2).

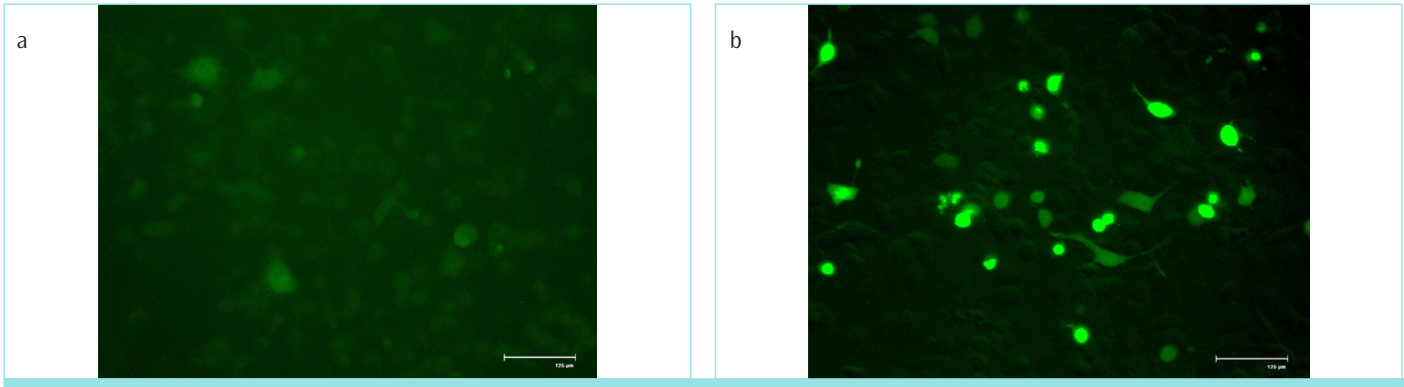


Figure 1. GFP fluorescence 24 hours after transfection with (a) TN-C CRISPR/Cas9 and (b) Scrambled CRISPR/Cas9 plasmids.

GFP: Green fluorescent protein, TN-C: Tenascin-C, CRISPR: Clustered regularly interspaced short palindromic repeats.

TN-C Genome Editing Induces Apoptosis in the Cancer Cells

The effect of TN-C genome editing by CRISPR/Cas9 on apoptosis was investigated 48 hours after transfection. With the onset of apoptosis, phosphatidyl serine, normally found in the inner layer of the cell membrane, is exposed on the outer layer, where Annexin-V can bind to it. Therefore, Annexin-V positive cells show the cell population in the early apoptotic phase. With the progression of apoptosis, PI can enter the cell due to increased cell permeability. Thus, double staining with Annexin-V and PI indicates late apoptosis. A representative result of four independent experiments is shown in Figure 3. TN-C genome editing increased the percentage of cells both in the early and late apoptotic stages, where the percentage was almost doubled for the late apoptotic cells.

Cell Cycle Distribution

As the literature shows that TN-C can affect the cell cycle, experiments were carried out to investigate whether reducing TN-C expression with the TN-C CRISPR/Cas9 plasmid alters cell distribution within the cell cycle phases. TN-C downregulation led to G0/G1 cell cycle arrest as the percentage of cells at this phase and in apoptosis (Figure 4, far left) increased, and the percentage of the cells in the later phases, namely S and G2/M phases decreased, relative to the scrambled CRISPR/Cas9 control.

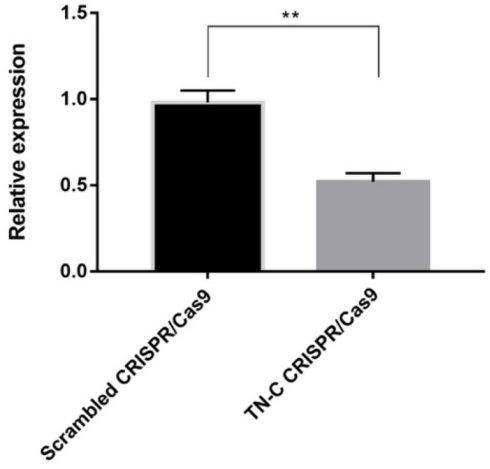


Figure 2. TN-C mRNA levels 3 days after transfection in the control and the experimental groups.

TN-C: Tenascin-C.

Transfection with the TN-C CRISPR/Cas9 Plasmids Decreased TNBC Cell Proliferation

Three days after the transfection, cell proliferation was examined using the MTT assay. Comparing the cell proliferation of the scrambled control transfected cells and TN-C CRISPR/Cas9 transfected TNBC cells revealed that the proliferation/cell numbers were halved by *TN-C* gene editing (p=0.0022) (Figure 5).

DISCUSSION

It has become increasingly apparent that the TME plays an enabling role in the maintenance and propagation of tumors. There is a two-way relationship between the tumor and its microenvironment, such that they reciprocally shape each other via chemokines, proteins, growth factors, and remodeling enzymes.[8] In light of this understanding, treatment strategies that regard the tumor as a "complex organ" and target the TME constituents have a promising therapeutic potential.

Breast cancer is projected to remain an important public health concern worldwide. TNBC specifically is an important threat to women's health globally since it has been refractory to the developments in diagnostic and therapeutic approaches. Addressing women's health issues such as breast cancer is integral to meeting the United Nation's Sustainable Development Goals (specifically Goals 3 and 5). Furthermore, research also shows that improving women's well-being alleviates the emotional and physical burden of disease and contributes to national productivity and economic advancement.[26] Therefore, we sought to provide a foundation for a new TNBC gene therapy approach by combining TME-targeting and the latest breakthrough in molecular biology, CRISPR/Cas9 technology. TN-C is one of the proteins found in the TME which sculpts the microenvironment to the benefit of the breast cancer cells, both in the primary site and the metastatic niche.[27,28] As TN-C expression is highly specific to cancer cells, it was chosen as the ideal knock-down target for the CRISPR/Cas9 system. Apoptosis evasion is a key mechanism

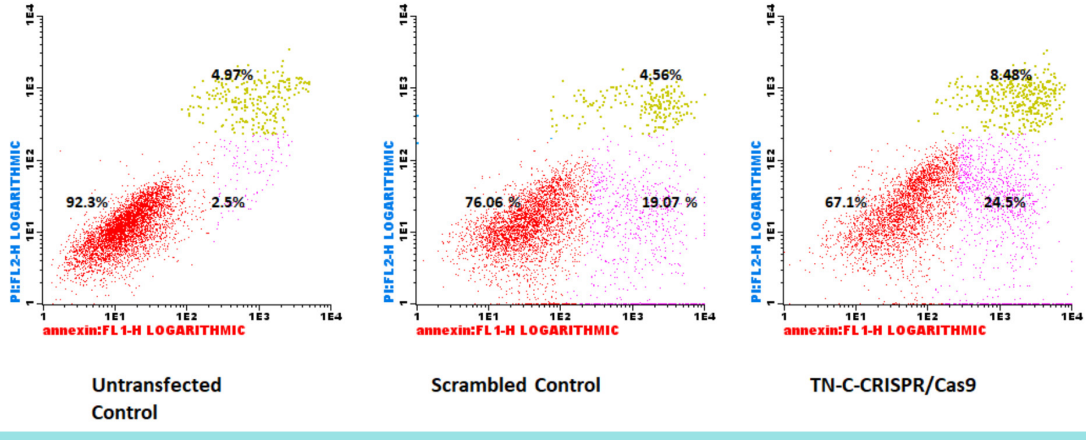


Figure 3. The percentage of cells alive (Annexin-V-, PI-) in early apoptosis (Annexin-V+, PI) and late apoptosis (Annexin-V+, PI+) in the control and the experimental groups. (Note: Annexin staining is depicted on the x-axis, and PI staining, the y-axis).

PI: Propidium iodide.

to target because it enables both tumor survival and therapy resistance, so reversing this evasion concomitantly opens up many therapeutic windows. In this study, we showed that TN-C downregulation by this system induced apoptosis in the aggressive MDA-MB-231 cell line and led to G1 arrest with a statistically significant decrease in cell proliferation. This shows that this system is viable for testing in more advanced studies, alone or in combination with other therapies.

CRISPR/Cas9 plasmids are usually challenging to transfect because of their large size due to the Cas9 enzyme and the strong promoters used. The TN-C-specific CRISPR/Cas9 plasmid and the control scrambled CRISPR/Cas9 plasmid used in this study were both large (10 kb). However, by increasing the amount of the complexation agent p3000 in the transfection kit, successful transfection was achieved, as shown by the GFP fluorescence images of the cells 24 hours post-transfection. The difference in the intensity of GFP fluorescence between the two plasmids can be attributed to the different GFPs coded on the plasmids, namely enhanced GFP on TN-C CRISPR/Cas9 plasmid and turbo GFP (tGFP) on the scrambled control under the control of CMV promoter and the promoter of the elongation factor-1 alpha (EF-1 alpha), respectively. EF-1 alpha has been shown to express the *GFP* gene more strongly than the CMV; therefore, the fluorescence from the scrambled control cells was stronger.[29]

Within the 3 days of transfection, there was approximately a 49% decrease in TN-C mRNA levels, demonstrating that two sgRNAs on the CRISPR/Cas9 plasmid can successfully target the *TN-C* gene. Two sgRNAs that target the initial exons of the large *TN-C* gene were used due to the size of the gene, which has 29 exons. The choice of exons to be targeted is especially important for genes such as *TN-C* because 9 of its exons can be alternatively spliced. Given that those sgRNAs targeting loci closer to the transcription start site result in better editing efficiency, the initial exons were successfully targeted by the sgRNAs used in this study.[30] It is known that the higher the number of cell cycles that the cell populations undergo, the higher the editing efficiency. Since MDA-MB-231 is a rapidly proliferating cell line, the high editing efficiency we observed is expected. The silencing efficiency during the later days can also be investigated to examine whether the edited cells have a selective disadvantage in the cell population pool. The latest addition to the chemotherapeutic arsenal against metastatic TNBC are poly (ADP-ribose) polymerase (PARP) inhibitors, such as Olaparib.[20] These inhibitors direct the cells to error-prone DNA damage repair (non-homologous end-joining), creating lethal mutations in the cell.[31] Since the CRISPR/Cas9-based knock-down and knock-out studies also rely on the mistakes made during the DNA-damage repair in non-homologous

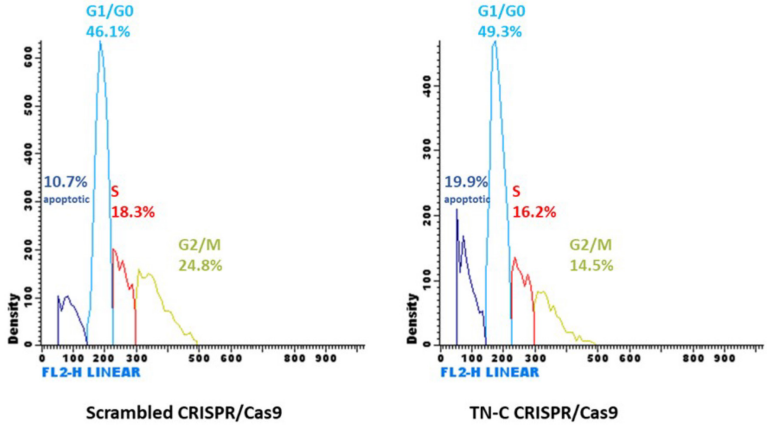


Figure 4. The distribution of the cells that are apoptotic and in different phases of the cell cycle, as evaluated by flow cytometric staining.
TN-C: Tenascin-C, CRISPR: Clustered regularly interspaced short palindromic repeats.

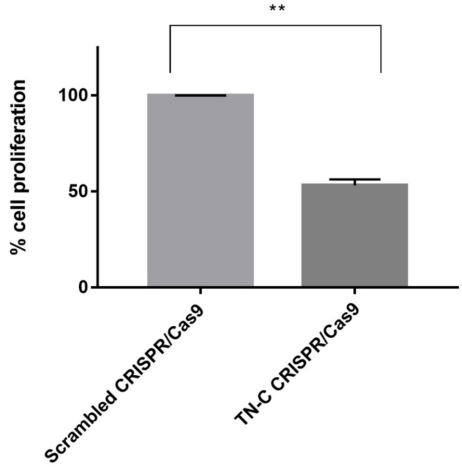


Figure 5. The effect of transfection with the control and TN-C CRISPR/Cas9 on cell proliferation.
TN-C: Tenascin-C, CRISPR: Clustered regularly interspaced short palindromic repeats.

end-joining, it is highly probable that combining PARP inhibitors with this CRISPR/Cas9-based therapy would enhance knock-down efficiency, leading to a synergistic anti-tumor response in TNBC.

A higher percentage of cells were found in early apoptosis in the scrambled control and TN-C CRISPR/Cas9 transfected cell groups than in the untransfected cells. The cell death and decrease in cell proliferation observed in the scrambled control transfected cells can be attributed to the expression of Cas9 and GFP which put extra strain on the cellular resources, which can lead to a decrease in proliferation and even apoptosis. Nevertheless, the early and late apoptotic cell percentages in the TN-C edited cell population were markedly higher than in the scrambled control cells. In future studies, using multicaspase flow cytometric staining or demonstrating caspase-3 activity would further support these findings. However, it is clear from the apoptosis and cell cycle analysis that TN-C downregulation induced apoptosis which yielded meaningful decreases in the number of TNBC cells and this is one of the ultimate goals of oncotherapies. TN-C can mediate anti-apoptotic effects by binding to growth factor receptors through its epidermal growth factor-like domain and induction of the ERK/NF-κB pathway.[24,32] This pathway can then lead to the activation of anti-apoptotic cells and cyclin D1 which reduce apoptosis and induce cell cycle progression.[33] Metastatic disease is the major cause of morbidity in TNBC, and metastatic TNBC cells have been shown to be more resistant to apoptosis. Therefore, the TN-C genome editing system could also be studied for use against metastatic TNBC.

The observed difference in cell proliferation can potentially be attributed to increased apoptosis and decreased proliferation since the pleiotropic effects of TN-C include cell cycle modulation. A study on the pancreatic ductal adenocarcinoma cells showed that TN-C addition to the cultures prompted G1/S transition.[24] Another study showed that TN-C promotes progression into the S phase via AKT signaling, leading to enhanced cyclin D1 expression. Cyclin D1 then complexes with CDK4 to free E2F from retinoblastoma protein to enable G1/S transition.[10] From these data, it could be deduced that the downregulation of TN-C would decrease G1/S transition. In the present study, this deduction has been confirmed. TN-C downregulation increased the number of cells in the G0/G1 phase, and the percentage of cells in the other phases decreased, consistent with previous studies. Drug-resistant disease is one of the primary challenges in treating TNBC, resulting in high mortality. Previous research has shown that TN-C can aid in doxorubicin resistance by reversing G1/S arrest via p21 inhibition.[23] We demonstrated that TN-C downregulation led to G1 arrest, and we propose that this approach could be assessed for reversing doxorubicin resistance and testing a doxorubicin combination therapy.

Study Limitations

The main limitation of this study is that only one TNBC cell line was used and another TN-C expressing TNBC should be used in order to confirm our findings. Evidence is also needed to show that different levels of silencing correlate with the amount of apoptosis induced. Furthermore, the silencing efficiency for the later time-points could be investigated in order to determine whether the edited cancer cell percentage would decrease due to the reduced fitness of these cells.

CONCLUSION

This study demonstrated for the first time that TN-C downregulation by the CRISPR/Cas9 system successfully induced apoptosis and G1 arrest in the human TNBC cell line. These results warrant further testing via the examination of combinatorial therapies with chemotherapeutic drugs and studies on reversing drug resistance. By formulating a viral or a non-viral vector, this approach could also be tested within *in vivo* gene therapy settings in an animal cancer model.

MAIN POINTS

- TNBC is an important problem for women's health globally.
- A tumor-promoting matricellular protein, TN-C, was successfully downregulated using CRISPR/Cas9 technology in TNBC cells, for which there is an urgent need for new therapeutic targets.
- TN-C downregulation induced apoptosis and inhibited G1/S transition, leading to a significant reduction in TNBC cell numbers.
- These data provide a strong foundation for further studies focusing on designing a new *TN-C*-based gene therapy approach using the powerful CRISPR/Cas9 system in TNBC cells.

ETHICS

Ethics Committee Approval: The study does not require ethics committee approval since it does not involve any human or animal subject.

Informed Consent: The study does not require informed consent approval since it does not involve any human or animal subject.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: H.B., E.S., S.Ö., Design: H.B., E.S., S.Ö., Data Collection and/or Processing: H.B., E.S., Analysis and/or Interpretation: H.B., E.S., S.Ö., Literature Search: H.B., Writing: H.B.

DISCLOSURES

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

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

References

1. Thun MJ, DeLancey JO, Center MM, Jemal A, Ward EM. The global burden of cancer: priorities for prevention. Carcinogenesis. 2010; 31(1): 100-10.
2. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021; 71(3): 209-49.
3. Hanahan D. Hallmarks of Cancer: New Dimensions. Cancer Discov. 2022; 12(1): 31-46.
4. Singh R, Letai A, Sarosiek K. Regulation of apoptosis in health and disease: the balancing act of BCL-2 family proteins. Nat Rev Mol Cell Biol. 2019; 20(3): 175-93.
5. Mohammad RM, Muqbil I, Lowe L, Yedjou C, Hsu HY, Lin LT, et al. Broad targeting of resistance to apoptosis in cancer. Semin Cancer Biol. 2015; 35: S78-103.
6. Bożyk A, Wojas-Krawczyk K, Krawczyk P, Milanowski J. Tumor Microenvironment-A short Review of Cellular and Interaction Diversity. Biology. 2022; 11(6): 929.

7. Nallanthighal S, Heiserman JP, Cheon DJ. The Role of The Extracellular Matrix in Cancer Stemness. Front Cell Dev Biol. 2019; 7: 86.
8. Hui L, Chen Y. Tumor microenvironment: Sanctuary of the devil. Cancer Lett. 2015; 368(1): 7-13.
9. Chong HC, Tan CK, Huang RL, Tan NS. Matricellular proteins: a sticky affair with cancers. J Oncol. 2012; 2012: 351089.
10. Cai J, Lu W, Du S, Guo Z, Wang H, Wei W, et al. Tenascin-C Modulates Cell Cycle Progression to Enhance Tumour Cell Proliferation through AKT/FOXO1 Signalling in Pancreatic Cancer. J Cancer. 2018; 9(23): 4449-62.
11. Guttery DS, Shaw JA, Lloyd K, Pringle JH, Walker RA. Expression of tenascin-C and its isoforms in the breast. Cancer Metastasis Rev. 2010; 29(4): 595-606.
12. Midwood KS, Orend G. The role of tenascin-C in tissue injury and tumorigenesis. J Cell Commun Signal. 2009; 3(3-4): 287-310.
13. Ming X, Qiu S, Liu X, Li S, Wang Y, Zhu M, et al. Prognostic Role of Tenascin-C for Cancer Outcome: A Meta-Analysis. Technol Cancer Res Treat. 2019; 18: 1533033818821106.
14. Hirakawa MP, Krishnakumar R, Timlin JA, Carney JP, Butler KS. Gene editing and CRISPR in the clinic: current and future perspectives. Bioscience Rep. 2020; 40(4): BSR20200127.
15. Yang Z, Zhang C, Feng Y, Quan M, Cui Y, Xuan Y. Tenascin-C predicts poor outcomes for patients with colorectal cancer and drives cancer stemness via Hedgehog signaling pathway. Cancer Cell Int. 2020; 20: 122.
16. Hongu T, Pein M, Insua-Rodríguez J, Gutjahr E, Mattavelli G, Meier J, et al. Perivascular tenascin C triggers sequential activation of macrophages and endothelial cells to generate a pro-metastatic vascular niche in the lungs. Nat Cancer. 2022; 3(4): 486-504.
17. Insua-Rodríguez J, Pein M, Hongu T, Meier J, Descot A, Lowy CM, et al. Stress signaling in breast cancer cells induces matrix components that promote chemoresistant metastasis. EMBO Mol Med. 2018; 10(10): e9003.
18. Tucker RP, Degen M. Revisiting the Tenascins: Exploitable as cancer targets? Front Oncol. 2022; 12: 908247.
19. Li ZL, Zhang HL, Huang Y, Huang JH, Sun P, Zhou NN, et al. Autophagy deficiency promotes triple-negative breast cancer resistance to T cell-mediated cytotoxicity by blocking tenascin-C degradation. Nat Commun. 2020; 11(1): 3806.
20. Bergin ART, Loi S. Triple-negative breast cancer: recent treatment advances. F1000Res. 2019; 8: 1342.
21. Deepak KGK, Vempati R, Nagaraju GP, Dasari VR, SN, Rao DN, Malla RR. Tumor microenvironment: Challenges and opportunities in targeting metastasis of triple negative breast cancer. Pharmacol Res. 2020; 153: 104683.
22. Wawrzyniak D, Grabowska M, Głodowicz P, Kuczyński K, Kuczyńska B, Fedoruk-Wyszomirska A, et al. Down-regulation of tenascin-C inhibits breast cancer cells development by cell growth, migration, and adhesion impairment. Languino LR, editor. PLoS One. 2020; 15(8): e0237889.
23. Wang B, Liu K, Lin HY, Bellam N, Ling S, Lin WC. 14-3-3Tau regulates ubiquitin-independent proteasomal degradation of p21, a novel mechanism of p21 downregulation in breast cancer. Mol Cell Biol. 2010; 30(6): 1508-27.
24. Shi M, He X, Wei W, Wang J, Zhang T, Shen X. Tenascin-C induces resistance to apoptosis in pancreatic cancer cell through activation of ERK/NF-κB pathway. Apoptosis. 2015; 20(6): 843-57.
25. Seo Y, Shin TH, Ahn JS, Oh SJ, Shin YY, Yang JW, et al. Human Tonsil-Derived Mesenchymal Stromal Cells Maintain Proliferating and ROS-Regulatory Properties via Stanniocalcin-1. Cells. 2020; 9(3): 636.
26. Onarheim KH, Iversen JH, Bloom DE. Economic Benefits of Investing in Women's Health: A Systematic Review. PLoS One. 2016; 11(3): e0150120.
27. Oskarsson T, Acharyya S, Zhang XH, Vanharanta S, Tavazoie SF, Morris PG, et al. Breast cancer cells produce tenascin C as a metastatic niche component to colonize the lungs. Nat Med. 2011; 17(7): 867-74.
28. Sun Z, Velázquez-Quesada I, Murdamoothoo D, Ahowesso C, Yilmaz A, Spenlé C, et al. Tenascin-C increases lung metastasis by impacting blood vessel invasions. Matrix Biol. 2019; 83: 26-47.
29. Teschendorf C, Warrington KH, Siemann DW, Muzyczka N. Comparison of the EF-1 alpha and the CMV promoter for engineering stable tumor cell lines using recombinant adeno-associated virus. Anticancer Res. 2002; 22(6A): 3325-30.
30. Radzisheuskaya A, Shlyueva D, Müller I, Helin K. Optimizing sgRNA position markedly improves the efficiency of CRISPR/dCas9-mediated transcriptional repression. Nucleic Acids Res. 2016; 44(18): e141.
31. Patel AG, Sarkaria JN, Kaufmann SH. Nonhomologous end joining drives poly(ADP-ribose) polymerase (PARP) inhibitor lethality in homologous recombination-deficient cells. Proc Natl Acad Sci USA. 2011; 108(8): 3406-11.
32. Iyoda T, Fujita M, Fukai F. Biologically Active TNIIIA2 Region in Tenascin-C Molecule: A Major Contributor to Elicit Aggressive Malignant Phenotypes From Tumors/Tumor Stroma. Front Immunol. 2020; 11: 610096.
33. Qian S, Tan X, Liu X, Liu P, Wu Y. Exosomal Tenascin-c induces proliferation and invasion of pancreatic cancer cells by WNT signaling. Onco Targets Ther. 2019; 12: 3197-205.

RESEARCH ARTICLE

CYPRUS
JOURNAL OF MEDICAL SCIENCES

DOI: 10.4274/cjms.2021.3522
Cyprus J Med Sci 2023;8(1):27-33

Polycystic Ovary Syndrome Among Patients of a University Hospital in Nicosia: A Retrospective Study

Özen Asut, Songül Vaizoğlu, Gulifeiya Abuduxike, Sanda Cali

Department of Public Health, Near East University Faculty of Medicine, Nicosia, North Cyprus

Abstract

BACKGROUND/AIMS: Polycystic ovary syndrome (PCOS) is a heterogeneous endocrine disorder, characterized by hyperandrogenism, ovulatory dysfunction and polycystic ovarian morphology leading to health complications including infertility, hirsutism and metabolic syndrome. The aim of this study was to determine the status, features and treatments implemented for PCOS patients diagnosed in a university hospital of Nicosia, North Cyprus, where no previous data on this issue was available in the medical literature.

MATERIALS AND METHODS: This descriptive study was administered using the records of 45,677 patients presenting to the gynecology and obstetrics clinic for the time period of 2015-2019. The study group included 819 patients diagnosed as PCOS. The data were collected using a data collection form designed by the researchers and analyzed by IBM-SPSS 18.0 program, with a significance level accepted as $p<0.05$.

RESULTS: European Society for Human Reproduction and Embryology/American Society for Reproductive Medicine Rotterdam Conference-2003 or the Androgen Excess and PCOS Society (AE-PCOS) Conference-2006 criteria were used for the diagnosis. The 819 patients diagnosed as PCOS comprised a frequency of 1.8%, lower than the expected average value reported among gynecologic patients. Family history existed in 0.7% of the cases. The characteristics of the patients were similar to those of other studies and treatments initiated were in accordance with PCOS consensuses in general, with the exception of a lower use of metformin.

CONCLUSION: The frequency of PCOS among North Cyprus women was low, indicating the disease was an under-recognized condition in this tertiary hospital compared to rates estimated in community samples globally. Further research is recommended to establish more valid data in order to improve women's health.

Keywords: Polycystic ovary syndrome, ovulation irregularity, hyperandrogenism, treatment, North Cyprus

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a heterogeneous endocrine disorder characterized by hyperandrogenism, ovulatory dysfunction and polycystic ovarian morphology, with excessive androgen production by the ovaries.[1] PCOS is the most frequent endocrinological disorder among women of reproductive ages leading to a number of health complications.[1,2] An international consensus definition of PCOS has been published, which defines PCOS as at least two of the following criteria: Reduced or no ovulation; clinical and/or biochemical signs of excessive secretion of androgens; and/or polycystic ovaries (the presence of at least 12 follicles measuring 2-9 mm in diameter, an ovarian volume in excess of 10 mL, or both).[3]

In terms of ovarian functions, PCOS is a syndrome of ovarian dysfunction characterized by an accumulation of incompletely developed follicles in the ovaries because of anovulation. Clinical manifestations include menstrual dysfunction, oligomenorrhea or amenorrhea, infertility, hirsutism and acne.[4] Insulin resistance affects 50-70% of women with PCOS, gradually ending up with co-morbidities such as hypertension, dyslipidemia, glucose intolerance, leading to diabetes and metabolic syndrome.[5]

To cite this article: Asut Ö, Vaizoğlu S, Abuduxike G, Cali S. Polycystic Ovary Syndrome Among Patients of a University Hospital in Nicosia: A Retrospective Study. Cyprus J Med Sci 2023;8(1):27-33

ORCID IDs of the authors: Ö.A. 0000-0002-9604-4037; S.V. 0000-0001-9279-1740; G.A. 0000-0002-9798-7459; S.C. 0000-0001-9929-2637.

Address for Correspondence: Özen Asut
E-mail: ozen.asut@neu.edu.tr
ORCID ID: orcid.org/0000-0002-9604-4037

Received: 23.11.2020
Accepted: 18.03.2021

Although the physiopathology of PCOS is unknown, most studies imply familial heritage. Some clinical genetic studies indicate an autosomal dominant inheritance, while others reveal a complex trait of oligogenic basis. On the other hand, the heterogeneity of phenotypic features point to the contribution of environmental factors.[6] Genetically transmitted β-cell dysfunction and high androgens, dyslipidemia and insulin resistance serum markers in families of women with PCOS, including the male population have been demonstrated.[7,8] The majority of PCOS patients exhibit metabolic dysfunction with insulin resistance, hyperinsulinaemia and an increased risk for type 2 diabetes mellitus, gestational diabetes and other pregnancy-related complications, cardiovascular events and endometrial cancer.[9-13]

PCOS is classified into four separate phenotypes (A-D), according to the presence or absence of three characteristics: hyperandrogenism (either biochemical or clinical), ovulatory dysfunction and polycystic ovarian morphology. Only phenotype A requires all three features of PCOS to be present. The various diagnostic criteria currently available for PCOS include a greater or fewer number of PCOS phenotypes.[11]

The prevalence of PCOS is remarkably similar worldwide. The prevalence of clinically evident PCOS in women of reproductive ages from the United States, Europe, Asia and Australia ranges between 5% and 9% based on the original 1990 US National Institutes of Health (NIH) diagnostic criteria. Using the broader 2003 Rotterdam criteria now accepted internationally, the prevalence of PCOS ranges from 5.5% to 19.9%.[11]

PCOS is reported to be diagnosed in 4-10% of women attending gynecology clinics in high income countries.[14] In some European studies, the prevalence of PCOS has been reported as being 6.5-8%.[14,15] The prevalence of PCOS may be in the range of 15-20% using the criteria of the European Society for Human Reproduction and Embryology/American Society for Reproductive Medicine (ESHRE/ASRM).[5] However, among unselected general female populations of different ethnicities, PCOS rates using other criteria were found as follows: Caucasians 5.5% (NIH: 1990); African-American and Afro-Brazilian 7.4% (NIH: 1990); Chinese 2.2% (NIH: 1990), 5.6% (ESHRE/ASRM, Rotterdam 2003); and Middle East (Iranian and Turkish) 6.1% (NIH: 1990), 16.0% (ESHRE/ASRM, Rotterdam 2003), 12.6% (AES, 2006).[4,16] Diagnostic criteria for PCOS include clinical or biochemical hyperandrogenism, oligo/anovulation and polycystic ovarian morphology according to ESHRE/ASRM 2012 criteria while clinical or biochemical hyperandrogenism and persistent oligo-/anovulation according to Endocrine Society 2013 criteria.[16] As a consequence, the worldwide prevalence of PCOS shows variations according to the diagnostic criteria used. Greater estimates of PCOS prevalence with the ESHRE/ASRM Rotterdam 2003 and Androgen Excess and PCOS Society (AE-PCOS) 2006 criteria are largely attributed to their more expansive definition and inclusion of additional phenotypes.[16] However, the results of PCOS studies largely depend on how the study population and the PCOS phenotypes were defined. Since the assessment of the PCOS phenotype is a complex process, study results may reflect underreporting due to the limitations of more intensive investigations.[15,16]

Aim: The aim of this study was to determine the status and features of PCOS and to investigate the treatments provided to patients diagnosed in the Near East University Hospital in Nicosia, North Cyprus, where no previous data on this issue was available in a search of the literature. The time period of this study was set as 1st September, 2015 to 31st August, 2019.

MATERIALS AND METHODS

Study type: The study was a descriptive records-based study.

Diagnostic and inclusion criteria: In order to establish a diagnosis of PCOS, the criteria of the ESHRE/ASRM Rotterdam Conference of 2003 or the AE-PCOS Conference 2006 were used in the Near East University Hospital. According to these criteria, the following parameters were considered sufficient for the diagnosis of PCOS: Clinical and biochemical hyperandrogenism, ovulation irregularity and/or polycystic ovary morphology.[10]

Study design and setting: The study was conducted between the 2nd and 9th of August, 2019 via an investigation of the patient records of the Gynecology and Obstetrics Clinics of the Near East University Hospital. The study group included those patients diagnosed as PCOS starting 1st September, 2015 until 31st August, 2019. All of the patients registered with this diagnosis were admitted into this study without exclusion. The data were obtained from the information system of the Near East University Hospital with the permission of the chief physician of the hospital.

Study tool: The data were collected and recorded by a data collection form designed and structured by the researchers. The details of the records of each patient were entered into the data collection form individually.

Statistical Analysis

The data were analyzed using IBM-SPSS (Statistical Package for the Social Sciences) version 18 (SPSS Inc., Chicago, IL, USA). Descriptive statistics including frequency, percentage, mean, and standard deviation were calculated to describe the characteristics of the study sample. Bivariate analysis using the chi-squared test was performed to examine the relationships between independent variables with a significance level set at $p<0.05$.

Permission from the Near East University Gynecology and Obstetrics Department and the Chief Physician of the Near East University Hospital was obtained and consent and approval of the Near East University Ethics Committee with report number YDU/2019/71-869 was provided.

RESULTS

In this descriptive study, the records of 819 patients diagnosed as PCOS among 45,677 patients presenting to the Gynecology and Obstetrics Clinics of the Near East University Hospital between 1st September, 2015 and 31st August, 2019 were studied. The detailed history of 80 patients were not recorded in the system, other than their diagnosis as PCOS.

The results are given under the headings of age distribution, family history, symptoms and findings, diagnosis and treatments. The age distribution, the status of family history and prior drug use history of the patients followed up with the diagnosis of PCOS are presented in Table 1.

Of the total patients, 41.5% were in the 20-24 year age group. The mean age of the PCOS patients was 27 years, with a minimum age of 15 and maximum age of 50. Only 5 patients (0.7%) had a family history according to their records. Of the 232 patients under drug therapy prior to admission, 42.2% were reported as taking contraceptive drugs and 78.0% as using other medications (Table 1).

The analysis of the patients followed up with the diagnosis of PCOS at the Near East University Hospital showed that 8.4% of the patients (69 patients) had undergone surgical interventions, while 2.2% (18 patients) of them had experienced gynecologic/obstetric operations prior to admission.

Table 2 indicates the presenting and later symptoms and signs of the PCOS patients diagnosed at the Near East University Hospital. Of the patients with recorded data, 45.9% were admitted to the hospital with complaints of menstrual irregularity, followed by the desire for offspring, hirsutism and dermatological problems as the major causes of presentation to the hospital. Leading symptoms at a later stage of the disease included oligomenorrhea, hirsutism and acne. In addition, data from the records showed that 78.6% of the patients diagnosed as PCOS had ultrasonographic findings of polycystic ovaries (Table 2).

The laboratory investigations requested of the patients followed up with the diagnosis of PCOS at the Near East University Hospital are given in Table 3. The patient records revealed that hormone profile, vaginal smear, thyroid function tests and HOMA-IR (insulin resistance) were the laboratory tests most commonly requested for those patients with PCOS, the leading test among the total being hormone profile with 83.5%, followed by vaginal smear with 14.4% (Table 3).

The details about drug therapies provided for the PCOS patients diagnosed at the Near East University Hospital are shown in Table 4. Among the records of the 819 PCOS patients registered, 759 patient records had pharmacotherapy information, 431 of whom were stated as receiving drug therapy and 328 as receiving no drug therapy. Of the total 431 records with information regarding pharmacologic therapies, 69.4% were taking oral contraceptives and progesterone. Oral contraceptive medications were prescribed for 39.2% of the patients while 29.0% received progesterone therapy. Antibiotics, metabolic drugs and supplements were the following groups of medications in order of frequency recommended for the PCOS patients.

Metformin was reported to be prescribed for 5.3% of the patients. Other ovulation stimulators used included clomiphene, dopamine agonists, estrogen agonists and aromatase inhibitors. Dermatologic and other medications were prescribed for 6% of the patients (Table 4).

Reporting of a PCOS symptom at first presentation to the hospital according to age groups is given in Table 5. A significant difference was illustrated between the age groups regarding the existence of symptoms at first presentation. Those patients under 25 years of age had symptoms regarding PCOS at their first visit to the hospital at significantly higher levels than those patients 25 years or older ($\chi^2=13.0$, $p<0.01$) (Table 5).

DISCUSSION

This study was conducted using the patient registration system of a university hospital in North Cyprus.

The frequency of PCOS was established as 1.8% among the gynecologic patients, lower than reported in the literature for the same age group of patients in similar settings. PCOS was reported to be diagnosed in 4-10% of women attending gynecology clinics in high income countries.[14]

According to our review of the literature, the prevalence of PCOS among women had not been investigated in North Cyprus until the present time. Therefore, a comparison with previous research was not possible and our study may be part of the initial research on this issue in this country.

The incidence and prevalence of PCOS among women seeking healthcare at primary healthcare settings was found to be low, indicating that PCOS is an under-recognized condition at the broadest level of the healthcare systems.[13] Prevalence studies from several countries have reported PCOS frequencies, such as 6.6% for Spain, 5% for Türkiye, 2.2% for the Peoples Republic of China and 1.6% for the USA.[9] A study in the UK found an overall incidence rate of 1.84% for the time period 2004-2014, with women aged 20-24 years and women living in deprived areas having the highest incidences. The prevalence of PCOS for the year 2014 was estimated to be approximately 2%, reflecting a lower level of this syndrome, similar to our study.[17]

Although our study group included gynecologic patients, the frequency of PCOS was 1.8%, lower than the reported 4-10% in the literature for the same age group of patients in similar settings.[14] In a cross-sectional study representing the South Cyprus population, the overall prevalence

Table 1. Age distribution, family history and drug use history of those patients with a diagnosis of PCOS at Near East University Hospital (Nicosia: August, 2019)

Age group (n=814)			**n**	**%**
<20			36	4.4
20-24			338	41.5
25-29			218	26.8
30-34			137	16.8
35-39			61	7.5
≥40			24	3.0
Mean ± SD: 27±5.8	**Median: 25**	**Minimum: 15**	**Maximum: 50**	
Family history (n=756)				
Yes			5	0.7
No			751	99.3
Drug use history on record (n=232)*				
PCOS-related drugs**			98	42.2
Other drugs (none PCOS-related)			181	78.0

*Limited to information in the patient records, **PCOS related drugs: Contraceptive drugs (cyproterone and estrogen, dienogest and ethinylestradiol, ulipristal, dienogest and estrogen, medroxyprogesterone, drospirenone and ethinylestradiol), PCOS: Polycystic ovary syndrome, SD: Standard deviation.

of PCOS among women was found to be 6.1%, much higher than the results of our study. The age group with the highest rate in this study of the South Cyprus population was the 25-44 age group women with 16.0%, followed by the 18-24 age group with 8.6%.[18]

Additionally, the prevalence of PCOS was found to be higher using the ESHRE-ASRM Rotterdam Conference 2003 and AE-PCOS Society conference 2006 criteria, as compared to the NIH 1990 criteria. Since the former two criteria were used in our clinics and the setting is a tertiary university hospital, the present frequency finding was much lower than the expected values, indicating an under-diagnosis of this syndrome, which may be attributed to the private status of the university hospital and also some missing data in the records. As PCOS is more a condition of deprived populations, more patients probably seek health

Table 2. The presenting and later symptoms and signs of patients followed up with a diagnosis of PCOS at the Near East University Hospital (Nicosia: August, 2019)

Presenting symptom (n=758)	**n**	**%**
Menstrual irregularity	348	45.9
Desire for offspring	57	7.5
Hirsutism	47	6.2
Dermatological problem	47	6.2
Pain	41	5.4
Amenorrhea	41	5.4
Vaginal secretion	35	4.6
Check-up	31	4.8
Pregnancy monitoring	27	3.5
Hemorrhage	25	3.2
PCOS control	20	2.6
Dysmenorrhea	13	1.7
Metabolic disorder	12	1.5
Infertility	5	0.7
Urinary infection	4	0.5
Other infection	2	0.3
Abdominal mass	2	0.3
Abortion	1	0.1
Later symptoms and signs		
Oligomenorrhea (n=759)	424	55.9
Hirsutism (n=759)	192	25.3
Acne (n=759)	87	11.5
Polycystic ovaries on ultrasound (n=760)	597	78.6

PCOS: Polycystic ovary syndrome.

Table 3. The records of the laboratory investigations requested of the patients followed up with a diagnosis of PCOS at the Near East University Hospital (Nicosia: August, 2019)

Investigation (n=480)	**n**	**%**
Hormone profile	401	83.5
Vaginal smear	69	14.4
Thyroid function tests	27	5.6
HOMA-IR (insulin resistance)	26	5.4
Complete blood count test	21	4.4
Urinary analysis	14	2.9
Hepatic function tests	7	1.5
Fasting blood glucose	7	1.5
Tumor markers	6	1.3
Glucose tolerance test	5	1
Magnetic resonance imaging	3	0.7
Chorionic villus sampling	3	0.7
Hysterosalpingography	2	0.4

PCOS: Polycystic ovary syndrome.

services at state institutions in Cyprus or in Türkiye. However, the socio-demographic features of the patients were not recorded in sufficient detail in the records used in the current study. Thus, further analysis in regard to other sociodemographic features other than age was not possible. Regarding family history, no evidence was found suggesting familial transmission among the majority of the patients.

Additionally, the assessment of the PCOS phenotype is a complex process and due to the unavailability of some investigations, study results may reflect lower prevalences.[15,16] This may be one of the other reasons for the lower prevalence found in our study.

The findings of the current study were in agreement with previous studies conducted in other studies.[18] Among the patients diagnosed as PCOS, two or more of the PCOS criteria of menstrual irregularity, polycystic ovary morphology, and/or hyperandrogenism-hirsutism were present in 80% of the cases. Similarly, 75% of the patients had two or more of these criteria in another study conducted in Denmark.[19]

According to the European survey of diagnosis and management of PCOS among European Society of Endocrinology members, NIH criteria were utilized for PCOS diagnosis by the majority of the respondents.[20] The respondents were most likely to select menstrual irregularity as the most frequent criteria used for PCOS diagnosis, followed by hirsutism and biochemical hyperandrogenism, similar to our study as menstrual irregularity was the first and hirsutism the third ranked findings reported (Table 2).

The most frequent biochemical parameters in the differential diagnosis of hyperandrogenism were total testosterone or free androgen index, contrary to our findings regarding laboratory tests, which did not include all of these tests.[20]

Treatment is recommended to be in alignment with the complaints and needs of the patients and should involve lifestyle changes targeting metabolic abnormalities, medications and surgery for the management of excess weight, androgen suppression and/or blockade, endometrial protection, reproductive therapy and the treatment of psychological features.[11,20]

In the current study, 40.4% of the 431 patients prescribed medications were reported as receiving oral contraceptives and 29% as receiving progesterone. Totally, 16.6% of the patient records with pharmacotherapy information indicated the use of ovulation stimulators.

Metformin use was only 5.3% in our study, considerably less than reported in the literature. In a study from the USA, metformin comprised 75% of the drugs administered to PCOS patients.[9] However, the rate of metformin use was lower in the UK study covering 2004-2014 at 10.2%. In this UK study, the proportion of women with a prescription in the 24 months after their PCOS index date varied by drug type: 10.2% metformin, 15.2% combined oral contraceptives, 18.8% acne-related treatments, and less than 5% for clomiphene, spironolactone, cyproterone and eflornithine.[17] The use of oral contraceptives was much higher in our study than was found in the UK study, although metformin use was lower.

In the European survey, the most common treatments for patients with PCOS were metformin (33%), lifestyle modification (25%), oral contraceptives (22%), antiandrogens (13%), cosmetic procedures for hirsutism (8%) and a number of different combinations of these agents or methods. More direct treatments of infertility included clomiphene citrate alone or in combination with metformin, prescribed at rates of 9% and 23%, respectively, whereas only 6% used other methods for the induction of ovulation.[20] The treatment options in the present study were similar to the current literature, except for the low rate of metformin use.

Table 4. Drug therapies provided for the patients followed up with a diagnosis of PCOS at the Near East University Hospital (Nicosia: August, 2019)

Pharmacotherapies (n=431)	**n**	**%**
Oral contraceptives	174	40.4
Progesterone	125	29.0
Antibiotics	81	18.8
Metabolic drugs (anti-diabetics, thyroid hormones…)	54	12.5
Supplements (vitamins, ferrum...)	53	12.3
Ovulation stimulators		
Metformin	23	5.3
Dopamine agonists	19	4.4
Estrogen agonists (tamoxifen...)	17	3.9
Other ovulation stimulators (clomiphene, aromatase inhibitors)	13	3.0
Other drugs		
(Dermatologic agents and others)	26	6.0

Table 5. The distribution by age groups of the PCOS patients according to the existence of symptoms at first presentation to the hospital (Nicosia: August, 2019)

Age group						
PCOS symptom at first presentation (n=758)	**Under 25 years**		**25 years of age or over**		**Total**	
	n	**%**	**n**	**%**	**n**	**%**
Existent	309	88.5	322	78.7	631	83.2
Non-existent	40	11.5	87	21.3	127	16.8

X^2=13.0, p<0.01. PCOS: Polycystic ovary syndrome.

Regarding the lack of population level data on the prevalence and distribution of common benign women's health diseases, including PCOS in North Cyprus, the Cyprus Women's Health Research initiative will be the first cross-sectional study to evaluate these conditions, aiming to recruit 8,000 women of reproductive age and establish a cohort of women in North Cyprus. The results of this large-scale study are expected to shed light on the definite data on this condition of women as well as others.[21]

The current study has shown that only half of the patients diagnosed as PCOS continued their follow-up for this disease as recommended. Under-diagnosis and lack of monitoring may lead to rapid conversion of metabolic disorders for PCOS patients. This lack of treatment results in increased risks of type 2 diabetes, atherosclerosis and endometrium cancer.[10,11] Therefore, there is a need for programs to effectively monitor and treat patients diagnosed as or suspected of PCOS through accurate data keeping and registration systems. These patients should be informed and monitored closely and regularly regarding the possible risks of this syndrome.

Study Limitations

The lack of full data of the patients' records in the system is considered to be a limitation of this study. The data and results of this study are limited by the information covered in the records. There was considerable missing data in the system. There is definitely a need for a more comprehensive record-keeping and monitoring system in order to allow for a better follow-up of these patients. Due to the descriptive nature of this study, the results may show hints of the actual situation, which may only be derived from representative studies. The lower PCOS rate may be attributed to the private status of the hospital, as well as the insufficiency of the records.

CONCLUSION

Based on the NIH's diagnostic criteria, there is a similar prevalence of PCOS across countries. Some studies have shown some differences between geographical locations and ethnic groups. However, the existing data is not conclusive enough to decide whether or not there are any significant differences in the prevalence of PCOS across geographical locations, racial or ethnic groups.[9]

Different diagnostic criteria may be one of the factors for the considerable variations in the prevalence of PCOS. In spite of the efforts for diagnosis, a large percentage of women are anticipated to remain undiagnosed even after visiting multiple health care providers. This finding points to the need for ethnicity-specific guidelines for PCOS in order to prevent under- or over-diagnosis of this condition because under-diagnosis may lead to rapid conversion of metabolic disorders for patients, whereas over-diagnosis may cause negative psychological effects on patients, worsening the major symptoms of PCOS.

This was a descriptive record-based study, covering only the patients of a university hospital; thus it was not representative of the North Cyprus population, but it shed light on the features of a group of women with PCOS in this community. Our findings point to the probability of under-diagnosis compared to the international data presented in the medical literature. There is a need for representative studies of the whole country for a full picture of this issue and to prevent patients from being undiagnosed. Further knowledge about this specific women's health problem in the population of this geography may enlighten the road map in order to take effective measures for this issue and may be of assistance for interventions in similar communities.

What this paper contribues

The results of this study imply under-recognition of PCOS among the population of women in North Cyprus, which may lead to rapid conversion of metabolic disorders.

Only half of the patients diagnosed as PCOS continued their follow-up for this disease as recommended.

Under-diagnosis and a lack of monitoring result in increases in risks for type 2 diabetes, atherosclerosis and endometrium cancer.

Therefore, the results of this study point to a need for programs to effectively diagnose, treat and monitor those patients diagnosed as or suspected of PCOS, including accurate data keeping and registration systems, in order to improve women's health.

MAIN POINTS

- In the current study, the status of polycystic ovary syndrome (PCOS) was investigated retrospectively among patients of a university hospital in North Cyprus, where no previous research on this issue had been conducted.
- Among the 819 PCOS patients found for the time period of 2015-2019, 78.5% displayed polycystic ovary morphology via ultrasonography and a minority (0.7%) had a family history.
- PCOS patients comprised 1.8% of the total patients of the gynecology clinic, lower than the expected value, although ESHRE/ASRM Rotterdam Conference 2003 and AE-PCOS Conference 2006 criteria were used.
- The results point to the need for further research and effective interventions in this community for this specific women's health problem in order to prevent complications.

ETHICS

Ethics Committee Approval: Permission from the Near East University Gynecology and Obstetrics Department and the Chief Physician of the Near East University Hospital was obtained and consent and approval of the Near East University Ethics Committee with report number YDU/2019/71-869 was provided.

Informed Consent: It was obtained.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: Ö.A., S.V., G.A., S.C., Design: Ö.A., S.V., G.A., S.C., Supervision: Ö.A., S.V., G.A., S.C., Fundings: S.C., Materials: Ö.A., S.V., G.A., S.C., Data Collection and/or Processing: S.V., S.C., Analysis and/or Interpretation: Ö.A., S.V., G.A., S.C., Literature Search: Ö.A., S.V., G.A., Writing: Ö.A., S.V., Critical Review: S.V., G.A., S.C.

DISCLOSURES

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

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

References

1. Bozdag G, Mumusoglu S, Zengin D, Karabulut E, Yildiz BO. The prevalence and phenotypic features of polycystic ovary syndrome: a systematic review and meta-analysis. Hum Reprod. 2016; 31(12): 2841-55.
2. Varanasi LC, Subasinghe A, Jayasinghe YL, Callegari ET, Garland SM, Gorelik A, et al. Polycystic ovarian syndrome: Prevalence and impact on the wellbeing of Australian women aged 16-29 years. Aust N Z J Obstet Gynaecol. 2018; 58(2): 222-33.
3. Rotterdam ESHRE/ASRM-sponsored PCOS consensus workshop group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertil Steril. 2003; 1(1): 19-25.
4. Ding T, Hardiman PJ, Petersen I, Wang FF, Qu F, Baio G. The prevalence of polycystic ovary syndrome in reproductive-aged women of different ethnicity: a systematic review and meta-analysis. Oncotarget. 2017; 8(56): 96351-8.
5. Sirmans SM, Pate KA. Epidemiology, diagnosis, and management of polycystic ovary syndrome. Clin Epidemiol. 2014; 6: 1-13.
6. Prapas N, Karkanaki A, Prapas I, Kalogiannidis I, Katsikis I, Panidis D. Genetics of polycystic ovary syndrome. Hippokratia. 2009; 13(4): 216-23.
7. Shuvani S. Polycystic ovary syndrome, insulin resistance, and obesity: Navigating the pathophysiologic labyrinth. Medical News Today 5th January 2018. (Accessed: 30 Jul 2019). Available from: https://www.medicalnewstoday.com/articles/265309.php
8. Krul-Poel YHM, Koenders PP, Steegers-Theunissen RP, ten Boekel E, Wee MMt, Louwers Y, et al. Vitamin D and metabolic disturbances in polycystic ovary syndrome (PCOS): A cross-sectional study. PLoS One. 2018; 13(12): e0204748.
9. Wolf W, Wattick R, Kinkade O, Olfert M. Geographical prevalence of polycystic ovary syndrome as determined by region and race/ethnicity. Int J Environ Res Public Health. 2018; 15(11): 2589.
10. Anagnostis P, Tarlatzis BC, Kauffman RP. Polycystic ovarian syndrome (PCOS): Long-term metabolic consequences. Metabolism. 2018; 86: 33-43.
11. Azziz R, Carmina E, Chen Z, Dunaif A, Laven JSE, Legro RS, et al. Polycystic ovary syndrome. PRIME PubMed. NatRev Dis Primers. 2016; 2: 16057.
12. Dunaif A. Hyperandrogenic anovulation (PCOS): A unique disorder of insulin action associated with an increased risk of non-insulin-dependent diabetes mellitus. Am J Med. 1995; 98(1A): S33-9.
13. Rojas J, Chávez M, Olivar L, Rojas M, Morillo J, Mejías J, et al. Polycystic ovary syndrome, insulin resistance, and obesity: Navigating the pathophysiologic labyrinth. Int J Reprod Med. 2014; 2014: 719050.
14. Lucidi RS. Polycystic ovarian syndrome. (Accessed: 2 Oct 2019). https://emedicine.medscape.com/article/256806-overview#a5
15. Cahill DJ. PCOS. BMJ Clin Evid. 2009; 2009: 1408.
16. Lizneva D, Suturina L, Walker W, Brakta S, Gavrilova-Jordan L, Azziz R. Criteria, prevalence, and phenotypes of polycystic ovary syndrome. Fertil Steril. 2016; 106(1): 6-15.
17. Ding T, Baio G, Hardiman P J, Petersen I, Sammon C. Diagnosis and management of polycystic ovary syndrome in the UK (2004-2014): a retrospective cohort study. BMJ Open. 2016; 6(7): e012461.
18. Kyprianidou M, Panagiotakos D, Faka A, Kambanaros M, Makris KC, Christophi CA. Prevalence of multimorbidity in the Cypriot population; A cross-sectional study (2018-2019). PLoS One. 2020; 15(10): e0239835.
19. Lauristen MP, Bentzen JG, Pingborg A, Loft A, Forman JL, Thuesen LL, et al. The prevalence of polycystic ovary syndrome in a normal population according to the Rotterdam criteria versus revised criteria including anti-Müllerian hormone. Hum Reprod. 2014: 29(4): 791-801.
20. Conway G, Dewailly D, Diamanti-Kandarakis E, Escobar-Morreale HF, Frans S, Gambineri A, et al; ESE PCOS Special Interest Group. European survey of diagnosis and management of the polycystic ovary syndrome: results of the ESE PCOS Special Interest Group's Questionnaire. Eur J Endocrinol. 2014; 171: 489-98.
21. Hocaoglu MB, Gurkas S, Karaderi T, Erguler K, Barin B, Bilgin EM, et al. Cyprus Women's Health Research (COHERE) initiative: determining the relative burden of women's health conditions and related co-morbidities in an Eastern Mediterranean population. BMC Women's Health. 2019; 19(1): 50.

ORIGINAL ARTICLE

CYPRUS
JOURNAL OF MEDICAL SCIENCES

DOI: 10.4274/cjms.2021.3570
Cyprus J Med Sci 2023;8(1):34-39

The Effect of Infraclavicular Block on Tourniquet-Induced Ischaemia Reperfusion Injury: A Prospective Randomized Controlled Study

Özlem Taş[1], Faruk Çiçekci[2], Husamettin Vatansev[2], Esra Paydaş Hataysal[2], Mehmet Sargın[3], İnci Kara[3]

[1]Department of Anesthesiology, Kozluk State Hospital, Batman, Türkiye
[2]Department of Anesthesiology and Reanimation, Selçuk University Faculty of Medicine, Konya, Turkey
[3]Department of Biochemistry, Selçuk University Faculty of Medicine, Konya, Türkiye

Abstract

BACKGROUND/AIMS: Ischemia-reperfusion injury (IRI) occurs due to the release of free oxygen radicals after tourniquet usage. Following tourniquet application, parameters such as ischemia modified albumin (IMA), total antioxidant status (TAS) and total oxidant status (TOS) become more frequently studied in order to reveal IRI. The aim of this study was to compare the effects of both infraclavicular block (ICB) and general anaesthesia (GA) on IRI in a prospective randomized controlled manner.

MATERIALS AND METHODS: Sixty patients undergoing extremity surgery with tourniquet were randomized in two groups (the ICB group ICB and the GA group GA). In the group ICB, anaesthesia using USG linear probe was applied via a lateral-sagittal technique. Conversely, anaesthesia was inducted with propofol and was maintained with 2-3% sevoflurane, 50% O_2/air mixture in the group GA. Blood samples were drawn before ICB and the induction of GA (T1), and again 2 hours after tourniquet opening (T2). Serum TAS, TOS and IMA levels were calculated using diagnostic kits.

RESULTS: A total 47 patients were evaluated in both groups. There was no statistical significance within or between the two groups in terms of their IMA, TAS and TOS values at T1 and T2 ($p>0.05$). In addition, there was no statistical significance within or between either group in terms of IMA, TAS and TOS values according to their tourniquet times (0-60 and 61-120 min) ($p>0.05$).

CONCLUSION: Infraclavicular nerve block and GA were not superior to each other in preventing IRI associated with a tourniquet duration of up to 120 min.

Keywords: Tourniquet, ischemia-reperfusion injury, general anaesthesia, infraclavicular nerve block

INTRODUCTION

Tourniquets are commonly used in the proximal region for limb operations to control bleeding in orthopaedic surgery. A tourniquet can cause ischemic-reperfusion injury (IRI) when applied in extremity surgery, cardiac surgery, thromboembolic events, revascularization, organ transplantation, and ischaemia restoration-induced severe hypotension and hypovolemic shock.[1] Anaerobic glycolysis is induced during ischaemia, followed by the release of pro- and anti-inflammatory cytokines, polymorphonuclear neutrophil activation and reperfusion. Simultaneously, platelet adhesion to the vascular endothelium occurs due to the production of reactive oxygen species (ROS) and the release of vasoactive factors. These free oxygen radicals trigger enzymatic

To cite this article: Taş Ö, Çiçekci F, Vatansev H, Paydaş Hataysal E, Sargın M, Kara İ. The Effect of Infraclavicular Block on Tourniquet-Induced Ischaemia Reperfusion Injury: A Prospective Randomized Controlled Study. Cyprus J Med Sci 2023;8(1):34-39

ORCID IDs of the authors: Ö.T. 0000-0002-4299-001X; F.Ç. 0000-0002-3248-0745; H.V. 0000-0002-0230-3414; E.P.H. 0000-0002-3538-8135; M.S. 0000-0002-6574-273X; İ.K. 0000-0001-6546-4277.

Address for Correspondence: Faruk Çiçekci MD, Department of Anesthesiology and Reanimation, Selçuk University Faculty of Medicine, Konya, Turkey
E-mail: farukcicekci@yahoo.com
ORCID ID: orcid.org/0000-0002-3248-0745

Received: 02.07.2020
Accepted: 05.11.2021

reactions, such as the peroxidation of polyunsaturated fatty acids or plasma lipoproteins, leading to the oxidative destruction of cell membranes, the production of toxic reactive metabolites and damage to the structure of DNA, proteins and lipids.[2] The production of free radicals causes hypoxia, acidosis, sodium-calcium pump disturbance and tissue damage, leading to increased ischaemia-modified albumin (IMA) production.[3] Total antioxidant status (TAS), an indicator of antioxidant activity, counteracts oxidative stress and reperfusion damage. The production of ROS increases, whereas TAS decreases as a result of oxidative stress. Total oxidant status (TOS) is related to ROS production.[4]

During elective orthopaedic surgery, tourniquet-induced skeletal muscle ischaemia results in the oxidation of muscle proteins.[5] However, tourniquet-induced ischaemia and its immediate effects on human skeletal muscle cells have rarely been documented.[6] In this study, we investigated the effects of infraclavicular block (ICB) on tourniquet-induced ischaemia reperfusion upper extremity injury in a prospective study. These parameters were also compared between whole groups according to different tourniquet times during surgery (0-60 and 61-120 min).

MATERIALS AND METHODS

Study Group

This study was approved by the Ethics Committee of Selçuk University Faculty of Medicine (approval number: 07.03.2018/2018/05), and all the patients provided informed consent. The study group comprised 60 males and females, aged between 18 and 65 years with American Society of Anesthesiologists (ASA) physical status 3-3 undergoing routine upper extremity surgery with a pneumatic tourniquet. The exclusion criteria were as follows: cardiac, metabolic, renal, or hepatic diseases; systemic rheumatological diseases; inflammatory, autoimmune, or peripheral vascular diseases; symptomatic diabetic microangiopathy (diabetic foot, diabetic retinopathy, etc.); known limb ischaemia; deep vein thrombosis; or hemodynamic instability. Additional exclusion criteria were a history of cancer, a history of coronary artery disease within the previous year or extremity surgery within the previous 3 months, drug use which could impair acid-base balance, steroid drug use, alcohol consumption and smoking.

Sample Size Calculation and Group Allocation

The sample size in each group was determined according to the method of Omür et al.[7] According to a power analysis, to achieve a power of 0.830 and a significance of p=0.05, 22 patients were required in each group. However, to take into account possible data loss, a minimum of 30 patients were required per group.

The patients were given the choice of ICB or general anaesthesia (GA). Patient recruitment continued until there were 30 patients in each group. On reaching the required patient number (n=30) in one group, the patient were not enrolled into the other group, as the patient's choice of anaesthesia method was directed by the group allocation.

Anaesthesia Induction

The patients were taken to the operating room without any premedication. On arrival in the operating room, each patient was monitored using a standard electrocardiogram and pulse oximetry, capnography, respiratory rate and non-invasive arterial pressure measurements. Immediately prior to anaesthesia, all the patients received 0.9% sodium chloride solution administered via intravenous infusion.

Infraclavicular Block Group

Each patient in the ICB group was placed in a supine position, with the patient's head turned to the opposite side of the block. The skin over the interscalene block area was sterilized with 10% povidone iodine. The block was applied using a My Lab 30 USG (Esaote, Florence, Italy) multi-frequency linear probe (10-18 MHz). The USG linear probe was applied using a lateral-sagittal technique, when applying ICB. A 100 mm 22-G nerve stimulation needle relative to the artery in the USG image was directed at 3-6-9 clockwise. The position of the needle with the neurostimulator was confirmed by observing rhythmic contraction movements of the hand and wrist. Then, 10 mL of 2% prilocaine and 10 mL of 0.5% bupivacaine (total dose=20 mL) were given as an intermittent negative aspiration as a local anaesthetic mixture. The U-shaped spread of local anaesthesia around the three branches of the brachial plexus was confirmed by ultrasound.

General Anaesthesia Group

In the GA group, the patients were anesthetized with 2 mg/kg of propofol, 2 mg/kg of fentanyl and 0.6 mg/kg of rocuronium at the beginning of GA induction. The patients were intubated after 2 min of muscle relaxation. Anaesthesia was maintained by administering 2-3% sevoflurane, 50% O_2/air and 0.1 mg/kg/dk remifentanil infusion. At the end of the operation, the return of spontaneous breathing was provided with sugammadex (4 mg/kg), and the patients were extubated.

Data Collection and Measurements

Blood samples were drawn before ICB and the induction of GA, and these values were accepted as T1 values before tourniquet application. A pneumatic tourniquet routinely used in upper extremity procedures was inflated just before surgery to maintain the patient's mean systolic blood pressure above 100 mmHg. Blood samples were drawn again 2 hours after tourniquet opening, and these values were accepted as T1 values too. The blood samples were collected in ependurach tubes and stored at -20 °C. Serum IMA levels were measured spectrophotometrically using a spectrophotometer (Lambda, PerkinElmer, Massachusetts, USA) and the albumin-cobalt binding method. The results were recorded in absorbance units. Colorimetric method kits were used for the measurement of serum TAS and TOS levels. TAS and TOS levels were calculated (μmol H_2O_2 equivalent/L) using commercially available diagnostic kits. The TAS, TOS and IMA levels in the groups were also measured at different tourniquet times: 0-60 min and 61-120 min.

In both groups, intravenous ephedrine (5-10 mg) was administered if the systolic blood pressure decreased by more than 30% as compared with the baseline value. Atropine was administered when the patient's heart rate decreased below 50 beats/min.

Statistical Analysis

Statistical analysis of the data was performed using SPSS 21.0 (Statistical Program for Social Sciences, Chicago, IL, USA). The Kolmogorov-Smirnov test was performed to determine the normality of the data distribution. A t-test was used for comparisons of data with a normal distribution, and the non-parametric Mann-Whitney U test was used for data without a normal distribution. Demographic data and descriptive statistics of

continuous variables are presented as mean ± standard deviation. A chi-squared test was used to evaluate categorical data. A t-test was applied for between-group comparisons of measurements. A repeated measures analysis of variance was used to examine whether intra-group comparisons (T1 vs T2) showed a significant change, and a paired t-test was used for within-group comparisons. A Bonferroni correction was performed for evaluations of repeated measurements within groups. A value of $p<0.05$ was considered statistically significant.

RESULTS

In total, 60 patients were included in this study. In the ICB group, blood samples from four patients were not available because of deteriorated blood samples and/or changes in the anaesthesia method from block to GA due to the patients' pain. In the GA group, some samples were not available to use because of deteriorated blood samples and/or prolonged operation times. Thus, in the final analysis, 26 patients were included in the ICB group, and 28 patients were included in the GA group. Detailed information on the enrolment of the patients into this study is shown in the CONSORT flow diagram in Figure 1. When the demographic and clinical results of the patients were evaluated, there were no between-group differences in terms of age, gender, body mass index, ASA status, operation time, anaesthesia time or tourniquet time (Table 1). There was no statistical significance within or between the two groups in terms of TAS, TOS and IMA values at T1 and T2 ($p>0.05$) (Table 2). In addition, there was no statistical significance within or between the both groups in terms of their TAS, TOS and IMA values according to their tourniquet times (0-60 and 61-120 min) ($p>0.05$) (Table 3).

DISCUSSION

In our study, neither ICB nor GA was superior in terms of the prevention of IRI associated with tourniquet use, as determined by measurements of IMA, TAS and TOS levels in serum samples at different tourniquet times (0-60 min and 61-120 min).

The use of a tourniquet in extremity surgery can result in the temporary occlusion of blood flow and ischaemia, which causes rapid damage to metabolically active tissues. Paradoxically, restoration of blood flow (reperfusion) is associated with the generation of ROS, which damage cellular components. Various methods have been proposed to reduce tourniquet-induced IRI. Such methods include ischemic preconditioning, which involves intermittent loosening and inflating of the tourniquet.[8] Previous research reported that this practice significantly reduces the likelihood of capillary flow arrest or the "no-reflow phenomenon". In addition, some studies have also shown that the use of tourniquets in this way increases the ability of muscles to withstand ischaemia for some time, while not reducing ischaemia reperfusion, thereby allowing tourniquets to be used for longer periods.[9]

Immunosuppressive agents, antioxidant agents, steroids, vitamin E, vitamin C and glutamine have been used to prevent IRI related to tourniquet use.[10-12] Various studies have demonstrated the protective effects of these agents against IRI in GA. In one study on tourniquet-induced IRI in anaesthesia, propofol and total intravenous anaesthesia were administered to one group of patients undergoing limb surgery, and inhalation anaesthesia with isoflurane were administered to

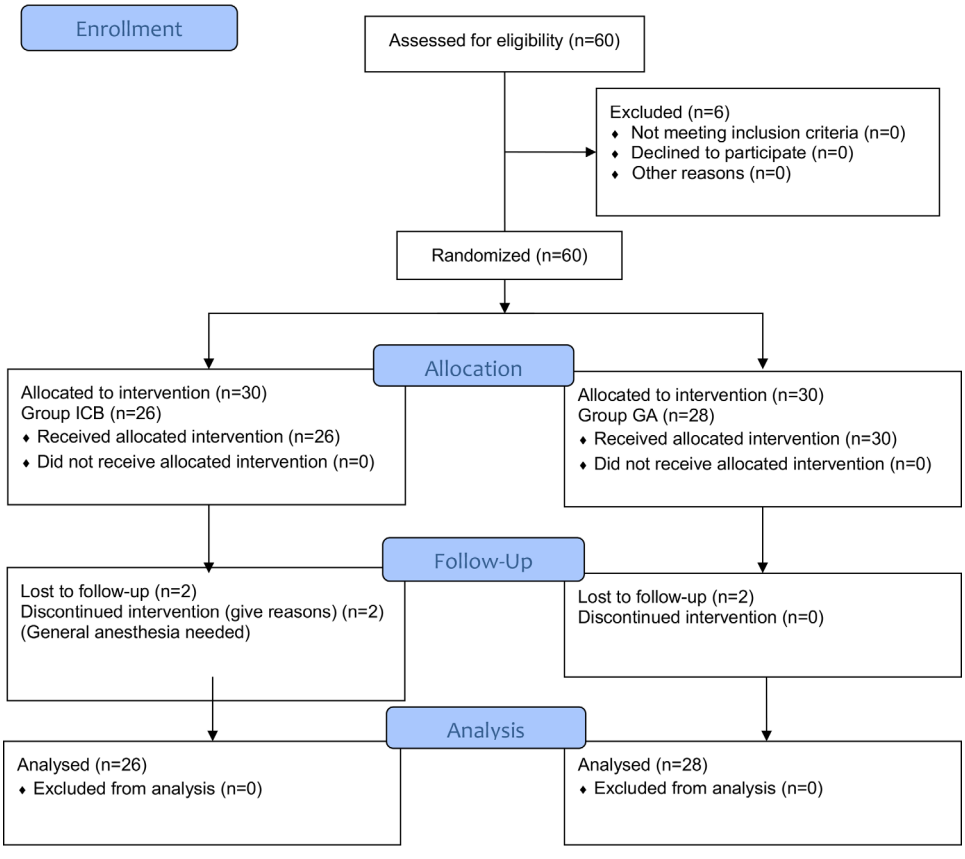


Figure 1. CONSORT flow diagram (group ICB, infraclavicular block, group GA, general anaesthesia).

another group. Malondialdehyde (MDA) levels, which are an important marker of reperfusion injury, were significantly lower in the propofol group.[13] Propofol is chemically similar to vitamin E, an endogenous antioxidant, and butylated hydroxytoluene, a free radical scavenger.[14] Previous research demonstrated that propofol protected erythrocytes against oxidative stress. Propofol accumulates in biomembranes with the rapid occurrence of radical scavenging activity due to the release of a hydrogen atom from the hydroxyl group.[15] Other studies have shown that propofol had high lipid solubility and that it accumulated in lipophilic membranes, which are very sensitive to oxidative damage, and it increased the antioxidant capacity of tissues.[16,17] Runzer et al.[18] demonstrated the antioxidant properties of propofol in different organs. Another study showed that the antioxidant effect of propofol was significantly greater than that of sevoflurane and that propofol reduced IRI caused by free oxygen radicals.[19] Studies have also shown that propofol reduced the pro-inflammatory response in sepsis.[20]

Another study showing the effects of volatile agents on IRI showed that inhalation anaesthetics protected IRI in cardiomyocyte cells by selectively activating ATP-sensitive potassium (K^{ATP}) channels via

Table 1. Characteristics of the patients

	Group ICB (n=26)	Group GA (n=28)	p-value
Age (year)	38.5±15.7	36.0±13.3	0.537
Gender (M/F)	16/10	17/11	0.788
BMI (kg/m²)	26.0±3.4	26.4±4.0	0.691
ASA status (I//II)	18/8	20/8	0.845
Surgery duration (min)	72.6±20.9	67.4±16.0	0.310
Aesthetic duration (min)	72.6±21.1	73.3±18.3	0.901
Tourniquet time (min)	52.7±13.3	51.0±14.1	0.652

Differences between groups are insignificant (p>0.05). Patients' characteristic and surgical data are given as mean ± standard deviation or number of patients. Group ICB: Infraclavicular block group, Group GA: General anaesthesia group. ASA: American Society of Anesthesiologists, BMI: Body mass index.

Table 2. Comparison of serum TAS, TOS and IMA levels between and within the groups according to T1 and T2 times

	Group ICB (n=26)	Group GA (n=28)	p-value
TAS (mmol Trolox Eq/L)			
T1	1.01±0.29	1.00±0.19	0.862
T2	0.92±0.17	0.92±0.20	0.911
TOS (mmol Trolox Eq/L)			
T1	7.22±4.99	10.16±5.15	0.051
T2	9.00±7.55	10.07±5.09	0.540
IMA (ABSUs)			
T1	0.89±0.37	0.90±0.31	0.954
T2	0.97±0.25	0.94±0.23	0.675

Plasma levels of total antioxidant status (TAS) (μmol H_2O_2 equivalent/L), total oxidant status (TOS) (μmol H_2O_2 equivalent/L) and ischemia-modified albumin (IMA) (absorbance units=ABSUs). From all patients, preoperatively (T1), 15 min before tourniquet inflation and 2 hours (T2) after tourniquet release. Group ICB: Infraclavicular block group, Group GA: General anaesthesia group. No statistically significant difference between or within the groups (p>0.05).

Table 3. Comparison of serum TAS, TOS and IMA levels between and within the groups according to the tourniquet times (0-60 and 61-120 minutes)

	Group ICB, (n=26)			Group GA, (n=28)		
	0-60 min, (n=11)	61-120 min, (n=15)	p-value	0-60 min, (n=13)	61-120 min, (n=15)	p-value
TAS (mmol Trolox Eq/L)						
T1	0.95±0.14	1.04±0.44	0.744	1.03±0.13	0.97±0.23	0.505
T2	0.91±0.18	0.95±0.14	0.617	0.95±0.20	0.88±0.20	0.539
TOS (mmol Trolox Eq/L)						
T1	7.93±4.97	5.29±4.86	0.239	9.55±4.26	10.86±6.12	0.431
T2	9.45±8.15	7.76±6.01	0.621	10.03±3.99	10.12±6.31	0.388
IMA (ABSU)						
T1	0.85±0.40	0.99±0.27	0.409	0.93±0.36	0.85±0.23	0.511
T2	0.92±0.21	1.10±0.31	0.109	0.97±0.28	0.91±0.18	0.963

Plasma levels of total antioxidant status (TAS) (μmol H_2O_2 equivalent/L), total oxidant status (TOS) (μmol H_2O_2 equivalent/L) and ischemia-modified albumin (IMA) (ABSUs), according to the tourniquet times (0-60 and 60-120 minutes). Group ICB: Infraclavicular block group, Group GA: General anaesthesia group. No statistically significant difference between and within the groups (p>0.05). ABSU: Absorbance units.

protein kinases.[21] Lucchinetti et al.[22] suggested that sevoflurane (0.5-1%) administered at sedative concentrations in healthy volunteers provided protection against endothelial damage by acting on leukocytes and preventing leukocyte activation and leukocyte adhesion. Another study on 20 patients who underwent elective coronary artery bypass surgery showed results which further supported the cardio-protective effects of sevoflurane.[23] In a study on thoracic surgery patients, at the 6th postoperative hour, the IMA level in a sevoflurane group was lower than that in a propofol group. Thus, the authors concluded that sevoflurane was superior to propofol in preventing IRI.[24] Julier et al.[25] also found that sevoflurane preserves myocardial and renal functions. Budić et al.[26] measured levels of MDA, a lipid peroxidation product, and the activity of an antioxidant enzyme, catalase, which is one of the first defence mechanisms against free radicals. They concluded that continuous propofol infusion and regional anaesthesia techniques attenuate tourniquet-induced IRI in paediatric extremity surgery.

Several studies have shown that remifentanil significantly reduced apoptosis in hepatic cells, protected mitochondrial cells against oedema and protected membranes against IRI, with the authors concluding that remifentanil had protective properties against IRI in liver and intestinal cells.[27,28]

Brachial plexus blockade increases blood flow in the extremity and in replanted digits by preventing neurally mediated spasm, and it is often used to provide sympathectomy and to improve blood flow when vascular insufficiency occurs.[29]

A previous study investigated ischaemia injury in patients undergoing elective knee surgery who received sevoflurane and GA or spinal anaesthesia. Similar tourniquet times were used in both groups. The authors reported that using GA with sevoflurane and nitrous oxide was associated with less oxidative stress than using spinal anaesthesia in their in vivo ischaemia-reperfusion model. In another study which compared sevoflurane and spinal anaesthesia, sevoflurane caused less oxidative stress than spinal anaesthesia.[19,20]

Some studies have also shown that lidocaine provided protection against oxidative stress in erythrocytes. However, only high doses of bupivacaine and ropivacaine provided protection against the harmful effects of oxidative stress.[30] In our study, we showed that there is no significant difference for tourniquet-induced IRI between the ICB group with the use of a mixture prilocaine and bupivacaine and the GA group.

In contrast to local anaesthesia and GA, brachial plexus blockade increases blood flow in the extremity and in replanted digits by preventing neurally mediated spasm. Brachial plexus blockade is often used to provide analgesia and sympathectomy and to improve blood flow when vascular insufficiency occurs. Brachial plexus blockade increases blood flow in the extremity and in replanted digits by preventing neurally mediated spasm.[31,32]

There are only a few studies on the levels of IMA, an indicator of IRI, in extremity surgery. In one study, MA and myoglobin levels in serum samples before knee arthroscopy using a tourniquet were significantly higher than those after surgery.[14] Another study suggested that the administration of both ketamine and lidocaine infusions may significantly decrease skeletal muscle IRI-related high lactate and IMA levels.[33] In our study, we found no significant difference in IMA levels in the pre-operative and post-operative samples.

In our ICB group, we used no systemic aesthetic drugs, and afterwards, similar TAS, TOS and IMA levels were observed in this group. In addition, in our GA group, which effectively represented the control group, we used sevoflurane as an inhaler agent during induction.

Study Limitations

In terms of strengths, standardization was achieved by selecting patients who had no history of trauma and no systemic inflammatory disorders or planned elective procedures. The limitations included the small number of patients, the single centre nature of this study and the measurements of post-operative TAS, TOS and IMA levels only once (i.e. 120 min) after surgery. We think that more significant results may be obtained in studies involving larger numbers of patient, and measuring TAS, TOS and IMA levels over longer post-operative time periods.

CONCLUSION

In this prospective randomized controlled study, ICB and GA were not superior to each other in preventing IRI associated with a tourniquet duration of up to 120 min. However, we believe that more clinical studies with a longer tourniquet times and a larger number of patients are needed in order to clarify these findings.

MAIN POINTS

- Following the opening of the tourniquet at the end of the operation, the re-entry of blood tissue into the general circulation causes a large release of oxygen radicals.
- However, tourniquet-induced ischemic-reperfusion injury (IRI) and its effects on human skeletal muscle cells have been poorly documented.
- Intraclavicular nerve block and general anaesthesia were not superior to each other in preventing IRI associated with a tourniquet duration of up to 120 min.

Ethics Committee Approval: This study was approved by the Ethics Committee of Selçuk University Faculty of Medicine (approval number: 07.03.2018/2018/05).

Informed Consent: All the patients provided informed consent.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: Ö.T., F.Ç., Design: Ö.T., F.Ç., Supervision: F.Ç., M.S., Materials: F.Ç., H.V., E.P.H., Data Collection and/or Processing: Ö.T., H.V., E.P.H., M.S., Analysis and/or Interpretation: H.V., E.P.H., M.S., Literature Search: Ö.T., F.Ç., İ.K., Writing: Ö.T., İ.K., Critical Review: H.V., İ.K.

DISCLOSURES

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

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

References

1. Meier WA, Marcus RL, Dibble LE, Foreman KB, Peters CL, Mizner RL, et al. The long-term contribution of muscle activation and muscle size to quadriceps weakness following total knee arthroplasty. J Geriatr Phys The. 2009; 32(2): 79-82.
2. Akyol A, Ulusoy H, Imamoğlu M, Cay A, Yuluğ E, Alver A, et al. Does propofol or caffeic acid phenethyl ester prevent lung injury after hindlimb ischaemia-reperfusion in ventilated rats? Injury. 2006; 37(5): 380-7.
3. Wudkowska A, Goch J, Goch A. Ischemia-modified albumin in differential diagnosis of acute coronary syndrome without ST elevation and unstable angina pectoris. Kardiol Pol. 2010; 68(4): 431-7.
4. LeBel CP, Bondy SC. Oxygen radicals: common mediators of neurotoxicity. Neurotoxicol Teratol. 1991; 13(3): 341-6.
5. Shadgan B, Reid WD, Harris RL, Jafari S, Powers SK, O'Brien PJ. Hemodynamic and oxidative mechanisms of tourniquet-induced muscle injury: near-infrared spectroscopy for the orthopedics setting. J Biomed Opt. 2012; 17(8): 081408-1.
6. Ratchford SM, Bailey AN, Senesac HA, Hocker AD, Smolkowski K, Lantz BA, et al. Proteins regulating cap-dependent translation are downregulated during total knee arthroplasty. Am J Physiol Regul Integr Comp Physiol. 2012; 302(6): 702-11.
7. Omür D, Hacivelioglu SÖ, Oguzalp H, Uyan B, Kiraz HA, Duman C, et al. The effect of anaesthesia technique on maternal and cord blood ischaemia-modified albumin levels during caesarean section: A randomized controlled study. J Int Med Res. 2013; 41(4): 1111-9.
8. Whetzel TP, Stevenson TR, Sharman RB, Carlsen RC. The effect of ischemic preconditioning on the recovery of skeletal muscle following tourniquet ischemia. Plast Reconstr Surg. 1997; 100(7): 1767-75.
9. Jerome SN, Akimitsu T, Gute DC, Korthuis RJ. Ischemic preconditioning attenuates capillary no-reflow induced by prolonged ischemia and reperfusion. Am J Physiol. 1995; 268(5 pt 2): H2063-7.
10. Mohler LR, Pedowitz RA, Ohara WM, Oyama BK, Lopez MA, Gershuni DH. Effects of an antioxidant in a rabbit model of tourniquet-induced skeletal muscle ischemia-reperfusion injury. J Surg Res. 1996; 60(1): 23-8.
11. Prem JT, Eppinger M, Lemmon G, Miller S, Nolan D, Peoples J. The role of glutamine in skeletal muscle ischemia/reperfusion injury in the rat hind limb model. Am J Surg. 1999; 178(2): 147-50.
12. Azari O, Kheirandish R, Azizi S, Abbasi MF, Chaman SGG, Bidi M. Protective Effects of Hydrocortisone, Vitamin C and E Alone or in Combination against Renal Ischemia-Reperfusion Injury in Rat. Iran J Pathol. 2015; 10(4): 272-80.
13. Cheng YJ, Wang YP, Chien CT, Chen CF. Small-dose propofol sedation attenuates the formation of reactive oxygen species in tourniquet-induced ischemia-reperfusion injury under spinal anesthesia. Anesth Analg. 2002; 94(6): 1617-20.
14. Refaai MA, Wright RW, Parvin CA, Gronowski AM, Scott MG, Eby CS. Ischemia-modified albumin increases after skeletal muscle ischemia during arthroscopic knee surgery. Clinica Chimica Acta. 2006; 366(1-2): 264-8.
15. Erturk E, Cekic B, Geze S, Kosucu M, Coskun I, Eroglu A, et al. Comparison of the effect of propofol and N-acetyl cysteine in preventing ischaemia-reperfusion injury. Eur J Anaesthesiol. 2009; 26(4): 279-84.
16. Cheng YJ, Wang YP, Chien CT, Chen CF. Small-dose propofol sedation attenuates the formation of reactive oxygen species in tourniquet-induced ischemia-reperfusion injury under spinal anesthesia. Anesth Analg. 2002; 94(6): 1617-20.
17. Turan R, Yagmurdur H, Kavutcu M, Dikmen B. Propofol and tourniquet induced ischaemia reperfusion injury in lower extremity operations. Eur J Anaesthesiol. 2007; 24(2): 185-9.
18. Runzer T, Ansley D, Godin D, Chambers G. Tissue Antioxidant Capacity During Anesthesia: Propofol Enhances In Vivo Red Cell and Tissue Antioxidant Capacity in a Rat Model Anesth Analg. 2002; 94(1): 89-93.
19. Carles M, Dellamonica J, Roux J, Lena D, Levraut J, Pittet JF, et al. Sevoflurane but not propofol increases interstitial glycolysis metabolites availability during tourniquet-induced ischaemia-reperfusion. Br J Anaesth. 2008; 100(1): 29-35.
20. Horlocker TT, Hebl JR, Gali B, Jankowski CJ, Burkle CM, Berry DJ, et al. Anesthetic, patient, and surgical risk factors for neurologic complications after prolonged total tourniquet time during total knee arthroplasty. Anesth Analg. 2006; 102(3): 950-5.
21. Zaugg M, Lucchinetti E, Spahn DR, Pasch T, Schaub MC. Volatile anesthetics mimic cardiac preconditioning by priming the activation of mitochondrial K(ATP) channels via multiple signaling pathways. Anesthesiology. 2002; 97(1): 4-14.
22. Lucchinetti E, Ambrosio S, Aguirre J, Herrmann P, Härter L, Keel M, et al. Sevoflurane inhalation at sedative concentrations provides endothelial protection against ischemia-reperfusion injury in humans. Anesthesiology. 2007; 106(2): 262-8.
23. Conzen PF, Fischer S, Detter C, Peter K. Sevoflurane Provides Greater Protection of the Myocardium than Propofol in Patients Undergoing Off-pump Coronary Artery Bypass Surgery. Anesthesiology. 2003; 99(4): 826-33.
24. Erturk E, Topaloglu S, Dohman D, Kutanis D, Beşir A, Demirci Y, et al. The comparison of the effects of sevoflurane inhalation anesthesia and intravenous propofol anesthesia on oxidative stress in one lung ventilation. Biomed Res Int. Inpress. 2014; 2014: 360936.
25. Julier K, da Silva R, Garcia C, Bestmann L, Frascarolo P, Zollinger A, et al. Preconditioning by sevoflurane decreases biochemical markers for myocardial and renal dysfunction in coronary artery bypass graft surgery: a double-blinded, placebo-controlled, multicenter study. Anesthesiology. 2003; 98(6): 1315-27.
26. Budić I, Pavlović D, Cvetković T, Djordjević N, Simić D, Milojević I, et al. The effects of different anesthesia techniques on free radical production after tourniquet-induced ischemia-reperfusion injury at children's age. Vojnosanit Pregl. 2010; 67(8): 659-64.
27. Novalija E, Kevin LG, Eells JT, Henry MM, Stowe DF. Anesthetic preconditioning improves adenosine triphosphate synthesis and reduces reactive oxygen species formation in mitochondria after ischemia by a redox dependent mechanism. Anesthesiology. 2003; 98(5): 1155-63.
28. Cho SS, Rudloff I, Berger PJ, Irwin MG, Nold MF, Cheng W, et al. Remifentanil ameliorates intestinal ischemia-reperfusion injury. BMC Gastroenterol 2013; 13: 69.
29. McGregor AD, Jones WK, Perlman D. Blood flow in the arm under brachial plexus anaesthesia. J Hand Surg Br. 1985; 10(1): 21-4.
30. Lenfant F, Lahet JJ, Courderot-Masuyer C, Freysz M, Rochette L. Lidocaine has better antioxidant potential than ropivacaine and bupivacaine: in vitro comparison in a model of human erythrocytes submitted to an oxidative stress. Biomed Pharmacother. 2004; 58(4): 248-54.
31. Su HH, Lui PW, Yu CL, Liew CS, Lin CH, Lin YT, et al. The effects of continuous axillary brachial plexus block with ropivacaine infusion on skin temperature and survival of crushed fingers after microsurgical replantation. Chang Gung Med J. 2005; 28(8): 567-74.
32. Kurt E, Ozturk S, Isık S, Zor F. Continuous brachial plexus blockade for digital replantations and toe-to-hand transfers. Ann Plast Surg. 2005; 54(1): 24-7.
33. Peker K, Ökesli S, Kıyıcı A, Deyişli C. The effects of ketamine and lidocaine on free radical production after tourniquet-induced ischemia-reperfusion injury in adults. Ulus Travma Acil Cerrahi Derg. 2019; 25(2): 111-7.

RESEARCH ARTICLE

CYPRUS
JOURNAL OF MEDICAL SCIENCES

DOI: 10.4274/cjms.2022.2022-46
Cyprus J Med Sci 2023;8(1):40-45

Role of Inflammatory Response Biomarkers, Monocytes, and Platelets as Prognostic Indicators in Lung Cancer Patients Presenting with Malignant Pleural Effusion

Filiz Çimen, Melike Aloğlu, Sevim Düzgün, Ayşegül Şentürk, Şükran Atikcan

Clinic of Chest Disease, University of Health Sciences Türkiye, Atatürk Chest Diseases and Chest Surgery Training and Research Hospital, Ankara, Türkiye

Abstract

BACKGROUND/AIMS: Malignant pleural effusion (MPE) patients do not have a good prognosis and are strongly associated with markers of systemic inflammation. The current research sought to investigate the association between systemic inflammation and prognosis in lung cancer patients with MPE.

MATERIALS AND METHODS: This retrospective analysis was carried out on cases of MPE and lung cancer which occurred between January, 2015 and December, 2019. The study also examined the association between prognosis and the hematological parameters recorded at the time of diagnosis.

RESULTS: This study enrolled 117 people with a median age of 63 years. Of the patients, ninety-two were male (78.6%), and 25 were female (21.4%). Following initial diagnosis, the patients were followed up on average for 12 months. Significantly higher levels of monocyte and platelet were observed in the group of deceased patients than in the group of living patients (monocyte 0.4±0.1 10^3/uL in living patients vs 0.6±0.5 10^3/uL in deceased patients, p=0.010, platelet 275.9±87.3 10^3/uL in living patients vs 339.1±113.010^3/uL in deceased patients, p=0.020). In the univariate model, survival time was strongly predicted by monocytes, neutrophils, platelets, C-reactive protein, albumin, and platelet/lymphocyte ratio values (p=0.012, p=0.038, p=0.004, p=0.040, p=0.011, p=0.022, respectively). Monocyte and platelet values were found to be independent risk factors for survival time, in the simplified multivariate model [monocyte heart ratio (HR): 1,382; 95% confidence interval (CI), 1,012-1,887, p=0.042, and platelet HR: 1,002; 95% CI, 1,001-1,004, p=0.009].

CONCLUSION: In cases of MPE accompanied by stage-4 lung cancer, high monocyte and platelet levels indicate a poor prognosis.

Keywords: Cancer, inflammatory markers, neoplasm

INTRODUCTION

In cancer cells, systemic inflammation can be triggered by oncogenic alterations, and it contributes to the development of cancer. In addition, inflammation indicators may increase in many cancer-related conditions such as anorexia, cachexia, and pain. Cancer patients at high risk can be identified, and the progression of the illness can be predicted using systemic inflammatory signs. These indicators are affordable and simply calculated using regular laboratory data. Lung cancer, which has a high mortality rate, has a similar incidence in men and women.[1] It is widely known that white blood cell (WBC) subtype counts and WBC counts are involved in systemic inflammations or infections. Tumor formation and growth depend on monocytes

To cite this article: Çimen F, Aloğlu M, Düzgün S, Şentürk A, Atikcan Ş. Role of Inflammatory Response Biomarkers, Monocytes, and Platelets as Prognostic Indicators in Lung Cancer Patients Presenting with Malignant Pleural Effusion. Cyprus J Med Sci 2023;8(1):40-45

ORCID IDs of the authors: F.Ç. 0000-0002-1983-2566; M.A. 0000-0002-2239-0324; S.D. 0000-0002-2973-8610; A.Ş. 0000-0002-1072-0592; Ş.A. 0000-0003-1915-1610.

Address for Correspondence: Filiz Çimen
E-mail: fhcimen@yahoo.com
ORCID ID: orcid.org/0000-0002-1983-2566

Received: 03.10.2022
Accepted: 24.01.2022

and platelets. The immune system's regulation of tumor growth and metastasis is essential. Wculek and Malanchi[2] proposed that changed leukocyte levels in tumor-bearing hosts' tissues might have an impact on particular subgroups of invasive cancer cells. More specifically, it has been shown that leukocytes build up in the lung before cancer cells invade nearby tissues, and they multiply via the metastatic process.[2] Studies have proven that systemic inflammatory response can predict prognosis in a variety of malignancies.[3] The majority of lung cancer patients had metastatic diseases at the time of diagnosis. It has been shown that markers such as low serum albumin levels and high plasma C-reactive protein (CRP) play an important role in cancer development and progression.[4] It has been discovered that in cases with a range of malignancies, especially lung cancer, a poor outcome can be predicted by neutrophil/lymphocyte ratio (NLR), a systemic inflammatory marker.[5]

In determining survival and the course of the disease in patients with lung cancer, lymphocyte/monocyte ratio (LMR), NLR, and platelet/lymphocyte ratio (PLR) are easily detectable laboratory tests which can be very helpful. The current research aimed to explore the prognostic value of systemic inflammation markers such as LMR, NLR, and PLR in stage-4 lung cancer accompanied by malignant pleural effusion (MPE).

MATERIALS AND METHODS

A retrospective analysis was carried out on cases diagnosed with stage-4 lung cancer from January, 2015 to December, 2019. The cases were examined in terms of LMR, NLR, PLR, and hematological systemic inflammation markers. Retrospective analyses were performed on age, sex, type of malignancy, hemogram, biochemical results, primary lesion, SUV_{max} for pleural fluid, and overall survival (OS) (OS was described as the length of time from the diagnosis to the last follow-up or death). A comparison was made between the living and deceased patients.

In our research, it was discovered that elevated platelet and monocyte counts independently predicted poor prognosis in cases of lung cancer accompanied by MPE.

Informed consent was not necessary because of the retrospective character of this study.

Study Population

The study population was selected consecutively from a patient group with MPE who had been histopathologically diagnosed with lung cancer at our medical center between 2015 and 2019. Patients who had malignant cells either in their tissue biopsy or pleural fluid cytology, patients with no other possible pleural effusion etiology, and patients who had suspected malignancy in pleural effusion pathologic examination together with massive or localized effusion causing ipsilateral lung volume loss were recruited. Between 2015 and 2019, 2,245 cases diagnosed in our hospital were screened. Of these, 302 had pleural fluid. The study enrolled 117 cases meeting the inclusion criteria.

The following criteria were the requirements for inclusion in this study: histologically confirmed lung cancer, proven MPE, and individuals who had been undergoing therapy for lung cancer.

The study's exclusion criteria were as follows: patients undergoing curative lung resection; a significant infection present at the time of cancer diagnosis; and having an underlying hematological illness.

Ethical Considerations

The Ethics Committee of the University of Health Sciences Ankara Atatürk Chest Diseases and Thoracic Surgery Training and Research Hospital gave its approval for this investigation (2019/653).

Statistical Method

The mean, standard deviation, frequency, ratio values median, lowest and highest values were calculated as descriptive statistics. The Kolmogorov-Smirnov test was used to examine the distribution of the variables. Mann-Whitney U test and t-test for independent samples were used to examine the quantitative data. The NLR, LMR, and PLR values were analyzed using the Mann-Whitney U test. When the Chi-squared test did not meet the criteria, Fisher's exact test was performed to examine qualitative data. Cox regression (univariate-multivariate) was used for survival analysis. The SPSS 27.0 application was used to analyze the data.

RESULTS

The study enrolled a total of 117 patients. The median age was 63 years. Of the patients, 92 were male (78.6%), and 25 were female (21.4%). Distribution according to the histological lung cancer types was found as follows: adenocarcinoma (69.2%), squamous cell carcinoma (25.6%), and not otherwise specified (5.1%). Following their initial diagnosis, the patients had an average of 12 months of follow-up care. Table 1 includes the hematological, radiological, and pathological data of the 117 patients and their causes of MPE.

The deceased and the living patient groups did not significantly differ from each other in terms of their age or gender distribution ($p>0.05$). In addition, there was no significant difference between the groups' cell type distribution, tumor N stage distribution, tumor diameter, or tumor SUV_{max} value ($p>0.05$). The percentage of positive biopsy results did not substantially differ between the groups ($p>0.05$). There was no difference in pleural effusion side distribution or pleural fluid cytology rate between the two groups ($p>0.05$) (Table 2).

There was no difference in lymphocyte, neutrophil, CRP, albumin, NLR, PLR, or LMR values between the deceased and living patient groups ($p>0.05$). The deceased patient group had significantly higher monocyte and platelet counts than the living patient group (Living patients' monocyte 0.4±0.1 vs Deceased patients' monocyte 0.6±0.5), (Living patients' platelets 275.9±87.3 vs Deceased patients' platelets 339.1± 113.0) ($p=0.010$, $p=0.020$ respectively) (Table 2).

In the univariate model, age, sex, cell type, tumor diameter, tumor SUV_{max}, T, tumor N stage, pleural fluid localization, pleural fluid cytology, pleural fluid FDG, pleural biopsy, lymphocyte, NLR, and LMR values were not statistically significant indicators of survival time ($p>0.05$). Monocytes [Heart ratio (HR): 1.420; 95% confidence interval (CI), 1.081-1.865, $p=0.012$], neutrophils (HR: 1.70; 95% CI, 1.23-2.35, $p=0.038$), platelets (HR: 1.002; 95% CI, 1.001-1.004, $p=0.004$), CRP (HR: 1.013; 95% CI, 1.001-1.025, $p=0.040$), albumin (HR: 0.666; 95% CI, 0.487-0.911, $p=0.011$), PLR (HR: 1.002; 95% CI, 1.000-1.004, $p=0.022$) values were found to be statistically significant in predicting survival time in a univariate model (Table 3).

Monocyte (HR: 1.382; 95% CI, 1.012-1.887, $p=0.042$) and platelet (HR: 1.002; 95% CI, 1.001-1.004, $p=0.009$) values were found as independent predictive factors in a reduced multivariate model (Table 3).

DISCUSSION

A very important role in the pathogenesis and progress of cancer is played by systemic inflammation. Studies have highlighted the link between systemic inflammatory biomarkers and the development of several cancer types. We also planned to study the function of systemic inflammation biomarkers in advanced lung cancers complicated by MPE. In our study, we found that high platelet and monocyte levels were independent predictors of a poor prognosis in cases of lung cancer accompanied by MPE.

A study which included 101 lung cancer cases with MPE found that the cytology exam of pleural fluid was positive in 60.4% of the cases. The authors underline pleural fluid cytology's sensitivity in MPE.[6] Another study involving 165 patients with pathologically proven MPE identified positive pleural cytology and positive histology as independent predictive factors for survival but found no significance in Cox regression.[7] Similarly, in our study, positive pleural cytology was found to have no significant effect on mortality or survival.

Biomarkers of an inflammatory response, such as LMR, PLR, and NLR, have frequently been cited in recent studies on lung cancer prognosis. Xu et al.[8] found that, apart from N3, PLR had a significant independent relationships with the T stage and N stage. NLR and PLR were found to be potential biomarkers for Non-small-cell lung cancer (NSCLC) in

Table 1. Demographic, radiological and pleural fluid characteristics

		Min.-Max.	Median	Mean ± SD/(n, %)
Age (years)		37.0-77.0	63.0	62.5±7.5
Sex	Female	-	-	25 (21.4%)
	Male	-	-	92 (78.6%)
Cell type	Adeno	-	-	81 (69.2%)
	SCC	-	-	30 (25.6%)
	NOS	-	-	6 (5.1%)
Tumor N	I	-	-	9 (7.7%)
	II	-		74 (63.2%)
	III	-		34 (29.1%)
Pleural	Biopsy (+)	-	-	26 (22.2%)
	Biopsy (-)	-		14 (12.0%)
	No biopsy	-		77 (65.8%)
Pleural fluid FDG	>2.5	-	-	57 (48.7%)
	<2.5	-		60 (51.3%)
Pleural fluid localization	Right	-	-	61 (52.1%)
	Left	-		56 (47.9%)
Pleural fluid cytology	(+)	-	-	64 (54.7%)
	(-)	-		53 (45.3%)
Tumor diameter (cm)		2.0-12.0	5.0	4.9±2.2
Tumor SUV_{max}		2.9-32.0	11.4	12.4±5.4
Lymphocyte (10^3/uL)		0.6-3.9	1.7	1.8±0.7
Monocyte (10^3/uL)		0.1-4.8	0.5	0.6±0.5
Neutrophil (10^3/uL)		2.1-16.7	6.1	6.6±2.7
Platelet (10^3/uL)		127.0-667.0	303.0	331.0±111.8
CRP (mg/dL)		0.1-98.2	4.1	8.0±13.5
Albumin (mg/dL)		2.3-5.1	3.8	3.8±0.6
NLR		0.9-15.5	3.4	4.1±2.7
PLR		65.1-561.6	180.0	209.1±106.0
LMR		0.2-12.3	3.6	4.0±2.3
Following time (months)		1.0-67.0	12.0	17.3±15.5
Alive		-	-	15 (12.8%)
Deceased		-	-	102 (87.2%)

Min.: Minimum, Max.: Maximum, SD: Standard deviation, SCC: Squamous cell carcinoma, NOS: Not otherwise specified, CRP: C-reactive protein, NLR: Neutrophil/lymphocyte ratio, PLR: Platelet/lymphocyte ratio, LMR: Lymphocyte/monocyte ratio

its early stages and could be useful in predicting stages 3 and 4. In both surgical and non-surgical NSCLC patients, elevated PLR was found to be strongly related to poor OS in a meta-analysis evaluating 5,314 patients from 13 studies, with a cut-off value of 160 for PLR; however, the same relationship was not discovered in the case of small cell lung cancer (SCLC).[9]

LMR was connected to both OS and disease-free survival (DFS) in another study of NSCLC patients.[10] Decreased progression-free survival and OS after systemic therapy were linked to higher NLR before treatment, according to a meta-analysis by Wang et al.[11] (chemotherapy, immunotherapy, and targeted therapy). In their retrospective study, Wang et al.[12] defined the inflammatory response biomarker (IRB) scores with 3 parameters, namely NLR, PLR, and LMR, and found that an IRB score of 2 or above was an independent predictive factor for poor OS and DFS.

High NLR and PLR levels were discovered to be linked to a worse OS in a study of 389 individuals with advanced-stage NSCLC taking chemotherapy, and a similar significant relationship was also identified for total lymphocyte count.[13] The results of our study contradict all this literature data, but this may be due to our smaller sample size when compared to the biomarker studies in the literature.

Previous studies determined that thrombocytosis before treatment was a poor indicator of prognosis in malignant mesothelioma, cervix, colon, and non-small cell carcinoma.[14,15] By generating growth and angiogenic factors, tumor-associated macrophages are known to promote angiogenesis, encourage the development of tumor cells, and facilitate invasion and metastasis.[16] According to Hamilton et al.[17], circulating tumor cells (CTC) interact with macrophages composed of monocytes. In SCLC, aggressive invasion of CTC as well as cytokines, chemokines, and growth factors are seen. As a result, a high monocyte count

Table 2. Data comparison between the living and the deceased group

		Living		Deceased		p-value
		Mean ± SD	Median	Mean ± SD	Median	
Age (years)		61.1±8.1	61.0	62.7±7.4	63.0	0.456
Sex	Female	2 (13.3%)	-	23 (22.5%)	-	0.416
	Male	13 (86.7%)	-	79 (77.5%)	-	
Cell type	Adeno	12 (80.0%)	-	69 (67.6%)	-	0.503
	SCC	3 (20.0%)	-	27 (26.5%)	-	0.526
	NOS	0 (0.0%)	-	6 (5.9%)	-	1.000
Tumor N	I	1 (6.7%)	-	8 (7.8%)	-	0.931
	II	9 (60.0%)	-	65 (63.7%)	-	
	III	5 (33.3%)	-	29 (28.4%)	-	
Pleural	Biopsy (+)	2 (50.0%)	-	24 (66.7%)	-	0.601
	Biopsy (-)	2 (50.0%)	-	12 (33.3%)	-	
	No biopsy	11	-	66	-	
Pleural fluid SUV_{max}	>2.5	8 (53.3%)	-	49 (48.0%)	-	0.702
	<2.5	7 (46.7%)	-	53 (52.0%)	-	
Pleural fluid localization	Right	10 (66.7%)	-	51 (50.0%)	-	0.228
	Left	5 (33.3%)	-	51 (50.0%)	-	
Pleural fluid cytology	(+)	6 (40.0%)	-	58 (56.9%)	-	0.221
	(-)	9 (60.0%)	-	44 (43.1%)	-	
Tumor diameter (cm)		4.9±2.4	5.0	4.9±2.2	5.0	0.843
Tumor SUV_{max}		12.3±7.2	10.4	12.4±5.1	11.9	0.441
Lymphocyte (10^3/uL)		1.6±0.6	1.6	1.9±0.7	1.8	0.274
Monocyte (10^3/uL)		0.4±0.1	0.4	0.6±0.5	0.5	***0.010***
Neutrophil (10^3/uL)		5.8±2.9	5.7	6.7±2.6	6.2	0.242
Platelet (10^3/uL)		275.9±87.3	252.0	339.1±113.0	310.0	***0.020***
CRP (mg/dL)		8.0±19.8	2.4	8.0±12.5	4.5	0.122
Albumin (mg/dL)		3.8±0.6	4.0	3.8±0.6	3.8	0.701
NLR		3.7±2.1	3.4	4.2±2.8	3.4	0.680
PLR		184.3±91.0	162.6	212.8±108.0	185.2	0.340
LMR		4.6±1.7	4.2	3.9±2.3	3.5	0.082
[t]t-test /[m]Mann-Whitney U test/[χ²]		Chi-squared test				

Bold and italic indicate significant values: p<0.05., SD: Standard deviation, SCC: Squamous cell carcinoma, NOS: Not otherwise specified, CRP: C-reactive protein, NLR: Neutrophil/lymphocyte ratio, PLR: Platelet/lymphocyte ratio, LMR: Lymphocyte/monocyte ratio

Table 3. Patient data in the univariate and multivariate model

	Univariate model			Multivariate model		
	HR	95% CI	p-value	HR	95% CI	p-value
Age (years)	1.016	0.988-1.044	0.270	-	-	-
Sex	1.129	0.703-1.812	0.616	-	-	-
Cell type	1.094	0.790-1.513	0.589	-	-	-
Tumor diameter (cm)	1.054	0.960-1.156	0.269	-	-	-
Tumor SUV_{max}	0.997	0.964-1.032	0.874	-	-	-
Tumor N stage	1.130	0.800-1.597	0.488	-	-	-
Pleural biopsy	1.079	0.855-1.362	0.522	-	-	-
Pleural fluid SUV_{max}	1.223	0.826-1.810	0.315	-	-	
Pleural fluid localization	1.059	0.713-1.571	0.777	-	-	-
Pleural fluid cytology	0.815	0.549-1.210	0.311	-	-	-
Lymphocyte (10^3/uL)	1.148	0.854-1.545	0.361	-	-	-
Monocyte (10^3/uL)	1.420	1.081-1.865	***0.012***	1.382	1.012-1.887	***0.042***
Neutrophils (10^3/uL)	1.075	1.004-1.151	***0.038***	-	-	-
Platelet (10^3/uL)	1.002	1.001-1.004	***0.004***	1.002	1.001-1.004	***0.009***
CRP (mg/dL)	1.013	1.001-1.025	***0.040***	-	-	-
Albumin (mg/dL)	0.666	0.487-0.911	***0.011***	-	-	-
NLR	1.060	0.987-1.137	0.108	-	-	-
PLR	1.002	1.000-1.004	***0.022***	-	-	-
LMR	0.905	0.814-1.005	0.062	-	-	-
Cox regression (forward LR)		-	-	-	-	-

Bold and italic indicate significant values: p<0.05. HR: Heart ratio, CI: Confidence interval, CRP: C-reactive protein, NLR: Neutrophil/lymphocyte ratio, PLR: Platelet/lymphocyte ratio, LMR: Lymphocyte/monocyte ratio

may contribute to tumor progression. In support of these findings, monocyte and platelet counts were discovered to be independent predictors of OS duration in our research.

Study Limitations

In our study, it was discovered that elevated platelet and monocyte levels are independent predictors of poor prognosis in cases of lung cancer accompanied by MPE. This suggests that more rapid and aggressive treatment should be applied to lung cancer patients with increased monocytes and platelets. In addition to conventional chemotherapy, developing adjunct therapies for monocyte and platelet functions (targeting monocyte-platelet receptors and the chemokines, cytokines, and the growth factors secreted from them) may facilitate the management of these patients and favorably influence their survival.

CONCLUSION

Patients with MPE generally have a poor prognosis. A correlation between prognosis and systemic inflammation marker levels has been emphasized in a number of previous studies in this patient group. This study showed a positive and independent correlation between high thrombocyte and monocyte levels and a poor prognosis in lung cancer patients with MPE. These are simple, widespread, and low-cost diagnostic tests which can help provide foresight toward future outcomes. In cases of advanced lung cancer with MPE, patients with high levels of thrombocytes and monocytes are subject to a poor prognosis.

MAIN POINTS

- Survival of patients with malignant pleural effusion is generally poor.
- In lung cancer patients, Neutrophil/lymphocyte ratio, platelet/lymphocyte ratio, lymphocyte/monocyte ratio and advanced lung cancer inflammation index show a strong correlation with prognosis.
- One hundred and seventeen patients were included in the study.
- Monocyte and platelet values were significantly higher in the deceased patients' group than in the living patients' group (p=0.010, p=0.020).
- High monocyte and platelet counts reflect poor prognosis in advanced-stage lung cancer cases with malignant pleural effusion.

ETHICS

Ethics Committee Approval: The Ethics Committee of the University of Health Sciences Türkiye, Ankara Atatürk Chest Diseases and Thoracic Surgery Training and Research Hospital gave its approval for this investigation (2019/653).

Peer-review: Externally peer reviewed.

Authorship Contributions

Concept: F.Ç., M.A., Ş.A., Design: F.Ç., Data Collection and/or Processing: F.Ç., M.A., S.D., A.Ş., Analysis or Interpretation: F.Ç., M.A., S.D., A.Ş., Literature Search: F.Ç., M.A., S.D., A.Ş., Ş.A., Writing: F.Ç., M.A.

DISCLOSURES

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

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

References

1. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin. 2015; 65(2): 87-108.
2. Wculek SK, Malanchi I. Neutrophils support lung colonization of metastasis-initiating breast cancer cells. Nature. 2015; 528(7582): 413-7.
3. Chen S, Guo J, Feng C, Ke Z, Chen L, Pan Y. The preoperative platelet-lymphocyte ratio versus neutrophil-lymphocyte ratio: Which is better as a prognostic factor in oral squamous cell carcinoma? Ther Adv Med Oncol. 2016; 8: 160-7.
4. Scott HR, McMillan DC, Forrest LM. The systemic inflammatory response, weight loss, performance status and survival in patients with inoperable non-small cell lung cancer. BJC. 2022; 87: 264-7.
5. Sarraf KM, Belcher E, Raevsky E. Neutrophil/lymphocyte ratio and its association with survival after complete resection in non-small cell lung cancer. J Thorac Cardiovasc Surg. 2008; 137(2): 425-8.
6. Brun C, Gay P, Cottier M, Karpathiou G, Patoir A, Tiffet O, et al. Comparison of cytology, chest computed and positron emission tomography findings in malignant pleural effusion from lung cancer. J Thorac Dis. 2018; 10(12): 6903-11.
7. Verma A, Abisheganaden J, Light RW. Identifying Malignant Pleural Effusion by A Cancer Ratio (Serum LDH: Pleural Fluid ADA Ratio). Lung. 2016; 194(1): 147-53.
8. Xu F, Xu P, Cui W, Gong W, Wei Y, Liu B, et al. Neutrophil-to-lymphocyte and platelet-to-lymphocyte ratios may aid in identifying patients with non-small cell lung cancer and predicting Tumor-Node-Metastasis stages. Oncol Lett. 2018; 16: 483-90.
9. Zhao QT, Yuan Z, Zhang H, Zhang XP, Wang HE, Wang ZK, Duan GC. et al. Prognostic role of platelet to lymphocyte ratio in non-small cell lung cancers: A meta-analysis including 3,720 patients. Int J Cancer. 2016; 139(1): 164-70.
10. Hu P, Shen H, Wang G, Zhang P, Liu Q, Du J. Prognostic significance of systemic inflammation-based lymphocyte- monocyte ratio in patients with lung cancer: based on a large cohort study. PLoS One. 2014; 9(9): e108062.
11. Wang Z, Zhan P, Lv Y, Shen K, Wei Y, Liu H, Song Y. et al. Prognostic role of pretreatment neutrophil-to-lymphocyte ratio in non-small cell lung cancer patients treated with systemic therapy: a meta-analysis. Transl Lung Cancer Res. 2019; 8(3): 214-26.
12. Wang Y, Hu X, Xu W, Wang H, Huang Y, Che G. Prognostic value of a novel scoring system using inflammatory response biomarkers in non-small cell lung cancer: A retrospective study. Thorac Cancer. 2019; 10(6): 1402-11.
13. Song X, Chen D, Yuan M, Wang H, Wang Z. Total lymphocyte count, neutrophil-lymphocyte ratio, and platelet-lymphocyte ratio as prognostic factors in advanced non-small cell lung cancer with chemoradiotherapy. Cancer Manag Res. 2018; 10: 6677-83.
14. Gao L, Zhang H, Zhang B, Zhang L, Wang C. Prognostic value of combination of preoperative platelet count and mean platelet volume in patients with resectable non-small cell lung cancer. Oncotarget. 2017; 8(9): 15632-41.
15. Schlesinger M. Role of platelets and platelet receptors in cancer metastasis. J Hematol Oncol. 2018; 11(1): 125.
16. Mantovani A, Marchesi F, Malesci A, Laghi L, Allavena P. Tumour-associated macrophages as treatment targets in oncology. Nat Rev Clin Oncol. 2017; 14: 399-416.
17. Hamilton G, Rath B, Klameth L, Hochmair MJ. Small cell lung cancer: Recruitment of macrophages by circulating tumor cells. Oncoimmunology. 2015; 5(3): e1093277.

RESEARCH ARTICLE

CYPRUS
JOURNAL OF MEDICAL SCIENCES

DOI: 10.4274/cjms.2021.2021-224
Cyprus J Med Sci 2023;8(1):46-52

Evaluation of Vaginal Discharge and Genital Hygiene Habits in Women: Turkish Republic of North Cyprus Example

Filiz Yarıcı, Betül Mammadov, Dilay Necipoğlu

Near East University Faculty of Health Sciences, Nicosia, North Cyprus

Abstract

BACKGROUND/AIMS: This research was conducted with the aim of evaluating vaginal discharge and genital hygiene in women between the ages of 18-49 who live in the Turkish Republic of Northern Cyprus.

MATERIALS AND METHODS: This research, which was planned as a descriptive study, was carried out on an online basis. The sample scope was determined as 95% confidence interval with a 5% margin of error with at least 382 women using a well-known sampling formula and 408 women volunteered for this study. The data were collected using a personal information form, vaginal discharge question form and Genital Hygiene Behaviors Inventory (GHBI). Number, percentage and average tests were used for the descriptive analysis. The conformity of the dependent and independent variables to the normal distribution was tested with the Kolmogorov-Smirnov test. The Independent t-test and One-Way ANOVA test were used to determine any differences between dependent and independent variables.

RESULTS: In this study, 36.5% of the participants were between the ages of 25-34, 59.8% were married, and in terms of their levels of education, 54.9% had university or higher-level qualifications. 8.3% of the women had a previous medical history of a sexually transmitted disease and this was continuing in 3.9% of them. It was determined that 46.8% of the women participating in this study had abnormal vaginal discharge. It was determined that the abnormal discharge of 14.2% of the women had lasted for 1 month and, for 13% of the women, it had lasted for 1 year or more. Also, it was seen that 30.2% of the women resorted to traditional methods for dealing with their abnormal vaginal discharge, including 11.5% of them who cleaned their vagina with vinegar, soda and/or lemon juice. The average score that the women obtained from the "GHBI" was 78.24±12.07 (minimum: 49.0, maximum: 108.0). A statistically significant difference was found between the GHBI mean score of the women participating in this study according to their past experiences of having abnormal vaginal discharges, the presence of previous sexually transmitted diseases and vaginal douching ($p<0.05$).

CONCLUSION: It was found that nearly half of the women had abnormal vaginal discharge (curd, green-yellow or grey in color and foul-smelling). In addition, it was determined that 30.2% of the women resorted to traditional methods for treating their abnormal vaginal discharge, including 11.5% of the participants who cleaned their vagina with vinegar, soda and/or lemon juice. Within the scope of primary health care services, it is recommended that midwives and nurses question the genital hygiene behaviors of women by both planning home visits and during the women's visits to health institutions, and by providing accurate information on reproductive health, genital hygiene, sexually transmitted diseases and family planning through brochures or training booklets.

Keywords: Vaginal discharge, genital hygiene, women's health

To cite this article: Yarıcı F, Mammadov B, Necipoğlu D. Evaluation of Vaginal Discharge and Genital Hygiene Habits in Women: Turkish of North Cyprus Example. Cyprus J Med Sci 2023;8(1):46-52

ORCID IDs of the authors: F.Y. 0000-0003-2922-8685; B.M. 0000-0002-9051-0279; D.N. 0000-0001-8213-5854.

Address for Correspondence: Filiz Yarıcı
E-mail: filiz_atis01@hotmail.com
ORCID ID: orcid.org/0000-0003-2922-8685

Received: 26.08.2021
Accepted: 26.10.2021

INTRODUCTION

Genital infections are recognized as the most common health problems seen in women in their reproductive stage between the ages of 18-49. Every year, one million women contract urogenital infections around the world and it has been reported that at least 75% of them have a history of vaginal infection.[1-3] Research conducted in Türkiye shows that the frequency of genital tract infections in women is between 52% and 92%.[4-9]

There are many reasons why genital infections are so common in women. The anatomic structure in which the urethra, vagina and anus are close together increases the risk of genital tract infections. Additionally, having multiple sexual partners, poor genital and menstrual hygiene, tight and synthetic underwear, bad nutrition, systemic diseases such as diabetes, poor obstetric history, stress, long term antibiotic and/or steroid use, incorrect genital hygiene applications, vaginal douche (VD), tampon use, poor environmental conditions, lack of education and many other factors can lead to genital infections.[9-13] Ensuring genital hygiene is one of the most important steps in preventing the serious problems which can be caused by genital infections, as well as protecting and maintaining women's health and their reproductive health. This is because when genital hygiene is neglected, the tendency for infection increases, and if the infection is not treated correctly and in a timely manner, this can have a negative impact on the woman's reproductive ability.[1,8,9] Additionally, genital infections can result in problems such as babies with low birth weight, abortion, fetal death, congenital infections in newborns, ectopic pregnancy, sepsis, cervical cancer, chronic pelvic pain etc.[1,9,13] In order to protect and enhance women's health, it is important to provide early diagnosis and treatment services along with protective measures against genital infections, and for health professionals to provide education and counselling for women.[9]

The way each woman describes and perceives vaginal discharge is different because many women do not know their body or how to identify abnormal vaginal discharge. Some women perceive their vaginal discharge as abnormal and consult health institutions, whereas even though others may have excessive discharge, they think that this is normal and take no action. Women's perception of genital infection risks differ according to their cultural structure, socio-economic situation, environmental conditions, stories they hear from their loved ones, traditional methods they may use for treatment, negative social exclusion and embarrassment with regard to gynecological examinations.[1,9] Due to these reasons, women often resort to traditional methods to solve their problems rather than using medical treatments. However genital infections which are not treated can cause serious reproductive health problems.[1,6] Many studies have examined the genital hygiene behaviors of women in Türkiye.[9,14,15] However, no studies have evaluated the genital hygiene behaviors of women living in the Turkish Republic of Northern Cyprus (TRNC). It is clear that midwives and nurses have an important role in protecting, maintaining and treating women's health conditions. Midwives in particular play an important role in determining the groups at risk of genital infections, evaluating the presence of genital infections in women and maintaining preventive health services in order to prevent their occurrence. For this reason, this study aimed to evaluate the abnormal vaginal discharge and genital hygiene behaviors of women between the ages of 18-49 living in the TRNC.

Research Questions

1. Is there a statistically significant difference between the mean scores of the Genital Hygiene Behaviors Inventory (GHBI) of women according to some socio-demographic characteristics and their vaginal discharge status?

2. Is there a significant difference between women's socio-demographic characteristics and their experiencing abnormal vaginal discharge?

3. Is there a significant difference between the socio-demographic characteristics of women and the practice of vaginal douching?

MATERIALS AND METHODS

Study Design and Sampling

This cross-sectional and descriptive study was carried out between 01.06.2021 and 30.06.2021 on an online basis. The universe of this study comprised n=77,920 women between the ages of 18-49 in accordance with the TRNC 2011 census. The sample, on the other hand, was calculated to be at least 382 women, with a 95% confidence interval (CI) with a 5% margin of error, according to a known sampling formula. The sample of this study consisted of n=408 women.

Data Collection

The data collection phase was conducted online. The data were collected by the researchers at a specified time on a web basis with an online questionnaire form. Invitations to participate in this study were shared via social media accounts and the WhatsApp application. Individuals who were directed to the link address were presented with an information and consent section. Individuals who provided their consent were then directed to the research form and participated in this study. Completing the online form took an average of 5 minutes.

Data Collection Tools

The data collection form consists of two sections. The first section includes all information regarding this study as well as a consent form. The second section consists of the data collection forms. In the first part of the information form, there are 20 questions including information about age, marital status, socio-economic characteristics and obstetric history, and some introductory features regarding vaginal discharge. The second part consists of 15 questions about vaginal discharge. These questions were prepared in line with the purpose of this study. Their validity and reliability were not determined. The scope validity was evaluated regarding the items on the question form. In order to achieve this, the expert opinions of five specialist lecturers of whom one specialized in the basics of nursing, one in public health nursing and three in the field of midwifery, were taken into account. Necessary changes were made to the data collection form according to the feedback from these experts. A pilot study of the data collection form was conducted with 20 women in the TRNC who were not included in the sample group. Any necessary changes were made to the data collection form according to the results of the pilot study.

Genital Hygiene Behaviours Inventory: GHBI is a one-dimensional scale consisting of 27 questions developed by Ege and Eryılmaz[16] in 2005. Every statement in the inventory is graded between 1 and 4. For the positive statements, one 1 point is given for the answer "never", 2 for "sometimes", 3 for "often" and 4 for "always" and vice versa for negative statements. Due to the fact that items 17, 26 and 27 in the

inventory include negative statements, they are reverse scored. The minimum score which can be obtained from the GHBI is 27 and the maximum is 108. The total scores obtained from the inventory indicate the genital hygiene behavior scores. As the scores obtained from the inventory increase, it is evaluated that genital hygiene behaviors improve. The Cronbach's alpha reliability coefficient was calculated to be 0.86.[16] In the current study, the Cronbach's alpha value of the scale was determined to be 0.77.

Statistical Analysis

The data collected online were evaluated by transferring to the Statistical Package for Social Sciences software version (SPSS 21.0- IBM SPSS Corp.; Armonk, NY USA) package program. The Cronbach's α-value was calculated in order to test the internal reliability of the scale. The number, percentage and test averages were used for descriptive analysis. The conformity of the dependent and independent variables to the normal distribution was tested with the Kolmogorov-Smirnov test. The Independent t-test and One-Way ANOVA test were used to determine the difference between the dependent and independent variables. The data were evaluated with a 95% CI and p=0.05 as the margin of error.

Approval from the Near East University Hospital Scientific Research Ethic Board was obtained before starting this research (approval number: 2021/1367). It was stated to the participants in the informed consent section that this study was voluntary, it could be completed at any time and the data would remain anonymous. No payment was made to or charged by the participants in this study. However, it was stated that they could receive summary information about the results of this study upon request.

RESULTS

The women's socio-demographic information is given in Table 1. 36.5% of the participants were between the ages of 25-34 years, 59.8% were married and the level of education of 54.9% was university or higher. The obstetric and gynecological details of the women are given in Table 2. It was determined that 46.8% of the women used family planning, 15.9% of the women used condoms, and 10.5% used intrauterine devices (IUD) for family planning. 8.3% of the participants had a previous sexually transmitted disease and 3.9% of them still had this disease. It was determined that 46.8% of the women in this study had abnormal vaginal discharge. It was determined that the discharge of 14.2% of the women had lasted for 1 month or less, and 13.0% of them had discharge which had lasted for 1 year or more. In addition, it was determined that 30.2% of the women resorted to traditional methods to treat their abnormal vaginal discharge, including 11.5% of the participants who cleaned their vagina with vinegar, soda and/or lemon juice.

The women who participated in this research had a mean score of 78.24±12.07 (minimum: 49.0, maximum: 108.0) from the "GHB". A comparison of the GHBI mean scores according to the participants' ages and levels of education is given in Table 3. According to this, a statistical difference was found between the GHBI mean scores of the women according to their ages and levels of education ($p<0.05$).

Table 4 shows a comparison of the GHBI mean scores of the participants according to certain obstetric and gynecological specifications and their discharge situations. It was found that there was a statistically significant difference between the mean scores of the "GHB" according to the women's status of distinguishing the normality of their vaginal discharge, their use of perfume, deodorant, or soap to prevent discharge, and keeping their vagina dry to prevent discharge ($p<0.05$). Additionally, a statistically significant difference was found between the GHBI mean scores of those who had abnormal vaginal discharge, those who had a previous history of sexually transmitted diseases and those who applied VD ($p<0.05$).

DISCUSSION

Genital tract infections, which are commonly seen in women, can be prevented and easily treated by changing certain hygiene habits and consulting health facilities in the presence of abnormal discharge. Therefore, the reproductive health, sexual health, emotional health and quality of life of women can be positively affected. According to the data of our study, in which the vaginal discharge and genital hygiene behaviors of 408 women in the TRNC were evaluated, it was determined that nearly half of the women had a history of abnormal vaginal discharge. When other studies were examined, it was seen that

Table 1. Socio-demographic characteristics of the women (n=408)

Socio-demographic characteristics		n	%
Age group	18-24 age	124	30.4
	25-34 age	149	36.5
	35-49 age	135	33.1
Marital status	Married	244	59.8
	Single	164	40.2
Educational status	Middle school or below	74	18.1
	High	110	27.0
	University or above	224	54.9
Working status	Working	234	57.4
	Not working	174	42.6
Economic status	Low	19	4.7
	Medium	268	65.7
	High	121	29.7
Child presence	Yes	201	49.3
	No	207	50.7

one out of every two women experience abnormal vaginal discharge due to poor genital hygiene habits.[3,17-20] Our study showed that this was not only related to hygiene habits, but it was also determined that about a third of the participants found it difficult to distinguish abnormal discharge. Additionally, it was determined that more than half of the women who experienced abnormal vaginal discharge did not consult health services and that about a third resorted to traditional methods (vinegar, soda, lemon juice etc.) in cases of abnormal vaginal discharge. The study conducted by Okumuş and Demirci[21] showed that although 22.8% of the women had abnormal vaginal discharge, they did not consult a health facility. The study conducted by Özcan et al.[22] showed that 26.6% of the women waited for their abnormal vaginal discharge to clear up on its own and that 13.9% of them resorted to traditional methods to treat themselves. Many other studies have shown that women use traditional applications for their abnormal vaginal discharge and urogenital tract infections, which are commonly seen in women.[23-25] In our study, the rates of applying to health services and using traditional methods in cases of abnormal vaginal discharge were found to be slightly higher compared to the literature. There are many reasons for this, such as delaying visiting health services for economic reasons, the exchange of information about health among women, considering an abnormal vaginal discharge to be an infection which heals easily, feelings of embarrassment about gynecological examinations, and regarding their situation as normal.

VD is a common method applied by women where the vagina is cleaned with water or antiseptic liquids.[26] In our study, about one third of the women stated that they applied VD. The study conducted by Yağmur[8] in 2007 showed that 57.2% of women applied VD, and in similar studies, it was reported that 88%, 59%, and 72.7% of women applied VD and that this practice increased the risk of infection by 2.5 times.[10,27,28] Although it is not as high as in the literature, the number of women who applied VD in the TRNC was also high. Approximately one out of three women stated that they applied VD. The most common reasons why women apply VD include "being hygienic",[2] as well as the belief that it is a

Table 2. Obstetric and gynecological details of the women (n=408)

Obstetric and gynecological features		n	%
Family planning usage status	Yes	191	46.8
	No	217	53.2
Using family planning method	Condom	65	15.9
	Intrauterine device	43	10.5
	Tablet	32	7.8
	Tube ligation	25	6.1
	Other (monthly injection and traditional methods)	26	6.5
	No	217	53.2
Past sexually transmitted disease status	Yes	34	8.3
	No	374	91.7
Vaginal douche application status	Yes	146	35.8
	No	262	64.2
Abnormal vaginal discharge	Yes	191	46.8
	No	217	53.2
The case of applying to the health service for abnormal vaginal discharge	Yes	158	38.7
	No	250	61.3
Traditional methods to treat abnormal vaginal discharge	Yes	123	30.2
	No	285	69.8
Used traditional methods to treat abnormal vaginal discharge	Cleaning the vagina with vinegar, soda or lemon juice	47	11.5
	Drink with onion, parsley, chamomile tea	28	6.8
	Cleaning the vagina with yoghurt	38	9.4
	Other (cleaning with garlic or nettle water)	10	2.5
	None	285	69.8

Table 3. Comparison of GHBI mean scores according to some socio-demographic specifications of participants (n=408)

Socio-demographic characteristics		GHBI (mean ± SD)	F	p
Age groups	18-24 age	73.61±12.19	17.53	0.001
	25-34 age	78.57±12.32		
	35-49 age	82.14±10.15		
Educational status	Middle school and below	75.70±11.62	11.581	0.001
	High school	74.80±11.87		
	University and above	80.77±11.76		

P<0.05; F: One-Way ANOVA, SD: Standard deviation, GHBI: Genital Hygiene Behaviors Inventory

contraceptive method and also due to religious views.[29] The results of our study show that vaginal douching is also a common practice in North Cyprus and it is a risk factor for vaginal infections. It is thought that this practice, which is adopted as a hygiene practice, is actually an application which affects the pH of the vagina and increases exposure to infection, but most women are not aware of its potential harm.

It is also thought that another factor which can increase the risk of vaginal infection is the family planning method they use as 10.5% of the women were using IUD, while 15.9% used condoms as protection. It is known that IUDs change the vaginal flora and create a suitable environment for anaerobic bacteria causing infection.[4] It is thought that the very low rate of condom use increases the risk of sexually transmitted infections in women whose spouses do not use condoms, and this may pave the way for the transmission of other infections which may cause abnormal vaginal discharge.

The mean score that the women obtained in this study from the GHBI was determined to be 78.24±12.07 (minimum: 49-maximum: 108). When it is considered that the highest score which can be obtained from this scale is 108, it can be said that the genital hygiene behaviors of the women are at the required level. In this study, it was found that the GHBI scores significantly increased with age (82.14±10.15) ($p<0.001$). It is known that most women experience genital infections at least once in their lifetime. Considering that 66.9% of the women participating in this study were young women under the age of 35, it is clear that these women have a higher risk of contracting genital tract infections during their lifetime. The reason that the older women received higher scores from the inventory may be due to them going to the hospital more often after experiencing birth, discharge and/or infection and trying to apply the information which they have obtained from health workers.

It was found that the GHBI scores (80.77±11.76) of the women with university or above levels of education were higher than the women in the other groups ($p<0.001$). Similar to our study, in a study conducted with women who applied to a health center, it was reported that their GHBI mean score was 80.28 (minimum: 48-maximum: 107). It has been reported that hygiene behavior is affected by education, being employed and having a higher income.[8,30] It has been proven that raising the level of education and making women more productive in working life have positive effects on women's health. Educated women have the awareness to seek health advice from the right sources, and to visit health institutions, instead of using traditional practices, in order to receive the right treatment in abnormal situations.

It was found that women who obtained higher scores in the GHBI could identify abnormalities in their discharge better than those who received lower scores ($p<0.001$), they understood the importance of keeping their vagina dry ($p<0.03$), they did not apply VD ($p<0.007$), and they avoided applying products such as soap, deodorant and/or perfume to their perineal area ($p<0.03$). Those women with a previous history of sexually transmitted diseases had significantly higher GHBI scores than those who did not ($p<0.005$), and this caused these women to be uncomfortable due to the negative effects which these infections had on their sexual health, reproductive health and quality of life, or they were afraid of the possibility that the infection would reoccur resulting in them having a more careful attitude towards their genital hygiene.

Study Limitations

Our study is limited to only those women who live in North Cyprus and who agreed to participate in this study and so increasing the sample size of the study would increase its power.

CONCLUSION

It was determined that about half of the women experienced abnormal vaginal discharge. Genital tract infections are diseases which can be prevented and quickly treated with the correct hygiene habits and timely treatment. However, when most women are faced with abnormal vaginal discharge, they do not apply to health establishments due to neglect, lack of education, embarrassment or economic reasons. Within the scope of primary health care services, it is recommended that midwives and nurses investigate the genital hygiene behaviors of women both by planning home visits and during women's visits to health institutions, and by providing accurate information on reproductive health, genital hygiene, sexually transmitted diseases and family planning with brochures or training booklets.

Table 4. Comparison of GHBI mean scores of participants according to certain obstetric and gynecological specifications and vaginal discharge situation (n=408)

Vaginal discharge condition		n	%	GHBI (mean ± SD)	t	p-value
Distinguishing normality of vaginal discharge	Yes	283	69.4	82.55±10.02	12.619	0.001
	No	125	30.6	68.48±10.54		
Use of perfume, deodorant, soap to prevent vaginal discharge	Yes	52	12.7	74.84±9.92	-2.184	0.03
	No	356	87.3	78.74±12.28		
In abnormal vaginal discharge, the vagina should be kept dry	Yes	220	53.9	79.47±10.06	2.233	0.03
	No	188	46.1	76.80±13.94		
Abnormal vaginal discharge	Yes	191	46.8	79.51±10.27	1.940	0.05
	No	217	53.2	77.19±13.31		
Past sexually transmitted disease status	Yes	34	8.3	83.79±1.46	2.824	0.005
	No	374	91.7	77.74±12.09		
Vaginal douche application status	Yes	146	35.8	76.11±11.53	-2.679	0.007
	No	262	64.2	79.43±12.21		

P<0.05; t: Independent t-test, SD: Standard deviation, GHBI: Genital Hygiene Behaviors Inventory

MAIN POINTS

- About half of the women participating in this study complained of abnormal vaginal discharge.
- About one-third of women used traditional methods to treat their abnormal vaginal discharge.
- Vaginal douche is a common practice among women.
- Increasing age and educational status of women were associated with an increase in the scores they obtained from the "GHBI".
- In this study, the GHBI mean scores of the women participating was determined to be high, and their Genital Hygiene Behaviors were determined to be at the desired level.

ETHICS

Ethics Committee Approval: Approval from the Near East University Hospital Scientific Research Ethic Board was obtained before starting this research (approval number: 2021/1367).

Informed Consent: Written informed consent was obtained from the who participated in this study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: F.Y., B.M., D.N., Design: F.Y., B.M., D.N., Supervision: F.Y., B.M., D.N., Resources: F.Y., B.M., D.N., Materials: F.Y., B.M., D.N., Data Collection and/or Processing: F.Y., B.M., D.N., Analysis and/or Interpretation: F.Y., B.M., D.N., Writing: F.Y., B.M., D.N., Critical Review: F.Y., B.M., D.N.

DISCLOSURES

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

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

REFERENCES

1. Daşıkan Z, Kılıç B, Baytok C, Kocairi H, Kuzu S. The Genital Hygiene Practices of Women Who Have Genital Discharge Gynecology Outpatient Clinic. Gümüşhane University Journal of Health Sciences. 2015; 4(1): 113-24.
2. Koştu N, Taşçı Beydağ K. Genital Hygiene Practices of Women who Present to a Gynecology Clinic. Ataturk University School of Nursing Journal. 2009; 12(1): 66-71.
3. Karatay G, Özvarış B. Evaluation of applications regarding the genital Hygyene of women living in barrel houses within a region existing a health center. Journal of the School of Nursing. 2006; 10(1): 7-14.
4. Zincir H, Temel AB. IUD Insertion According to Special IUD Consultation Principle and The Relation Between Genital Hygiene Training and The Incidence of Vulvovaginal Infections. J Heal Sci. 2010; 19(1): 60-7.
5. Arslan Özkan İ, Kulakaç Ö. Genital Hygiene Behaviors of Female Prisoners. J Anatolia Nurs Heal Sci. 2011; 14(2): 31-8.
6. Hacialioğlu N, Nazik E, Kiliç M. A descriptive study of douching practices in Turkish women. Int J Nurs Pract 2009; 15(2): 57-64.
7. Ünsal A, Özyazıcıoğlu N, Sezgin S. Attitudes About Genital Hygiene of Individuals Living in One Town and Nine Villages East Black Sea Region. Anadolu Hemşirelik ve Sağlık Bilimleri Dergisi. 2010; 13(2): 12-9 (Turkish).
8. Yağmur Y. The Genital Hygiene Behaviors of the Females Aged 15-49 Living at the Firat Health Clinic Neighborhood in Malatya. TAF Prev Med Bull. 2007; 6(5): 325-30.
9. Gözüyeşil E. Investigation of genital hygiene behavior: An example of a slum area. Ortadogu Med J. 2020; 12(2): 186-93.
10. Özkan S, Demir Ü. Determining the effectiveness of the nurse in the diagnosis of vajinitis and finding out the factors causing the formation of vaginitis in the fertile age women between 15-49. Heal Soc. 2002; 12(4): 54-61.
11. Hotun Şahin N, Bilgiç D, Kızılkaya Beji N. Women's Health and Diseases for Nurses and Midwives. In: Reproductive System Infections. 1st ed. Istanbul: Nobel Medicine Bookstores; 2015.
12. Can Gürkan Ö, Arslan Özkan H. Reproductive system infections and sexually transmitted infections. In: Women's Health and Diseases for Nursing and Midwifery. 1st ed. Ankara: Academician Bookstore; 2019.
13. Yağmur Y, Ergin İO. Life Conditions and Genital Hygiene Practices In Seasonal Agricultural Women. J Int Soc Res. 2017; 10(51): 614-20.
14. Cangöl E, Tokuç B. The Evaluation of Genital Infections and Genital Hygiene Practices of Women Who Applied to Gynecology Polyclinic. Florance Nightingale Nurs J. 2013; 21(2): 85-91.
15. Kisa S, Taskin L. Behavioral risk factors that predispose women to vaginal infections in Türkiye. Pak J Med Sci [Internet]. 2010; 26(4): 800-4.
16. Ege E, Eryılmaz G. The Effect of Planned Education Given to the Women on Genital Hygiene Behaviours. Ataturk University School of Nursing Journal. 2006; 9(3): 8-16.
17. Kısa S, Taşkın L. Behavioral risk factors that predispose women to vaginal infections in Türkiye. Pakistan J Med Sci. 2010; 26(4): 80-4.
18. Dalbudak S, Bilgili N. GATA The genital hygiene behaviors of women who applied to the obstetrics and gynecology outpatient clinic and the effect of these behaviors on vaginal infection. Gulhane Medical Journal. 2013; 55: 281-7.
19. Yılmaz Y, Kahraman S. The knowledge about the adolescent girls' genitals and hygiene who live in Sanliurfa, applications and factors that affect. J Hum Sci. 2019; 16(3): 823-32.
20. Srivastava L. Reproductive Tract Infections among Women of Rural Community in Mewat, India. J Health Manag. 2010; 12(4): 519-38.
21. Okumuş F, Demirci N. Health-seeking behaviors of women living in rural areas in case of vaginal discharge. Anadolu Nursing and Health Science Journal. 2015; 18(3): 204-13.
22. Özcan H, Arık S, Esen ÜG, Aslan N. Perception of Vaginal Discharge of Young Women and Traditional Practices for Vaginal Discharge. Gumushane University Health Science Journal. 2020; 9(3): 272-9.
23. Darvishi M, Jahdi F, Hamzegardeshi Z, Goodarzi S, Vahedi M. The Comparison of vaginal cream of mixing yogurt, honey and clotrimazole on symptoms of vaginal candidiasis. Glob J Health Sci. 2015; 7(6): 108-16.
24. Felix TC, de Brito Röder DVD, Dos Santos Pedroso R. Alternative and complementary therapies for vulvovaginal candidiasis. Folia Microbiol (Praha). 2019; 64(2): 133-41.
25. Li C, Han H, Lee J, Lee M, Lee Y, Kim M. Knowledge, behaviors and prevalence of reproductive tract infections: a descriptive study on rural women in hunchun, china. Asian Nurs Res (Korean Soc Nurs Sci). 2010; 4(3): 122-9.
26. Çalışkan D. Should Traditional Intravaginal Application "Vaginal Shower, Lavage" Be Done? Shouldn›t it be done? TTB Journal of Continuing Medical Education. 2005; 14(1): 15-8.
27. Yilmaz N, Saracoglu P, Egilmez P. Feminine Hygiene Practice. Turkish Gynecology Journal. 2003; 2(10): 10-5.

28. Büşra Ç, Akar Y. Prevalence of Vaginitis Among 15-49 Age Women Registered in a Family Health Center. J Midwifery Heal Sci. 2020; 3(2): 100-14.

29. Çalışkan D, Çöl M, Akdur R, Yavuzdemir Ş, Yavuz Y. Study on Vaginal Shower Frequency and Effective Factors in Women aged 15-49 in the Park Health Center Region. Ankara University Faculty of Medicine Journal. 1996; 49(2): 73-80.

30. Erbil N, Bölükbaş N, Kocabaş E. Genital Hygiene Behaviors of Married Women and Determination of Affecting Factors. In: Reproductive Health and Family Planning Congress Book. 2009.p.199-20.

RESEARCH ARTICLE

CYPRUS
JOURNAL OF MEDICAL SCIENCES

DOI: 10.4274/cjms.2022.2022-2
Cyprus J Med Sci 2023;8(1):53-59

Do Ectopic Pregnancy Treatment Choices Affect the Risk of COVID-19 Transmission?

Nilüfer Akgün[1], Mustafa Abdullah Demirel[1], Eylem Ünlübilgin[1], Mesut Akyol[2], Salim Erkaya[1], Yaprak Engin-Üstün[1]

[1]Clinic of Obstetrics and Gynecology, University of Health Sciences Türkiye, Ankara Etlik Zübeyde Hanım Training and Research Hospital, Ankara, Türkiye
[2]Department of Statistics, Yıldırım Beyazıt University Faculty of Medicine, Ankara, Türkiye

Abstract

BACKGROUND/AIMS: To investigate the effects of treatment choices in ectopic pregnancy (EP), either surgery or single-dose methotrexate approaches, to see whether it leads to the incidence of coronavirus disease-19 (COVID-19) disease after discharge, as well as to determine any correlations in changes in cell blood counts (CBC) with the progress of COVID-19 infections.

MATERIALS AND METHODS: This study was conducted with 95 patients who were diagnosed as having EP at University of Health Sciences Türkiye, Etlik Zübeyde Hanım Gynaecology Training and Research Hospital. COVID-19 swabs were taken from the patients with EP before hospitalization. Demographic parameters, CBCs, post-discharge COVID-19 disease occurrence rates, and infection progress were evaluated.

RESULTS: Ninety-five patients with tubal EP were separated into two groups; 20 patients who underwent surgical intervention (Group 1), and 75 patients who underwent single-dose methotrexate treatment (Group 2). There was no difference between the groups in terms of their demographic characteristics ($p>0.05$). The mass size was measured using transvaginal ultrasound and the mean mass diameter in Group 2 was significantly smaller than in Group 1 ($Z=4.123$; $p<0.001$). The hospital stay of Group 2 was longer than in group 1 ($Z=4.451$; $p<0.001$). No patients were infected with COVID-19 before the hospitalization period; however, three (3.1%) patients were COVID-19-positive in the 90-day post-treatment period, two of whom had surgical treatment, and the other who received medical treatment. The patient who had medical treatment went into self-isolation and used favipiravir medication at home and completely recovered. By contrast, the patients who had surgical treatment needed hospital care. The mean neutrophil-lymphocyte ratio (NLR) values were significantly higher for those patients with EP in Group 1 compared with Group 2, both pre-treatment and post-treatment ($Z=4.108$, $p<0.001$; $Z=4.783$; $p<0.001$). Also, significant differences were detected between the groups regarding their haemoglobin levels, and white blood cell, platelet, and neutrophil counts ($p=0.005$ $p=0.001$, $p=0.008$, and $p=0.001$ respectively).

CONCLUSION: Variances in EP treatment modalities and durations of hospitalization days did not increase the transmission of disease or mortality scores. It was concluded that methotrexate treatment could be chosen as the first-line treatment for ectopic pregnancy during the COVID-19 pandemic for patients. However, healthcare professionals must be aware that medical or surgical treatment approaches for patients with EP may change the NLR, which is an independent prognostic factor in COVID-19.

Keywords: COVID-19, ectopic pregnancy, methotrexate treatment, neutrophil-lymphocyte ratio

To cite this article: Akgün N, Demirel MA, Ünlübilgin E, Akyol M, Erkaya S, Engin-Üstün Y. Do Ectopic Pregnancy Treatment Choices Affect the Risk of COVID-19 Transmission?. Cyprus J Med Sci 2023;8(1):53-59

ORCID IDs of the authors: N.A. 0000-0003-0414-9470; M.A.D. 0000-0003-2654-4922; E.Ü. 0000-0002-1529-2523; M.A. 0000-0003-1449-7874; S.E. 0000-0002-0215-8552; Y.E.Ü. 0000-0002-1011-3848

Address for Correspondence: Nilüfer Akgün
E-mail: niluferakgun80@hotmail.com
ORCID ID: orcid.org/000-0003-0414-9470

Received: 31.01.2022
Accepted: 17.08.2022

INTRODUCTION

In ectopic pregnancy (EP), the fertilized ovum most frequently implants in the fallopian tubes (95%). The incidence of EP is 1% in patients aged 24-44 years.[1] Approaches for diagnosis [serum human chorionic gonadotropin (β-hCG) sensitive values, high-resolution ultrasounds (USG)] and treatment (experienced surgeons frequently use laparoscopic approaches) in EP are crucial for improving maternal morbidity and mortality. Treatment options for stable patients include expectant management, surgical treatment, and methotrexate (single or multiple-dose) administration. Methotrexate treatment is a non-invasive treatment approach used in patients with stable hemodynamics and smaller mass sizes. It is effective in the early stages of pregnancy as a folic acid antagonist, but it can cause anomalies immunodeficiency.[2] After administration, approximately 92% of methotrexate is excreted in the urine within the first 24 hours and 1-2% remains in the body.[3] The obstetrician's treatment approach selection can change because the benefits and harms of methotrexate must be balanced.

The coronavirus disease-2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was first identified in January, 2020 in a group of patients who developed severe acute respiratory symptoms (fever, cough, shortness of breath) in Wuhan Province, China. Although approximately 81% of patients with COVID-19 have mild symptoms such as a dry cough and fever, which do not require hospitalization, 14% have severe symptoms, and 5% have rapid-developing acute respiratory distress syndrome.[4] On March 11th, 2020, the first COVID-19 case in our country, Türkiye, was seen. As of September 26th, 2021, the mortality rate was almost 0.89%.[5] Governments had to focus on and change hospitalization advice for gynaecologic surgeries because of the intensity levels in hospitals. Governments had to change their recommendations for hospitalization for gynaecologic surgeries because of the COVID-19-related intensity levels of hospitals. Due to the hospital occupancy of patients during the COVID-19 pandemic, appropriate methods were determined in surgical indications to prevent mortality due to emergency surgeries and to manage oncological patients.[6] Although most patients exhibit mild symptoms, some patients have a worse prognosis[7] and studies focused on prognostic factors such as laboratory data.[8]

Severe inflammatory responses can provide a weak adaptive immune response, resulting in imbalances in the immune response. Therefore, circulating biomarkers which can represent inflammation and immune status may predict the likelihood and prognosis of COVID-19.[9] The peripheral white blood cell (WBC) count, neutrophil-to-lymphocyte ratio (NLR), and platelet-to-lymphocyte ratio are commonly investigated indicators of the systematic inflammatory response.[9] Also, NLR can be used as a prognostic marker for COVID-19 given the significant difference in NLR between those patients who died and those who recovered from COVID-19.[10]

Given the rapid spread and severe damage of COVID-19, it is important to improve and enrich clinical diagnostic and therapeutic research. Using methotrexate, which is an immunosuppressive agent, in the treatment of EP, has provoked the notion of the possibility of increased COVID-19 infections and transmission. In this study, our primary aim was to determine the effects of primary methotrexate or surgical treatment choices on patients with EP regarding the probability of having COVID-19 after treatment and its clinical severity, along with alterations in their descriptive laboratory parameters.

MATERIAL AND METHODS

This retrospective single-cohort study included patients who were admitted to University of Health Sciences Türkiye, Ankara Etlik Zübeyde Hanım Training and Research Hospital early pregnancy department with a diagnosis of EP and seronegative COVID-19 results between June 1st, 2020, and January 1st, 2021. Approval from the Ankara Etlik Zübeyde Hanım Training and Research Hospital Local Ethics Committee (approval number: 03, date: 26.02.2021) was received.

Selection and Description of Participants

This study included 95 patients with tubal EP who received either methotrexate or surgical treatment. All women were followed up through transvaginal USG and measurements of beta-hCG (β-hCG) and those with a diagnosis of EP received medical or surgical treatment. Maternal age, parity, gravidity, mode of conception (natural or assisted reproduction method), gestational age, smoking habits, ultrasonographic measurements, and previous EP history were recorded. We compared the effects of single-dose methotrexate treatment with surgical treatment for tubal EP with regards to the length of hospital stay, and CBC parameters. In addition, the effect of treatment types on whether patients had COVID-19 after discharge was evaluated retrospectively.

Methotrexate treatment was given to hemodynamically stable patients, if there were no contraindications for methotrexate administration, with β-hCG levels of <6,000 IU/L at the time of diagnosis, tubal EP diagnosis with an ectopic pregnancy mass size <45 mm, no history of tubal surgery, and no foetal heartbeat. Patients with a decline of 15% in β-hCG levels between days 4 and 7 were accepted as having a positive response and monitored on an outpatient basis weekly until their β-hCG levels were below 5 mU/mL. Surgery was performed on those patients who did not meet these criteria, whose hemodynamics deteriorated due to tubal rupture during follow-up, had a 15% decrease in β-hCG between the fourth and seventh day, and those who had hepatic or renal disease and did not respond to medical treatment. According to these criteria, the patients were separated into two groups, those who had success with methotrexate treatment, and those who required surgery. In addition, CBCs were performed after both methotrexate treatment and surgical treatment. All hematologic laboratory parameters were compared between the groups.

Technical Information

Methotrexate reversibly inhibits the enzyme dihydrofolate reductase. Further pregnancy attempts are allowed 3 months after methotrexate treatment. Patients with EP between January 1st, 2020 and January 1st, 2021 were included in this study. All patients were followed up until April 1st, 2021 (3 months later due to the continuation of the effects of methotrexate) to determine whether they had had COVID-19. The patients were screened retrospectively for COVID-19 test results via the report system after hospital discharge. After discharge from the hospital, COVID-19 test results were scanned via the Hospital Information Management System (HIMS) or by phone to inquire whether the patients had had COVID-19. Those patients whose personal data could not be accessed, who had positive COVID-19 test results when hospitalized, with systemic diseases which were contraindicated for methotrexate treatment and multiple-dose methotrexate regimes, and those who required methotrexate treatment after surgical treatment because of treatment failure were not included in this study.

Statistical Analysis

The data obtained within the scope of this research were statistically analysed using the IBM SPSS Statistics program Ver. 23.0 software package (IBM Corp. IBM SPSS Statistics for Windows, Armonk, NY: IBM Corp.). The normality of the data distribution was examined graphically and the Shapiro-Wilk test. Frequency (n) and percentage (%) values were calculated in the definition of categorical variables related to the medical history, and median [interquartile range (IQR)] values were used for the measurement variables. The Mann-Whitney test was used to compare non-normally distributed continuous variables and the chi-squared test was used for categorical variables. Pre-treatment and post-treatment comparisons in each group were made using the Wilcoxon signed-rank test. The level of significance was accepted as $p<0.05$ in all analyses.

RESULTS

This study included 95 patients with tubal EP who were divided into two groups: Group 1, those treated with single-dose 50 mg/m^2 intramuscular methotrexate therapy (n=75), and Group 2, patients who underwent surgery (n=20). The mean age of the patients was 31.8±6.0 (range, 19-46) years. Their mean BMI was 26.96±4.53 kg/m^2. Six (6.3%) patients had chronic diseases (asthma n=2, type 2 diabetes mellitus n=2, epilepsy n=1, multiple sclerosis n=1). Twenty-eight (29.5%) patients had undergone previous surgery. The most common surgery was caesarean section (22.1%). Twenty-two patients were smokers and none had allergies. None of the patients had received in vitro fertilization treatment before. Twenty-seven (28.4%) patients were nulliparous. Eight (8.4%) patients had had elective abortions (Table 1). In our study, the median overall hospital length of stay was 5 (IQR 3) (min: 3 max: 21) days. A statistically significant difference was detected between the groups in terms of hospital stay ($p<0.01$).

Most tubal masses were visualized in the right adnexal region (n=51; 60%); the left adnexal region was n=34, 40%. Ten patients with EP tubal masses were not detected using transvaginal USG. The mean tubal mass diameter was 21 (IQR=13) (range, 6-40) mm. Free abdominal fluid was visualized in 35 patients. The mean volume of abdominal free fluid was 41 (IQR=29) (range, 16-140) mm^3. The number of patients who had ruptured ectopic pregnancies was 22 (23.2%). The ASA PS classification of all 26 (27.4%) patients who underwent surgery was ASA II. Ten (50%) of these patients underwent left salpingectomy and the other 10 (50%) underwent right salpingectomy.

The masses, which were measured using transvaginal USG diameter, in Group 1 were significantly smaller than in Group 2 ($Z=4.123$; $p<0.001$). The hospital stay in Group 1 was longer than in Group 2 ($Z=4.451$; $p<0.001$). There was no difference between the groups in terms of the other demographic characteristics ($p>0.05$) (Table 2). Three (3.1%) patients had COVID-19 positive tests after their treatments. Table 3 shows which types of treatment were received by those patients who became COVID-19 positive, the progress of their infection, their survival rates, and their treatment modalities. The mean NLR values were significantly higher for those patients with EP in Group 2 compared with Group 1 both before and post-treatment ($Z=4.108$, $p<0.001$; $Z=4.783$; $p<0.001$). Although NLR decreased after treatment in

Table 1. Demographic features and obstetric histories of patients

	Surgery (n=20)	**Methotrexate (n=75)**	**Group comp.**
	Median (IQR)	**Median (IQR)**	**Z; p**
Age (year)[a]	31.00 (8.75)	32.50 (9.00)	Z:0.347; p=0.728
BMI (kg/m^2)[a]	28.58 (6.07)	26.50 (5.68)	Z=1.753; p=0.080
	n (%)	**n (%)**	**Z; p**
Chronical disease[b]	1 (5.0)	5 (6.7)	Z=0.78; p=0.780
Previous surgery[b]	2 (10.0)	26 (34.7)	Z=4.622; p=**0.032**
Smoker[b]	5 (25.0)	17 (22.7)	Z=0.048; p=0.826
Gravida[b]			
1	3 (15.0)	15 (17.6)	Z=0.257; p=0.612
2+	17 (85.0)	60 (82.4)	
Parity[b]			
0	5 (25.0)	21 (28)	Z=0.071; p=0.789
1+	15 (75.0)	54 (72.0)	
Before ectopic pregnancy[b]			
0	17 (85.0)	65 (86.7)	Z=0.037; p=0.847
1+	3 (15.0)	10 (13.3)	
Adnexal mass*	**Median (IQR)**	**Median (IQR)**	**Z; p**
Average size (mm)[a]	28.00 (17.00)	18.00 (10.25)	Z=4.123 p<**0.001**
Ectopic pregnancy site[b]	n (%)	n (%)	
Right	12 (60.0)	NA	
Left	8 (40.0)	NA	
Hospitalization	**Median (IQR)**	**Median (IQR)**	**Z, p**
Hospitalizations (day)	4.50 (1.75)	7.00 (3.00)	Z=4.451; p<**0.001**

[a]: Mann-Whitney test, [b]: Chi-squared test, BMI: Body mass index, IQR: Interquartile range

both groups, it did not change significantly during treatment (p=0.108 and p=0.431, respectively). Also, significant differences were detected between the groups regarding their haemoglobin levels, and WBC, platelet, and neutrophil counts (p=0.005, p=0.001, p=0.008, and p=0.001 respectively).

DISCUSSION

To the best of our knowledge, this study is the first to compare treatment options for EP, the probability of contracting the disease according to changes in blood parameters, and the length of hospital stay, and to calculate the COVID-19 positivity rates. The post-treatment COVID-19 rate was 3.1% for our patients with EP (two patients underwent surgery, and another who had methotrexate treatment). The methotrexate-treated patient went into self-isolation, only used favipiravir medication at home, and completely recovered. By contrast, the surgically treated patients required hospitalization. NLR values were significantly higher in those patients with EP in Group 2 compared with Group 1, both before and after treatment.

Medical treatment with methotrexate is used as an alternative to surgical treatment because of its potency, cost-effectiveness, and safer characteristics in EP.11 Methotrexate reversibly inhibits the enzyme dihydrofolate reductase, preventing the conversion of purine and pyrimidine synthesis, inhibiting cell growth.[12] It is hypothesized that patients who receive methotrexate treatment may be more prone to infection during the pandemic owing to their immunocompromised condition. Patients who use this treatment often need hospital monitoring. It may alter hematologic and biochemical parameters, and

Table 2. Biochemical parameters changes for the treatment modalities

Parameters	Surgery treatment (group 1) (n=20)			Methotrexate medication (group 2) (n=75)			Group 1 vs. group 2	
	Before treatment	After treatment	Before vs. after treatment	Before treatment	After treatment	Before vs. after treatment[a]	Before treatment[b]	After treatment[b]
Neutrophil to lymphocyte ratio (NLR)	5.87 (8.07)	4.7 (4.28)	Z=1.605; p=0.108	2.44 (1.53)	2.03 (1.41)	Z=0.787; p=0.431	Z=4.108; p<**0.001**	Z=4.783; p<**0.001**
Platelet to lymphocyte ratio (PLR)	167.92 (107.7)	116.89 (51.7)	Z=2.987; p=**0.003**	130.73 (61.83)	123.53 (62.26)	Z=0.158; p=0.874	Z=3.295; p<**0.001**	Z=0.529; p=0.596
Hb (gr/dL)	12.05 (2.43)	11.2 (2.3)	Z=1.755; p=0.079	12.5 (2.2)	12 (1.9)	Z=4.944; p<**0.001**	Z=1.845; p=0.065	Z=2.790; p=**0.005**
RDW (%)	13.3 (1.28)	13.3 (1.08)	Z=0.698; p=0.485	13.6 (1.7)	13.4 (1.7)	Z=3.039; p=**0.002**	Z=0.859; p=0.390	Z=0.324; p=0.746
MPV (fL)	9.6 (1.35)	9.65 (1.03)	Z=0.311; p=0.756	9.8 (1.6)	9.6 (1.4)	Z=1.514; p=0.130	Z=0.539; p=0.590	Z=0.005; p=0.996
WBC ($x10^9$/L)	11.675 (7.740)	11.675 (5.875)	Z=0.728; p=0.467	7.870 (3.090)	7.170 (2787.5)	Z=3.130; p=**0.002**	Z=4.802; p<**0.001**	Z=4.434; p<**0.001**
Lymphocyte (%)	1.520 (1.347)	1.925 (787.5)	Z=1.232; p=0.218	2.110 (750)	2.040 (800)	Z=1.240; p=093	Z=2.698; p=**0.007**	Z=1.269; p=0.204
Platelet ($x10^3$/L)	290.500 (79.000)	213.500 (71.500)	Z=3.734; p<**0.001**	273.000 (80.000)	256.500 (91.000)	Z=2.189; p=**0.029**	Z=0.854; p=0.393	Z=2657; p=**0.008**
Neutrophil (%)	8.470 (7082.5)	8.690 (6.835)	Z=0.243; p=0.808	5.030 (2.340)	4.460 (2.150)	Z=2.735; p=**0.006**	Z=4.364; p<**0.001**	Z=5.194; p<**0.001**

[a]: Wilcoxon signed rank test, [b]: Mann-Whitney test, RDW: Red distribution width, MPV: Mean platelet volume, WBC: White blood cell

Table 3. Patients characteristics who contracted COVID-19 after EG treatment

Patient	Age (years)	BMI (kg/m^2)	Smoking habits	Additional disease	Treatment type	Hospitalisation duration day	Time between COVID-19 diagnosis and treatment	Intensive care unit admission	Medical treatment for COVID-19	Progress	Recovery time (days)
1	30	25.9	No	No	Surgery	10	60	Yes	Favipiravir enoxaparine sodium	Complete recovery	18
2	34	28.5	No	No	Methotrexate	0	10	No	Favipiravir enoxaparine sodium	Complete recovery	12
3	35	28.6	No	No	Surgery	5	56	No	Favipiravir enoxaparine sodium	Complete recovery	10

BMI: Body mass index, COVID-19: Coronavirus disease-19

hence they may be at risk of COVID-19. Some studies which compared the clinical and laboratory components of methotrexate and surgical treatments showed that NLR was significantly increased in the surgical group.[13,14] Raised NLR is a prognostic marker for severe COVID-19, indicating immediate admission to the intensive care unit (ICU).[15] A meta-analysis of 15 studies including 1,653 patients with severe COVID-19 and non-survivor patients showed increased ICU admittance rates of RR 2.74 (95% CI: 0.98-7.66) compared with those patients with non-severe COVID-19 and those patients who recovered.[16] Yang et al.[9] also showed that NLR was an independent prognostic factor in COVID-19. According to Eslamijouybari et al.[10], NLR used as a prognostic marker for COVID-19 showed that an increase in NLR could be a marker for those who died from COVID-19 when blood analyses were compared between those who died and those who recovered from COVID-19. It may be the result of the discharge of numerous cytokines which cause tissue and/or organ damage; this situation reduces cellular immunity and alters the ability to contract COVID-19 and prevents disease progression.[9] In our study, the incidence of COVID-19 was 3.1% (n=3 patients). Two patients had surgical treatment and the other had medical treatment. The mean NLR values were significantly higher for those patients with EP in the surgical group compared with the methotrexate group both before and after treatment (Z=4.108, p<0.001; Z=4.783; p<0.001). None of these 3 patients were smokers or had additional disease. The patient who had medical treatment went into self-isolation, took Favipiravir medication at home, and completely recovered. By contrast, both surgically treated patients needed hospitalization. However, no patients died and there was no ICU need. The EG patients who were diagnosed with COVID-19 after surgical and medical treatment recovered completely and their medications were regulated to Favipiravir and Enoxaparin sodium. COVID-19 polymerase chain reaction (PCR) positivity was identified postoperatively on the 56th and 60th days after treatment. Since it is known that the incubation period of COVID-19 infection is approximately two weeks,[17] these infections diagnosed more than 30 days after surgery may be a community-acquired infection.

In addition, NLR was considered a usable prognostic factor when deciding on the most suitable EP treatment. Kan et al.[17] reported that patients with EP with tubal rupture had higher NLR than those without rupture, and they suggested that NLR could assist obstetricians in choosing a treatment approach. Also, in an investigation of 78 patients with EP with methotrexate treatment success and 37 with treatment failure, higher NLR was observed in those patients who had methotrexate treatment success. This study indicated that NLR was a predictor of methotrexate treatment efficacy.[18] However, in our study, NLR decreased after treatment in both groups, and did not change significantly during treatment (p=0.108 and p=0.431, respectively). Also, a significant difference was identified between the groups regarding haemoglobin levels, and WBC, platelet, and neutrophil counts.

Another problem in the treatment of EP during the COVID-19 pandemic is hospitalization. Thousands of patients worldwide have had difficulty accessing hospitals due to the high occupancy rates of hospitals and ICUs, leading to a decrease in elective procedures and outpatient visits.[19] With the delays in diagnosis of EP, emergency surgical procedures have increased, causing increased morbidity and mortality as an additional adverse effect of the COVID-19 pandemic. In their retrospective study, Casadio et al.[20] showed an increased proportion of ruptured tubal EPs requiring emergency surgical interventions during the COVID-19 lockdown period. Furthermore, Anteby et al.[21] reported that, in their tertiary medical centre in Israel, although the number of admissions to the emergency department considered as suspected extrauterine pregnancies did not change, the rate of ruptured extrauterine pregnancies was significantly higher during the COVID-19 pandemic (odds ratio: 2.40, 1.27-4.54). Despite there being no government restrictions in terms of emergency department presentations during the pandemic around the world, patients with EP presented later with significantly increased blood loss and were symptomatic.[22] The author explained this as being due to postponements in presenting to emergency departments due to a fear of socializing and/or overburdening healthcare systems. Also, in agreement with other studies,[23,24] significantly increased emergency surgical procedures were reportedly caused due to interruptions in routine scans and later presentations during the COVID-19 pandemic.

Another condition which changes treatment management of EP is hospital stay. Hospitalization days are accepted as relevant risk factor for COVID-19 infections because of the nature of the disease and the human-to-human contamination risk for droplet transmission.[25] A single institution reported that the hospital-acquired transmission rate was almost 6.7% in patients with cancer.[25,26] In our study, the median hospital stay was 5 (IQR 3) days. Furthermore, there was a statistically significant difference detected between the groups, with the methotrexate treatment group having a longer hospital stay (p<0.01). The underlying longer stay using immunosuppressive treatments can be explained by patients requiring at least four days of follow-up until a decline is observed in β-hCG levels in our hospital. However, in our study, hospitalization did not increase the rate of COVID-19 illness. This may be because patients had adapted to the social isolation rules, the restrictions on visitors, and compliance with the guidelines during hospitalization.

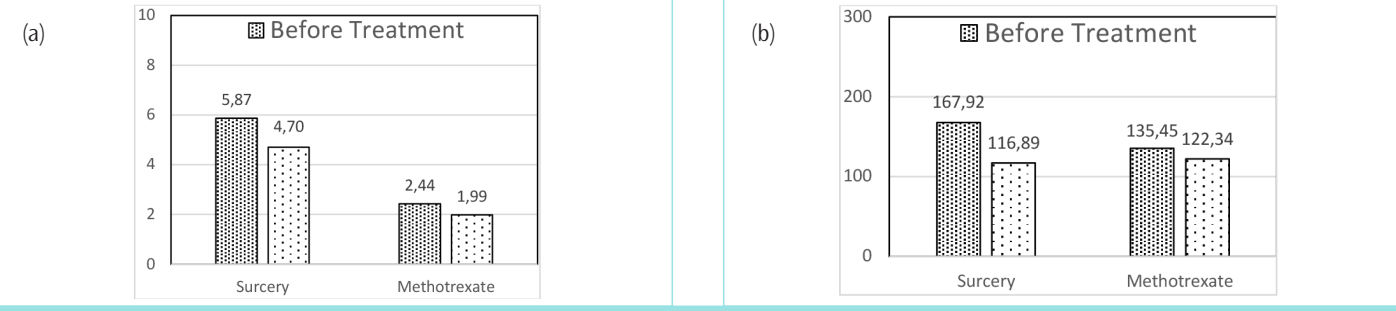


Figure 1. Relationship between NLR and PLR levels before and after single-dose methotrexate treatment and surgery. (a) Median NLR ratio in groups (before and after treatment), (b) median PLR ratio in groups (before and after treatment)

NLR: Neutrophil-to-lymphocyte ratio, PLR: Platelet-to-lymphocyte ratio.

Study Limitations

There are several notable limitations to this paper. First, the data were obtained from a single clinical trial centre, not from multiple centres. Secondly, the experimental data are limited and the data analyses are retrospective from the patient records. In addition, only three patients developed COVID-19, which may differ from the results of other research. Also, pregnant patients are a special group regarding COVID-19; they may be more attentive to social distancing in order to protect themselves during the COVID-19 pandemic. Social distancing may have an impact on the volume and distribution of illness and the assessment of exposure situations for COVID-19. Also, several other factors such as whole-family characteristics may be important, for example, families of more than five people living together at home, the demographic characteristics at home, and asymptomatic patients may have been included in the population. Screening tests for COVID-19 in our hospital were performed using PCR analyses as soon as possible upon hospitalization.

CONCLUSION

Methotrexate treatment may be chosen as the first-line treatment for EP during the COVID-19 pandemic. Also, it may be useful during other infectious diseases apart from the COVID-19 pandemic. The mean NLR values were significantly higher among those patients with EP who underwent surgery compared with those who had methotrexate treatment both before and post-treatment. Although NLR decreased after treatment in both groups, it did not change significantly during treatment. The length of hospital stay and immunosuppressive treatment procedure did not negatively affect EP treatment. Healthcare professionals must be aware that medical or surgical treatment approaches in patients with EP may change NLR, which is an independent prognostic factor for COVID-19.

MAIN POINTS

- Ectopic pregnancy treatments include expectant management, surgical treatment, and methotrexate (single or multiple-dose) administration. Methotrexate treatment is a non-invasive treatment approach and it is used in patients as the first line treatment but it can cause immunodeficiency.
- The COVID-19 disease has an effect on imbalances in the immune response. Blood laboratory parameters are commonly investigated indicators of this systematic inflammatory response. NLR can be used as a prognostic marker for COVID-19.
- We discussed the ectopic pregnancy treatment choices, either surgery or single doses of methotrexate approaches, and hospitalization days effects on the risk of COVID-19 transmission. Secondly, we aimed to investigate whether the treatment approaches have an effect on the clinical severity after treatment, and compared correlations with alter cell blood counts.

ETHICS

Ethics Committee Approval: Approval from the University of Health Sciences Türkiye, Ankara Etlik Zübeyde Hanım Training and Research Hospital Local Ethics Committee (approval number: 03, date: 26.02.2021) was received.

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Data Collection and/or Processing: N.A., M.D., M.A., Literature Search: Y.Ü., E.Ü., Writing: N.A., M.D., E.Ü., S.E., Y.Ü.

DISCLOSURES

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

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

REFERENCES

1. Bouyer J, Coste J, Fernandez H, Pouly JL, Job-Spira N. Sites of ectopic pregnancy: a 10 year population-based study of 1800 cases. Hum Reprod. 2002; 17(12): 3224-30.
2. Medicine PCotASfR. Medical treatment of ectopic pregnancy: a committee opinion. Fertil Steril. 2013; 100(3): 638-44.
3. Chan ES, Cronstein BN. Molecular action of methotrexate in inflammatory diseases. Arthritis Res. 2002; 4(4): 1-8.
4. Wu Z, McGoogan JM. Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: summary of a report of 72 314 cases from the Chinese Center for Disease Control and Prevention. JAMA. 2020; 323(13): 1239-42.
5. (https://covid19.tubitak.gov.tr). 2021
6. El Boghdady M, Ewalds-Kvist BM. Laparoscopic Surgery and the debate on its safety during COVID-19 pandemic: A systematic review of recommendations. Surgeon. 2021; 19(2): e29-e39.
7. Li Q, Guan X, Wu P, Wang X, Zhou L, Tong Y, et al. Early transmission dynamics in Wuhan, China, of novel coronavirus-infected pneumonia. N Engl J Med. 2020; 382(13): 1199-207.
8. Jang JG, Hur J, Choi EY, Hong KS, Lee W, Ahn JH. Prognostic factors for severe coronavirus disease 2019 in Daegu, Korea. J Korean Med Sci. 2020; 35(23): e209.
9. Yang AP, Liu JP, Tao WQ, Li HM. The diagnostic and predictive role of NLR, d-NLR and PLR in COVID-19 patients. Int Immunopharmacol. 2020; 84: 106504.
10. Eslamijouybari M, Heydari K, Maleki I, Moosazadeh M, Hedayatizadeh-Omran A, Vahedi L, et al. Neutrophil-to-Lymphocyte and Platelet-to-Lymphocyte Ratios in COVID-19 Patients and Control Group and Relationship with Disease Prognosis. Caspian Journal of Internal Medicine. 2020; 11(Suppl 1): 531.
11. Barnhart KT, Gosman G, Ashby R, Sammel M. The medical management of ectopic pregnancy: a meta-analysis comparing "single dose" and "multidose" regimens. Obstet Gynecol. 2003; 101(4): 778-84.
12. Tian H, Cronstein BN. Understanding the mechanisms of action of methotrexate. Bull NYU Hosp Jt Dis. 2007; 65(3): 168-73.
13. Akkaya H, Uysal G. Can hematologic parameters predict treatment of ectopic pregnancy? Pak J Med Sci. 2017; 33(4): 937.
14. Kanmaz AG, Inan AH, Beyan E, Budak A. Role of various complete blood count parameters in predicting the success of single-dose Methotrexate in treating ectopic pregnancy. Pak J Med Sci. 2018; 34(5): 1132.
15. Liu J, Liu Y, Xiang P, Pu L, Xiong H, Li C, et al. Neutrophil-to-lymphocyte ratio predicts severe illness patients with 2019 novel coronavirus in the early stage. MedRxiv. 2020.
16. Simadibrata DM, Calvin J, Wijaya AD, Ibrahim NAA. Neutrophil-to-lymphocyte ratio on admission to predict the severity and mortality of COVID-19 patients: a meta-analysis. Am J Emerg Med. 2021; 42: 60-9.

17. Kan Ö, Gemici A, Alkilic A, Cetindag EN, Cakir C, Dur R, et al. The effect of preoperative neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio on predicting rupture risk in tubal ectopic pregnancies. Gynecologic and obstetric investigation. 2019; 84(4): 378-82.

18. Cekmez Y, Göçmen A, Sanlıkan F, Türkmen S. Role of mean platelet volume and neutrophil/lymphocyte ratio to predict single-dose methotrexate treatment success in tubal ectopic pregnancy. Clin Exp Obstet Gynecol. 2016; 43(4): 509-11.

19. Søreide K, Hallet J, Matthews JB, Schnitzbauer AA, Line PD, Lai P, et al. Immediate and long-term impact of the COVID-19 pandemic on delivery of surgical services. Journal of British Surgery. 2020; 107(10): 1250-61.

20. Casadio P, Youssef A, Arena A, Gamal N, Pilu G, Seracchioli R. Increased rate of ruptured ectopic pregnancy in the COVID-19 pandemic: an analysis from the North of Italy. Ultrasound in Obstetrics & Gynecology. 2020.

21. Anteby M, Van Mil L, Michaan N, Laskov I, Grisaru D. Effects of the COVID-19 pandemic on timely care for extrauterine pregnancies: A retrospective analysis. Lancet Reg Health Eur. 2021; 2: 100026.

22. Nina la Cour Freiesleben N, Nielsen HS. Timely care for extrauterine pregnancies during the COVID-19 pandemic is needed. Lancet Reg Health Eur. 2021; 2: 100037.

23. Orthopoulos G, Santone E, Izzo F, Tirabassi M, Pérez-Caraballo AM, Corriveau N, et al. Increasing incidence of complicated appendicitis during COVID-19 pandemic. Am J Surg. 2021; 221(5): 1056-60.

24. Fisher JC, Tomita SS, Ginsburg HB, Gordon A, Walker D, Kuenzler KA. Increase in pediatric perforated appendicitis in the New York City metropolitan region at the epicenter of the COVID-19 outbreak. Ann Surg. 2021; 273(3): 410.

25. Yu J, Ouyang W, Chua ML, Xie C. SARS-CoV-2 transmission in patients with cancer at a tertiary care hospital in Wuhan, China. JAMA Oncology. 2020; 6(7): 1108-10.

26. Ayhan A, Oz M, Ozkan NT, Aslan K, Altintas MI, Akilli H, et al. Perioperative SARS-CoV-2 infection among women undergoing major gynecologic cancer surgery in the COVID-19 era: A nationwide, cohort study from Türkiye. Gynecol Oncol. 2021; 160(2): 499-505.

RESEARCH ARTICLE

CYPRUS
JOURNAL OF MEDICAL SCIENCES

DOI: 10.4274/cjms.2022.2022-13
Cyprus J Med Sci 2023;8(1):60-65

Clinical and Pathological Features of Adrenal Myelolipoma and Myelolipomatous Metaplasia Cases in Our Hospital Over 13 Years

Sibel Şensu[1], Aylin Ege Gül[2], Sevinç Hallaç Keser[2], Yeşim Saliha Gürbüz[1], Mehmet Altıntaş[3], Cem Cahit Barışık[4], Nagehan Özdemir Barışık[2], Nusret Erdoğan[1]

[1]Clinic of Pathology, İstinye University Faculty of Medicine, İstanbul, Türkiye
[2]Clinic of Pathology, University of Health Sciences Türkiye, Kartal Dr. Lütfi Kırdar City Hospital, İstanbul, Türkiye
[3]Clinic of Surgical Medical Sciences, University of Health Sciences Türkiye, Kartal Dr. Lütfi Kırdar City Hospital, İstanbul, Türkiye
[4]Department of Radiology, Medipol University Hospital, İstanbul, Türkiye

Abstract

BACKGROUND/AIMS: The development and widespread use of abdominal imaging techniques has increased the incidence of unexpected adrenal tumors called adrenal incidentaloma. Adrenal myelolipomas are the second most common incidentalomas. Similar myelolipomatous morphology appears as a secondary degenerative change in other adrenal lesions and is called myelolipomatous metaplasia. This study investigated the adrenal entities of the last 13 years which had myelolipomatous components.

MATERIALS AND METHODS: In this retrospective observational study, cases diagnosed as adrenal myelolipoma or myelolipomatous metaplasia between January, 2009 and January, 2022 were re-examined regarding their age, gender, localization, lesion size, and secondary histopathological changes, accompanying pathological diagnoses as well as clinical and radiological data.

RESULTS: Eleven adrenal myelolipoma cases and 6 myelolipomatous metaplasia cases were detected. In myelolipomas, the mean age was 55.45 years, 73% were female and 82% were located on the right side. The lesions were encapsulated and their mean size was 7 cm. One case had a diagnosis of subclinical Cushing's syndrome and the others were hormonally inactive. Some cases were accompanied with hypertension (27%), type 2 diabetes (18%), and asthma (18%). All myelolipomatous metaplasias, which are non-encapsulated, were detected in adrenocortical adenomas. The mean age was 58 years; nonencapsulated and 67% were located on the right side with no gender predilection. Concomitant hypertension (50%), diabetes /33%, and asthma (33%) were frequent.

CONCLUSION: Adrenal myelolipoma and myelolipomatous metaplasia both contain adipose and myeloid components. Myelolipoma is a benign and encapsulated neoplasia which is usually detected incidentally. They frequently coexist with chronic diseases such as hypertension, diabetes and asthma.

Keywords: Adrenal, myelolipoma, myelolipomatous metaplasia

To cite this article: Şensu S, Ege Gül A, Hallaç Keser S, Gürbüz YS, Altıntaş M, Barışık CC, Özdemir Barışık N, Erdoğan N. Clinical and Pathological Features of Adrenal Myelolipoma and Myelolipomatous Metaplasia Cases in Our Hospital Over 13 Years. Cyprus J Med Sci 2023;8(1):60-65

ORCID IDs of the authors: S.Ş. 0000-0002-4607-780X; A.E.G. 0000-0003-0640-7218; S.H.K. 0000-0002-6987-0333; Y.S.G. 0000-0002-6189-2474; M.A. 0000-0002-1522-8687; C.C.B. 0000-0003-3540-6492; N.Ö.B. 0000-0002-6606-1848; N.E. 0000-0002-0333-8600.

Address for Correspondence: Sibel Şensu
E-mail: sibel.sensu@istinye.edu.tr
ORCID ID: orcid.org/0000-0002-4607-780X

Received: 01.04.2022
Accepted: 17.08.2022

INTRODUCTION

The development and widespread use of abdominal imaging techniques have increased the incidence of unexpected adrenal tumors called adrenal incidentaloma.[1,2] These cases, with a reported incidence of 10-15% today, are mostly biochemically nonfunctional, small, and benign. However, since one in ten cases can be functional or show malignancy, their detection and pathological diagnosis are important.[3-5] Adrenocortical adenomas constitute the majority (60-70%), while adrenal myelolipomas are the second most common adrenal incidentalomas (6-16%).[6-9]

Adrenal myelolipoma is a benign neoplasm composed of hemopoietic and adipose tissues.[9] Similar myelolipomatous morphology appears as a secondary degenerative change in other adrenal neoplastic and non-neoplastic entities, especially in adrenocortical adenoma, and is called myelolipomatous metaplasia.[10] Lipomatous tumors are rare among adrenal tumors. Although myelolipoma is the most common tumor in this group, a differential diagnosis should be made with myelolipomatous changes in lipomas, teratomas, angiomyolipomas, and adrenocortical lesions.

Myelolipoma was first described by Giercke in 1905 and named by Oberling in 1929.[5,11] The 2017 classification by the World Health Organization (WHO) included myelolipoma in the category of mesenchymal and stromal tumors of the adrenal cortex, with its current clinical and pathological details.[7] The 2022 World Health Organization bluebook covers a new separate chapter on myelolipoma.[12] According to one of the hypotheses on the origin of myelolipoma, it is thought that cancer, Cushing's disease, hypertension, diabetes, obesity, and/or stressful life conditions lead to necrosis or inflammation in the adrenal cortex tissue, causing prolonged endogenous adrenocorticotropic hormone stimulation and chronic adrenal stimulation. As a result, metaplasia may occur in reticuloendothelial cells, leading to the formation of adrenal myelolipoma.[1,7,13] According to another hypothesis, adipocytes develop from mesenchymal stem cells in the vessel walls in the stromal adipose tissue of the adrenal cortex under the influence of various stimuli. As they differentiate, these adipocytes become inflammatory, stimulating the adrenal cortex to release substances which recruit hematopoietic progenitors to the environment.[6] These also seem to be responsible for the development of myelolipomatous metaplasia.[10]

In this study, which was conducted with our data of the last 13 years, incidental entities of the adrenal gland which are adrenal myelolipoma with adipose and hematopoietic components and adrenocortical adenoma with myelolipomatous metaplasia were reviewed and discussed in the light of findings in the literature.

MATERIALS AND METHODS

Cases reported as adrenal myelolipoma and myelolipomatous metaplasia between January, 2009 and January, 2022 in the University of Health Sciences Türkiye, Kartal Dr. Lütfi Kırdar City Hospital, Clinic of Medical Pathology were included in this retrospective observational study. Paraffin blocks, hematoxylin-eosin-stained slides, pathology reports, laboratory data, and patient files were extracted from the archive and re-examined. Data regarding these cases such as age, gender, location, lesion size and secondary histopathological changes, pathological, clinical, and radiological diagnoses were recorded. For statistical analysis, using GraphPad Prism 8.0.1, descriptive data analysis (mean and percentage values) was performed for quantitative data and Independent t-test and chi-squared test were used for comparisons. A value of $p<0.05$ was considered statistically significant.

Ethics Committee Approval: University of Health Sciences Türkiye, Kartal Dr. Lütfi Kirdar City Hospital Clinical Research Ethical Committee (approval number: 2022/514/220/13, date: 22.02.2022).

Statistical Analysis

Statistical analysis between myelolipoma and myelolipomatous metaplasia cases did not reveal any statistically significant relationship between gender, age, or the location of the lesions ($p>0.05$).

RESULTS

Myelolipoma

Eleven cases diagnosed as adrenal myelolipoma and 6 cases of myelolipomatous metaplasia were included in this study. Adrenal myelolipoma cases were between 31 and 72 years old (mean: 55.45); 8 cases (72.7%) were female, and 3 cases (27.3%) were male; 9 of the cases (81.8%) were located in the right and 2 (18.2%) in the left adrenal; macroscopically, the cases were round or elliptical and encapsulated, with their sizes ranging from 0.6 cm to 11.5 cm (mean: 7.18 cm). There were yellow and red-brown areas in the sections (Figure 1). Microscopically, the yellow areas consisted of dense adipose tissue, and the red-brown areas consisted of erythroid, granulocytic, lymphoid, and megakaryocytic cells (Figure 2, 3). Hemorrhagic areas were seen in three of the cases with a hemorrhagic infarction in one of them. Other degenerative findings were dystrophic calcification and hyalinization. Concomitant adrenal cortex adenoma was found in two cases (18.2%). The patients presented with abdominal and back pain, abdominal swelling, and lower urinary tract complaints, and some cases were found incidentally during radiological examination (Table 1). One case had a diagnosis of subclinical Cushing's syndrome. The most common diseases accompanying the cases were type 2 diabetes (2 cases, 18.2%), asthma (2 cases, 18.2%), and hypertension (3 cases, 27.3%) (Table 1). Sarcomatoid urothelial carcinoma of the bladder was reported in one case and benign prostatic hypertrophy (BPH) in another case.

Figure 1. Macroscopy of myelolipoma.

Myelolipomatous Metaplasia

Six myelolipomatous metaplasia cases were detected and all of the cases were located in adrenal cortex adenomas. The patients were between 50-76 years of age (mean: 58); three (50%) were male and three were female. Four of the cases (66.7%) were located on the right, and two (33.3%) were on the left side. Microscopically, scattered fat and hematopoietic tissue elements were detected within the adenomas (Figure 4). Three (50%) cases had a history of hypertension, two (33.3%) had diabetes, and two (33.3%) had asthma (Table 2).

DISCUSSION

Adrenal lesions with lipomatous components are usually detected incidentally upon radiological examinations of patients for unrelated complaints or investigations for other tumors. These unexpected lesions are mostly removed due to a suspicion of malignancy.[10] Two of these lipomatous lesions which are myelolipoma and myelolipomatous metaplasia include hematopoietic elements in addition to adipose tissue. Although both have similar components, myelolipoma forms a well-circumscribed encapsulated/pseudocapsular mass, whereas myelolipomatous metaplasia is seen as diffuse, poorly circumscribed adipose and myeloid tissue foci within another tumor.[10] Although several authors report similar etiopathogenetic, clinical, and microscopic features, and suggest a developmental link between them, for now, they are considered two different entities.[10] According to some current cytogenetic studies, myelolipoma is a clonal tumor based on nonrandom X chromosome inactivation.[1,14] A study by Chang et al.[15] defined balanced f(3;21) (q25; p11) translocation in tumor cells in one case. New genetic studies in this field will illuminate the origin of these tumors and contribute to the management of these patients.

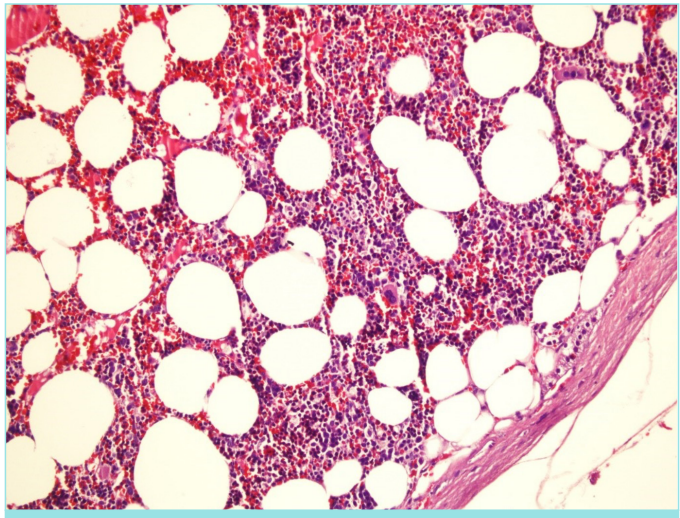


Figure 2. Histopathological appearance of myelolipoma composed of mature fat cells mixed with hematopoietic elements. (H&E, x200).

H&E: Hematoxylin & eosin

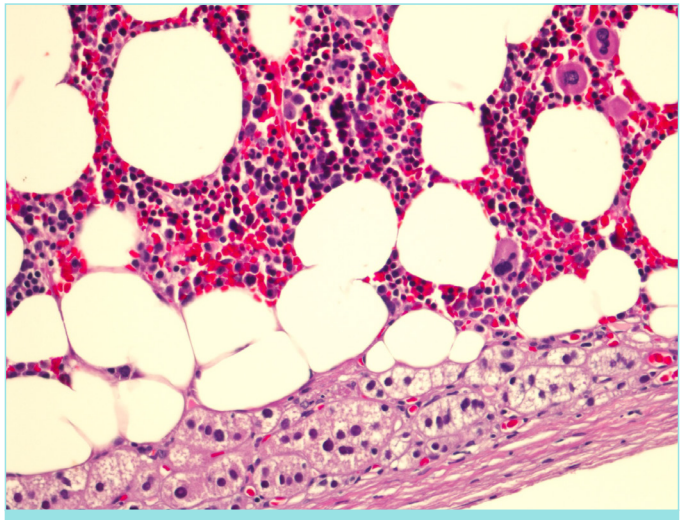


Figure 3. Histopathological appearance of myelolipoma composed of mature fat cells mixed with hematopoietic elements. (H&E, x400).

H&E: Hematoxylin & eosin

Clinical Features

Anbardar et al.[10] reported that adrenal myelolipomas occurred with equal frequency in both sexes while in a large series by Lin et al.[16], 58% of the patients were female. The tumors more frequently involved the right adrenal gland.[10] In our group, female predominance was more prominent (73%) and similarly, right adrenal predilection was higher (82%).

According to the literature, myelomatous change (metaplasia), which is considered a secondary degenerative change, had a female predilection; was mostly on the left adrenal, and the mean age was 52.5 years.[10] Contrarily, our cases were evenly distributed between both genders; mostly located in the right adrenal, and our mean age was higher.

The most common complaints of patients with myelolipoma were reported as abdominal, hypochondrial, and flank pain.[6] Spontaneous retroperitoneal hemorrhage was the most important complication, especially in large myelolipomas and could progress with local back, epigastrium, or flank pain, nausea, vomiting, hypotension, and anemia.[17] Our cases, apart from than the incidental ones, were also admitted to the hospital with abdominal pain and/or distention. In our series, all right-sided lesions were asymptomatic. This might be because, in these anatomical locations, the tumors are more distant from the neural plexuses and peritoneal structures which are sensitive to pressure and irritation.

Investigations on myelolipoma have shown that large and bilateral tumors develop due to external erythropoietin stimulation in hematological disorders such as hereditary spherocytosis, thalassemia intermedia, thalassemia major, or sickle cell anemia.[7,13] Although in our series, we had five large cases of myelolipoma ≥10 cm, these patients did not have a history of hematological disease. Hemorrhage is more common in large tumors, and probably, intratumoral hemorrhages cause tumors to enlarge. However, adrenal myelolipoma cases reported in the literature were generally small (<5 cm) and so far, only two giant cases, 31 cm/6,000 g and 34 cm/5,900 g have been reported.[1,18,19] The myelolipomas in our series were slightly larger than those reported in the literature, possibly related to the high prevalence of hemorrhagic areas.

According to the literature, up to 10% of adrenal myelolipoma cases had hormone hypersecretion.[2,5-7,17] Cases with Cushing's syndrome, primary hyperaldosteronism, pheochromocytoma, or congenital adrenal hyperplasia have been reported.[1,6,11,17] We had one case (0.6 cm)

with a diagnosis of subclinical Cushing's syndrome. Although hormonal hyperactivity was not detected in the majority of our cases, this relationship should be kept in mind in those patients with hormonal pathologies.

In our series, the frequency of hypertension, diabetes, and asthma was high in both myelolipoma and myelolipomatous metaplasia cases similar to the literature.[10] In addition, one of our patients with a myelolipoma was operated on for sarcomatoid urothelial carcinoma of the bladder. In our study, there were BPH patients in both groups. These histories support the hypothesis that chronic diseases, previous operations, and stress states might be associated with these entities.

According to the literature, 5-6% of myelolipoma cases were seen together with adrenocortical adenomas.[6,10,17] This coexistence rate was much higher (18%) in our series. We plan to observe and re-analyze this rate in larger groups.

Myelomatous change was usually observed in adrenocortical adenomas and more rarely, in adrenocortical carcinomas, pheochromocytomas, and ganglioneuromas.[6,20] Adrenal pathology was reported as adrenocortical adenoma in 70% of the cases examined by Anbardar et al.[10] In our group, all myelomatous metaplasia cases were associated with adrenocortical adenomas. The rest of the cases in Anbardar et al.'s[10]

Table 1. Clinicopathological features of myelolipoma cases

Case no	Age, gender	Location	Size	Patient complaints and history	Surgical procedure	Radiological and other findings
1	64, F	Right adrenal	11 cm	No complaints.	Right adrenalectomy	Diffuse hemorrhagic areas on microscopy.
2	47, F	Right adrenal	2.7 cm	Abdominal and back pain.	Right adrenalectomy	Association with adrenal cortex adenoma.
3	31, F	Right adrenal	10 cm	No complaints. The mass was detected in the right adrenal gland during follow-ups 2.5 years previously; no surgical intervention was made. A diagnosis of asthma.	Right adrenalectomy	Radiological preliminary diagnosis: Exophytic angiomyolipoma in the upper pole of the right kidney? Hamartoma tumor of right adrenal origin? Secondary degeneration (hemorrhagia and hyalinization).
4	55, F	Right adrenal	0.6 cm	A diagnosis of asthma, DM and HT. Upon routine endocrine follow-up, bilateral adrenal adenoma was detected. Diagnosis of subclinical Cushing's syndrome.	Laparoscopic right adrenalectomy	Abdominal US: appearance thought to be compatible with adenoma. Association with adrenal cortex adenoma.
5	52, F	Right adrenal	4.5 cm	No complaints.	Right adrenalectomy	Radiological diagnosis: Adenolipoma? Hemorrhagic areas.
6	72, F	Left adrenal	10 cm	Abdominal pain and swelling. History of HT, DM, CAD.	Left adrenalectomy	Whole abdomen and diffusion MRI: Adenoma? Diffuse hemorrhagic infarction, dystrophic calcification, significant hyalinization, and lymphocyte infiltration in capsule.
7	50, M	Right adrenal	8 cm	Lower urinary tract symptoms. History of HT.	Right laparoscopic adrenalectomy	Radiological diagnosis: Left adrenal lipoma? Angiomyolipoma?
8	56, M	Right adrenal	10 cm	Abdominal pain.	Right adrenalectomy	Radiological diagnosis: Adrenal adenoma?
9	46, F	Right adrenal	11.5 cm	No complaints.	Right adrenalectomy	Radiological diagnosis: Myelolipoma?
10	67, F	Right adrenal	4 cm	No complaints. History of surgery for sarcomatoid urothelial bladder carcinoma.	Right adrenalectomy	MRI: Heterogeneous mass lesion measuring approximately 3.6x4.4 cm with subcentimetric calcifications in the right adrenal gland, without F18-FDG uptake.
11	70, M	Left adrenal	4.5 cm	No complaints. History of BPH.	Left adrenalectomy	US preliminary diagnosis: Adenoma? Adrenal gland CT. Radiologic diagnosis: Neoplasm of the adrenal gland with unclear or unknown behavior.

BPH: Benign prostatic hypertrophy, CAD: Coronary artery disease, CT: Computerized tomography, DM: Diabetes mellitus, F: Female, FDG: Fluorodeoxyglucose, HT: Hypertension, M: Male, MRI: Magnetic resonance imaging, US: Ultrasound.

study were oncocytoma, cavernous hemangioma, adrenal hyperplasia, or adrenocortical neoplasms with unknown malignant potential. In our study, there was one patient who had undergone cholecystectomy and one patient with BPH in the myelolipomatous metaplasia group.

Radiological Features

Ninety percent of adrenal myelolipoma cases are detected by computed tomography (CT) and magnetic resonance imaging. A radiological diagnosis of at least 50% of them depends on observing an adrenal mass consisting of fat.[1,10] Lipomatous retroperitoneal lesions like lipoma, teratoma, angiomyolipoma, well-differentiated liposarcoma, adrenocortical adenoma/carcinoma, and extramedullary hematopoiesis are included in the radiological differential diagnosis.[8,11] Among our cases, the preliminary diagnoses in those who underwent radiological examination were angiomyolipoma developing exophytically from the upper pole of the right kidney; hamartomatous tumor of right adrenal origin; adrenal adenoma; and adrenal lipoma. The exclusion of pheochromocytoma, the suspicion of malignancy, and the fact that biopsy findings change the treatment are generally accepted indications for adrenal biopsy.[1] CT-guided fine-needle aspiration biopsy (FNAB) is recommended for diagnosis by some authors.[11] FNAB was not performed on any of our myeloma cases, and the precise diagnosis was achieved after the histological examination of the resection materials.

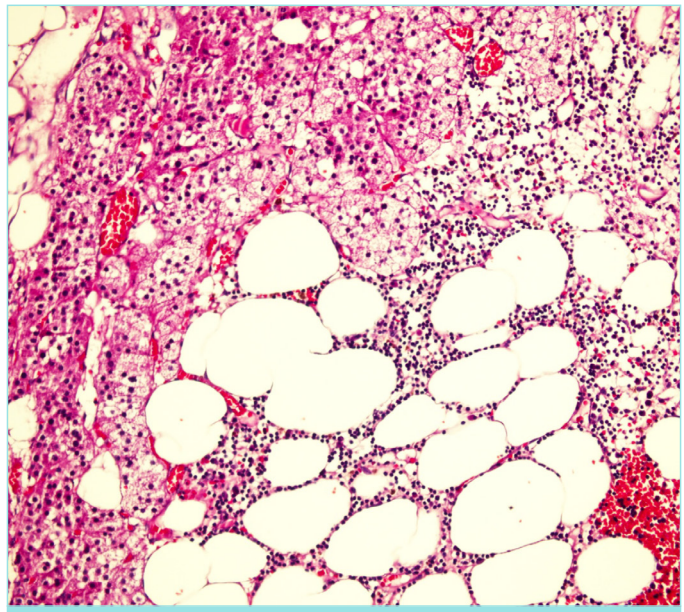


Figure 4. Histopathological appearance of myelolipomatous metaplasia (H&E, x100).

H&E: Hematoxylin & eosin

Table 2. Clinicopathological features of the cases with myelolipomatous metaplasia

Case no	Age, gender	Location	Size	Patient complaints and history	Surgical procedure	Adrenal neoplasia and radiological findings
1	56, M	Left adrenal	9 cm	No complaints.	Left adrenalectomy	Adrenal cortex adenoma
2	51, M	Right adrenal	6 cm	No complaints. Mass detected in the right adrenal gland upon routine check-up.	Right adrenalectomy	Adrenal cortex adenoma
3	50, F	Left adrenal	10 cm	No complaints. History of HT and DM.	Left adrenalectomy	Adrenal cortex adenoma MRI: mass lesion in the left adrenal gland measuring 40x33 mm, showing intense suppression; may be compatible with adenoma.
4	76, F	Right adrenal	5.7 cm	Palpitations and sweating. History of DM.	Right adrenalectomy	Adrenal cortex adenoma Whole abdomen and diffusion MRI: heterogeneous-appearing mass lesion with cystic areas measuring 29x53x46 mm and contrasting heterogeneously observed in the right adrenal gland.
5	67, M	Right adrenal	5.5 cm	No complaints. History of BPH, HT, asthma, cholecystectomy, and lumbar hernia intervention (surgical).	Right adrenalectomy	Adrenal cortex adenoma
6	48, F	Right adrenal	7.5 cm	Headache. History of asthma and HT.	Right adrenalectomy	Right adrenal cortex adenoma measuring 5 cm. Radiological diagnosis: Pheochromocytoma?

BPH: Benign prostatic hypertrophy, DM: Diabetes mellitus, F: Female, HT: Hypertension, M: Male, MRI: Magnetic resonance imaging.

Management

Current guidelines on the management of adrenal incidentalomas do not require hormonal investigations when the definite diagnosis is myelolipoma.[8,17] However, an endocrinological examination is useful in younger patients with hypertension, diabetes, or prediabetes, and for bilateral cases.[1] Surgery is recommended in myelolipomas presenting with complaints such as pain and swelling or symptoms related to hormone secretion, and in cases larger than 5 cm due to the risk of spontaneous rupture and retroperitoneal hemorrhage.[1,7,9,11] Myelolipomas smaller than 5 cm are usually asymptomatic, have no indication for surgical intervention, and are followed up with imaging studies.[21]

CONCLUSION

Adrenal myelolipoma are rare entities which are usually detected incidentally. They have a similar histopathological appearance and probably a similar origin to myelolipomatous metaplasia. In lipomatous adrenal lesions which develop based on chronic diseases, myelolipoma and an adrenal entity with myelolipomatous metaplasia should be kept in mind.

MAIN POINTS

- Adrenal myelolipoma is a rare entity which is usually detected incidentally.
- It is composed of adipose and hematopoietic tissue, and has a similar histopathological appearance and probably a similar origin to myelolipomatous metaplasia.
- Adrenal myelolipoma is encapsulated, while myelolipomatous metaplasia is a diffuse lesion mostly located in an adrenal adenoma.
- They both frequently coexist with chronic diseases such as hypertension, diabetes, and asthma.

ETHICS

Ethics Committee Approval: University of Health Sciences Türkiye, Kartal Dr. Lütfi Kıdar City Hospital Clinical Research Ethical Committee (approval number: 2022/514/220/13, date: 22.02.2022).

Informed Consent: Retrospective study.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: M.A., C.C.B., Concept: S.Ş., A.E.G., S.H.K., Y.S.G., M.A., C.C.B., N.Ö.B., N.E., Design: S.Ş., A.E.G., S.H.K., Y.S.G., M.A., C.C.B., N.Ö.B., N.E., Data Collection and/or Processing: A.E.G., S.H.K., N.Ö.B., Analysis and/or Interpretation: S.Ş., A.E.G., S.H.K., Y.S.G., M.A., C.C.B., N.Ö.B., N.E., Literature Search: S.Ş., Y.S.G., Writing: S.Ş., A.E.G., S.H.K., Y.S.G., M.A., C.C.B., N.Ö.B., N.E.

DISCLOSURES

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

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

REFERENCES

1. Adapa S, Naramala S, Gayam V, Gavini F, Dhingra H, Hazard FKG, et al. Adrenal Incidentaloma: Challenges in Diagnosing Adrenal Myelolipoma. J Investig Med High Impact Case Rep. 2019; 7: 2324709619870311.
2. Samimagham H, Kazemi Jahromi M. Bilateral Adrenal Myelolipoma, A Case Presentation and Brief Literature Review. Iran J Kidney Dis. 2020; 14(1): 62-4.
3. Moreira SG Jr, Pow-Sang JM. Evaluation and management of adrenal masses. Cancer Control. 2002; 9(4): 326-34.
4. Chatzellis E, Kaltsas G. Adrenal incidentalomas. [Updated: 2019 Nov 7]. In: Feingold KR, Anawalt B, Blackman MR, Boyce A, Chrousos G, Corpas E, et al, (editors). Endotext [Internet]. South Dartmouth (MA): MDText.com, Inc.; 2000.
5. Gadelkareem RA, Moeen AM, Khalil M, Reda A, Farouk M, Abdelkawi IF, et al. Experience of a Tertiary-Level Urology Center in Clinical Urological Events of Rare and Very Rare Incidence. V. Urological Tumors: 1. Adrenal Myelolipoma. Curr Urol. 2020; 14(2): 85-91.
6. Decmann Á, Perge P, Tóth M, Igaz P. Adrenal myelolipoma: a comprehensive review. Endocrine. 2018; 59(1): 7-15.
7. Lam AK. Update on Adrenal Tumours in 2017 World Health Organization (WHO) of Endocrine Tumours. Endocr Pathol. 2017; 28(3): 213-27.
8. Mhammedi WA, Ouslim H, Ouraghi A, Irzi M, Elhoumaidi A, Elhoumaidi A, et al. Adrenal myelolipoma: from tumorigenesis to management. Pan Afr Med J. 2019; 34: 180.
9. Bokhari MR, Zulfiqar H, Garla VV. Adrenal Myelolipoma. [Updated: 2022 Jan 7]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022.
10. Anbardar MH, Soleimani N, Nikeghbalian S, Mohebbi M. Adrenocortical adenoma with myelolipomatous metaplasia: a potential diagnostic pitfall: a case report and review of the literature. J Med Case Rep. 2021; 15(1): 333.
11. Rkik M, Fadil Y, Elidrissi O, Dakir M, Debbagh A, Aboutaieb R. Geant adrenal myelolipoma: A case report with literature review. Urol Case Rep. 2020; 35: 101541.
12. Mete O, Erickson LA, Juhlin CC, de Krijger RR, Sasano H, Volante M, et al. Overview of the 2022 WHO Classification of Adrenal Cortical Tumors. Endocr Pathol. 2022; 33(1): 155-96.
13. Barman S, Mandal KC, Mukhopadhyay M. Adrenal myelolipoma: An incidental and rare benign tumor in children. J Indian Assoc Pediatr Surg. 2014; 19(4): 236-8.
14. Bishop E, Eble JN, Cheng L, Wang M, Chase DR, Orazi A, et al. Adrenal myelolipomas show nonrandom X-chromosome inactivation in hematopoietic elements and fat: support for a clonal origin of myelolipomas. Am J Surg Pathol. 2006; 30(7): 838-43.
15. Chang KC, Chen PI, Huang ZH, Lin YM, Kuo PL. Adrenal myelolipoma with translocation (3;21)(q25;p11). Cancer Genet Cytogenet. 2002; 134(1): 77-80.
16. Lin L, Gong L, Cheng L, Liu Z, Shen S, Zhu Y, et al. Adrenal Myelolipoma: 369 Cases from a High-Volume Center. Front Cardiovasc Med. 2021; 8: 663346.
17. Hamidi O, Raman R, Lazik N, Iniguez-Ariza N, McKenzie TJ, Lyden ML, et al. Clinical course of adrenal myelolipoma: A long-term longitudinal follow-up study. Clin Endocrinol (Oxf). 2020; 93(1): 11-8.
18. Akamatsu H, Koseki M, Nakaba H, Sunada S, Ito A, Teramoto S, et al. Giant adrenal myelolipoma: report of a case. Surg Today. 2004; 34(3): 283-5.
19. Boudreaux D, Waisman J, Skinner DG, Low R. Giant adrenal myelolipoma and testicular interstitial cell tumor in a man with congenital 21-hydroxylase deficiency. Am J Surg Pathol. 1979; 3(2): 109-23.
20. Fırat C, Eryigit S, Yener S, Demir T, Bozkurt O, Demir O, et al. Myelolipomatous changes within adrenocortical adenoma. Cukurova Med J. 2019; 44(3): 1135-8.
21. Omura D, Nakano Y, Takase R, Otsuka F. Symptomatic Adrenal Myelolipoma. Intern Med. 2019; 58(8): 1181.

RESEARCH ARTICLE

CYPRUS
JOURNAL OF MEDICAL SCIENCES

DOI: 10.4274/cjms.2021.2021-54
Cyprus J Med Sci 2023;8(1):66-73

Opinions and Attitudes of Nursing Students Towards Distance Education During the COVID-19 Pandemic

Seda Cevheroğlu[1], Sevinç Taştan[2], Seda Sümer Dalkıran[3]

[1]Department of Nursing, İstanbul University-Cerrahpaşa, Florence Nightingale Faculty of Nursing, İstanbul, Türkiye
[2]Department of Nursing, Eastern Mediterranean University Faculty of Health Sciences, Famagusta, North Cyprus
[3]Department of Midwifery, University of Health Sciences Turkey, Hamidiye Faculty of Health Sciences, İstanbul, Türkiye

Abstract

BACKGROUND/AIMS: This study aimed to determine the opinions and attitudes of nursing students towards distance education during the coronavirus disease-19 (COVID-19) pandemic.

MATERIALS AND METHODS: This research was designed as a descriptive study. Two hundred ten students of a nursing department of a private university in the Turkish Republic of Northern Cyprus, who received distance education in the spring semester of 2019-2020 academic year due to the COVID-19 pandemic, constituted the sample of this study. The participants were asked to complete an online survey which included a descriptive information form, an Opinions on Distance Education Scale (ODES) and an Attitude Scale towards Distance Education (ASDE).

RESULTS: The mean age of the participants was 21.62±1.90 and 55.7% used their mobile phone to participate in distance education. The mean internet use was 6.58±0.27 hours per day and 74.4% had internet access problems. The mean scores obtained from the ODES and ASDE were 45.50±0.77 and 95.74±2.15, respectively. There was a positive and moderate correlation between the mean ODES and ASDE scores.

CONCLUSION: The findings of this study imply that lectures with lab and clinical practice are not appropriate for distance education and so any missed lab or clinical practice might be compensated for via face-to-face education after the COVID-19 pandemic ends.

Keywords: Distance education, nursing students, attitudes, opinions

INTRODUCTION

The World Health Organization declared a global pandemic on the 11th of March, 2020 as coronavirus disease-19 (COVID-19) had been spreading globally since late December, 2019. Since then, various measures to prevent the spread of this disease were taken in different sectors, including education.[1] Most countries temporarily closed down educational institutions, including, pre-schools, schools and universities.[2,3] In line with these global measures, the Ministry of Education and Culture in the Turkish Republic of North Cyprus (TRNC) decided to temporarily close down all educational institutions as the first COVID-19 case was reported in the country on the 10th of March, 2019.[4] Similarly, after the first COVID-19 case was announced, on the 11th of March, 2020, the Turkish government first decided to suspend face-to-face education starting on the 16th of March, 2020, which was followed by the decision to temporarily close all educational institutions on the 25th of March, 2020.[5] During this period, some of the universities and colleges in different countries decided to postpone the spring semester of the 2019-2020 academic year, whereas others decided to use distance education.[2,6] The Council of Higher Education (CoHE), the primary institution responsible for all higher education institutions in Türkiye and the TRNC, decided to continue the spring term of the 2019-2020 academic year via distance education.[5,7] Universities in the TRNC complied with the decision of the CoHE on distance education so that university students could graduate on time.[8]

To cite this article: Cevheroğlu S, Taştan S, Sümer Dalkıran S. Opinions and Attitudes of Nursing Students Towards Distance Education During the COVID-19 Pandemic. Cyprus J Med Sci 2023;8(1):66-73

ORCID IDs of the authors: S.C 0000-0002-4671-3095; S.T. 0000-0002-1647-6965; S.S.D. 0000-0001-5758-6514.

Address for Correspondence: Seda Cevheroğlu
E-mail: seda.cevheroglu@emu.edu.tr
ORCID ID: orcid.org/0000-0002-4671-3095

Received: 01.03.2021
Accepted: 17.07.2021

Distance education is an education system in which students and lecturers are not in the same physical environment but they can simultaneously or sequentially communicate for educational purposes.[9] This education system is frequently used in today's world parallel to the advances in communication and information technologies and the increasing importance of life-long learning.[10] Although distance education has been a widely used method of learning during the COVID-19 pandemic, its roots in nursing education can be dated back to the 1960s.[11,12] In Türkiye, the first associate degree program of nursing which used the distance education method was initiated in 1993 but it was only in 2009 that the graduates of these programs were granted the right to complete a bachelor's degree.[12] However, the global pandemic resulted in the temporary replacement of face-to-face learning by distance education.[12,13] Developments in internet technologies and their use in education enabled students to learn by themselves and provided a more flexible and individualized learning environment, which, in turn, resulted in more positive attitudes towards online education. In addition, distance education has several positive aspects, including lower costs than face-to-face learning and having better access to various sources of information in a relatively short time and to a geographically widespread population.[14] Despite these positive aspects, technical problems, communication deficiency, affective inadequacies, and problems with educational materials may have negative effects on the opinions and attitudes of students towards distance education.[15]

Attitude is defined by the Oxford Learner's Dictionaries as "the way that you think and feel about someone or something". As a positive or negative reaction towards a person or an object, attitude is an important factor which influences the efficiency of learning.[16] The success and effectiveness of distance education depends on the attitudes and opinions towards this education system. The attitude of an individual towards distance education is closely related with their success in learning. That is, people with positive attitudes towards distance education are more likely to have successful learning outcomes.[17] Developing positive attitudes towards distance education, on the other hand, depends on determining the opinions and feelings of the students towards this education method. Additionally, determining the opinions and attitudes of students towards distance education helps scholars to reveal the factors which lead to negative perceptions and to set up the learning environment in order to minimize these negative perceptions.[16] Consequently, this study aimed to reveal the opinions and attitudes of nursing students towards distance education during the COVID-19 pandemic. An analysis of the literature on distance education in nursing revealed that distance education had positive effects on the cognitive and psychomotor skills of nursing students,[18,19] and did not significantly differ from face-to-face learning.[20,21] Studies on distance education in nursing during the COVID-19 pandemic found, on the other hand, that student satisfaction was moderate or lower, and students experienced internet access problems or felt insufficient in terms of clinical practices.[13,22,23] In contrast to other studies, this study aimed to evaluate both the opinions of the nursing students and their attitudes towards online education.

MATERIALS AND METHODS

The Purpose of This Study

This descriptive study was conducted on nursing students who were enrolled in the department of nursing of a private university in the TRNC who received distance education during the spring semester of the 2019-2020 academic year.

The research questions included the following:

- What were the opinions of the nursing students on distance education during the COVID-19 pandemic?

- What were the attitudes of the nursing students towards distance education during the COVID-19 pandemic?

Participants

Two hundred and ten students in the second, third and fourth years of the nursing department constituted the study population of this study. Voluntary nursing students at the age of 18 or above, who were enrolled in the nursing department and who received distance education during the spring semester of the 2019-2020 academic year, were included in this study. The criteria for exclusion were the students' decision not to participate or being under the age of 18.

Data Collection Tools

We used a descriptive information form (11 items), the Opinions on Distance Education Scale (ODES) (18 items) and the Attitude Scale towards Distance Education (ASDE) (35 items) for data collection. The participants were asked to sign into the Moodle software, which was used to access distance education, and to complete the online survey. The first part of the survey provided information about the researchers and the aims of the research and included a statement regarding informed consent.

Descriptive Information Form

This form was developed by the researchers using the relevant literature.[24] It includes 11 questions on age, gender, class, computer and internet skills and the internet access of the participants.

Opinions on Distance Education Scale (ODES)

Developed by Yıldırım et al.[25], ODES is composed of 18 items which measure four dimensions of opinions on distance education, namely: personal suitability, effectiveness, instructiveness and familiarity, by using a five-point Likert scale, ranging from "strongly disagree" (1 point) to "strongly agree" (5 points). The maximum scores which can be obtained from the personal suitability, effectiveness, instructiveness and familiarity subscales are 30, 25, 20 and 15, respectively. The minimum and maximum scores which can be obtained range from 5 to 90. The Cronbach's alpha of the scale in the original study and in our study were 0.864 and 0.758, respectively.[25]

Attitude Scale towards Distance Education

Developed by Kışla, the ASDE has 35 items which are scored on a five-point Likert scale. The total scores can range between 35 and 175 points, with higher scores indicating positive attitudes towards distance education. The Cronbach's alpha of the scale in the original study and our study were 0.89 and 0.955, respectively.[10]

Data Collection

Following the preparation of the online survey, we asked for the permission of the academicians who had lectures with the 2nd, 3rd and 4th year students to conduct our study before their online course. After obtaining permission, we informed the students about the aims and the scope of the research and its voluntary nature. We then asked them to complete the online survey before the start of their courses via Moodle-

Microsoft Teams software. After obtaining their informed consent, the students were given 15 minutes to complete the survey.

We obtained permission from the Ethical Commission of the Eastern Mediterranean University where this study was conducted (approval number: ETK 00-2020- 0245) and institutional permission from the head of the department of nursing. All participants were informed before the study and their consent was obtained.

Statistical Analysis

SPSS 22.0 (SPSS Inc.; Chicago, IL, USA) was used for the statistical analysis of collected data. The Kolmogorov-Smirnov test was used to evaluate normality. The Mann-Whitney U and Kruskal-Wallis tests were used for data without normal distribution. Spearman's correlation analysis was used to evaluate the relationship between the age of the participants, the personal suitability, effectiveness, instructiveness and familiarity subscales of the ODES and the mean ODES and ASDE scores. Statistical significance was set at $p<0.05$.

RESULTS

Table 1 presents the findings on the descriptive characteristics of the participants and the relationship between their descriptive characteristics and the mean ASDE scores. This study was conducted on 210 nursing students who received distance education during the COVID-19 pandemic. Their mean age was 21.62±1.90. 62.1% had personal computers and 55.7% used mobile phones to participate in distance education. 52.7% had a moderate level of computer skills and 27.8% had moderate internet skills. Daily internet use was 7 hours or above for 38.9% of the participants and their mean internet use was 6.58±0.27 hours per day. 74.4% had internet access problems.

The mean ASDE scores of the participants aged 23 years or above (112.00±33.41) were statistically significantly higher than for those students aged 20 years or below (86.81±27.26) and between 21 and 22 years of age (93.19±27.69) ($p<0.001$). We did not find any statistically significant relationship between class standing, gender, the ownership of personal computer, the type of distance learning device, internet skills, daily internet use or the mean ASDE scores ($p>0.05$). The mean ASDE score of the participants with advanced computer skills (120.38±36.31) was significantly higher than for those participants with low (101.33±31.49), moderate (91.24±28.37) and high levels of computer skills (97.04±31.70) ($p<0.001$). Finally, the mean ASDE scores of the participants without internet access problems (108.40±32.65) was significantly higher than for those students with internet access problems (91.39±28.66) ($p<0.001$).

Table 2 shows the mean ODES and ASDE scores of the participants. The mean ODES score was 45.50±0.77. The mean scores obtained from personal suitability, effectiveness, instructiveness and familiarity subscales of ODES were 14.59±0.51, 10.16±0.41, 15.07±0.36 and 5.70±0.21, respectively. Finally, the mean ASDE score of the participants was 95.74±2.15.

Table 3 evaluates the relationship between the ODES scores and the descriptive characteristics of the participants. There was a statistically significant relationship between age, mean ODES scores and the scores obtained from the personal suitability, effectiveness, and instructiveness subscales of the ODES ($p<0.001$). In other words, the positive opinions of nursing students on distance education increased in parallel with their increase in age. Additionally, the relationship between gender and the instructiveness subscale of ODES was statistically significant ($p<0.021$). That is, female students considered distance education as more instructive, however, there was no significant relationship between gender, mean ODES scores and the other subscales of ODES ($p>0.05$). We found a statistically significant relationship between the computer skills of the participants and the mean scores obtained from the effectiveness ($p<0.033$) and instructiveness ($p<0.042$) subscales of the ODES. Students with advanced (16.38±7.27) and high (15.73±4.52) levels of computer skills had more positive opinions on the effectiveness of distance education. Regarding the relationship between internet access problems and their ODES scores, we found that students without internet access problems obtained significantly higher scores from the ODES (47.60±10.42; $p<0.023$) and its subscales of personal suitability (16.98±7.95; $p<0.009$) and effectiveness (12.69±6.41; $p<0.001$). On the other hand, there was a significant relationship between the instructiveness (15.69±4.78; $p<0.007$) and familiarity (6.05±3.10; $p<0.003$) scores of the participants with internet access problems. Although not shown in the table, no statistically significant relationship was found between the ASDE scores and some introductory student characteristics.

Table 4 shows the correlation between the mean ODES and ASDE scores of the nursing students. We found a moderate and positive correlation between their mean ASDE and ODES scores ($r= 0.491$; $p<0.001$). In this sense, higher ASDE scores of the participants correlated with higher scores obtained from the ODES.

DISCUSSION

Sufficient technological infrastructure is required for the effective participation of university students in distance education.[26,27] Within this context, Zan and Umut[27] analyzed the technological capabilities of students during the COVID-19 pandemic and found that 60.25% of the students had computers or tables but 40.3% had internet connection problems. The study of Li et al.[23] reported self-discipline, frequency to access the internet, support and help from the university and the use of course resources as the facilitators to improve success in the online education of nursing students. Our findings were also consistent with the literature. At this point, the 6 GB package provided by the CoHE to university students was an important institutional support.[28] However, in addition to internet, the need of university students to have computers or tablets might be met by the state or university-sponsored campaigns. In order to cope with internet access problems during synchronous lectures, we recommend that videos and lecture notes be shared before the lecture.[29]

Keskin and Özer Kaya[30] found that the mean internet use of university students increased from 2.98±2.12 hours to 5.27±2.98 hours per day after the COVID-19 pandemic. Armstrong-Mensah et al.[31] also found that daily internet use of university students increased to 4 hours during the COVID-19 pandemic. In our study, the mean internet use of the nursing students was 6.58±0.27 per day. These findings imply that daily internet use may have increased due to the transition to distance education during the COVID-19 pandemic. Various studies which analyzed the skills of using computers and other communication technologies found that university students had moderate or high levels of computer skills.[32-34] Similar to our study, university students in the studies of Düşünceli et al.[26], Abbasi et al.[24] and Karakuş et al.[35] had moderate computer skills. On the other hand, we found that most

of our students had internet access problems and used mobile phones for their distance education. The studies of Düşünceli et al.[26] and Abbasi et al.[24] had similar findings. Consequently, measures to reduce the negative effects of internet outages on distance education, such as asynchronous video lectures, might be taken.

During the COVID-19 pandemic, universities used distance education as a rapid response to cope with the crisis.[30,36] Due to the rapid pace of the transition to distance education, the number of studies on the opinions and attitudes of university students towards this new method of learning is limited.[36] Our study, which aimed to fill this gap, found that the opinions and attitudes of nursing students towards distance education was negative in general. Additionally, we found a positive relationship between the opinions on and attitudes towards distance education. In other words, students with positive opinions on distance education had also positive attitudes towards this education method. There were a number of studies on the opinions on and attitudes towards distance education in the literature. Altuntaş Yılmaz found that attitudes of physiotherapy and rehabilitation students towards distance education during the COVID-19 pandemic were positive.[37] Ali

Table 1. Descriptive characteristics of the participants and the relationship between the scale

Variables	Mean	SD	Total scale score		
Age	21.62	1.90			
Daily internet usage time	6.58	0.27			
	Number	Percentage	Mean (SD)	Test X^2/Z	p
Age group					
20 years and below	69	34.0	86.81±27.26	19.68	0.001
21-22 years	83	40.9	93.19±27.69		
23 years and above	51	25.1	112.00±33.41		
Gender					
Male	71	35	97.89±33.23	0.618	0.537
Female	132	65	94.60±29.11		
Class					
2nd class	71	35	90.45±32.13	4.65	0.098
3rd class	78	38.4	95.83±25.46		
4th class	54	26.6	102.59±34.23		
Owning a computer					
Yes	127	62.6	98.37±31.67	-1.406	0.160
No	76	37.4	91.45±28.35		
Device used in distance education					
Desktop computer	14	6.9	109.57±34.90	2.778	0.249
Laptop	76	37.4	95.51±31.33		
Mobile phone	113	55.7	94.19±39.33		
Computer skills					
Low level	40	19.7	101.33±31.49	8.615	0.035
Moderate level	107	52.7	91.24±28.37		
High level	48	23.6	97.04±31.70		
Advanced level	8	3.9	120.38±36.31		
Internet skills					
Low level	15	7.4	100.33±26.17	3.763	0.288
Moderate level	97	47.8	94.81±30.09		
High level	78	38.4	93.37±30.07		
Advanced level	13	6.4	111.69±39.23		
Daily internet usage time					
3 hours or below	48	23.6	92.77±28.98	2.099	0.350
4-6 hours	76	37.4	100.07±33.52		
7 hours or above	79	38.9	93.41±28.36		
Internet access status					
Hassle-free	52	25.6	108.40±32.65	-3.378	0.001
Problematic	151	74.4	91.39±28.66		

Z: Mann-Whitney U test, X^2: Kruskal-Wallis H test

et al.[38] found a favorable attitude towards e-learning among nursing students. Similarly, Balaman[39] found that vocational school students had positive perspectives on web-based distance education. In contrast to these, other studies reported negative findings. Nursing education, which depends on psychomotor skills, requires lab courses and clinical practices during the undergraduate studies.[40] Especially, existing studies on students of nursing and medicine during the COVID-19 pandemic showed that the students mostly preferred face-to-face learning and believed that distance education was insufficient in terms of lab courses and clinical practices.[24,30,40] Similarly, the university students in the study of Karakuş et al.[35] had negative opinions on distance education. In regards to this negative side, nursing students experienced difficulties in understanding applied courses[13] and expressed that distance education was not suitable for nursing practice and consequently for the department of nursing.[8,40] Based on these findings, we may suggest that measures to prevent infection from COVID-19 might be taken in order to start applied courses as soon as possible. Additionally, students might be informed that so any missed lab or clinical practice might be compensated for via in-person education at a convenient future date. Another reason for the negative opinions and attitudes of university students towards distance education may be related with the lack of necessary internet and communication infrastructure. Additionally, students may not be suited to distance education and may consider it as an ineffective method of learning. Students of health sciences are especially more anxious regarding distance education since they receive online applied courses. In order to overcome this problem, methods other than online courses might be preferred for applied courses and online courses should be supplemented with virtual simulation practice. Simulation in nursing has become one of the alternative methods of learning in nursing education due to the possible restrictions caused by pandemics, such as the COVID-19 pandemic. Simulation programs helps nursing students to analyze clinical cases, plan nursing care and evaluate their own performance.[29] Additionally, we believe that these educations might be complemented with face-to-face courses in order to reach program learning outcomes.

Determining students' opinions and attitudes towards distance education is crucial in order to reveal the factors which may lead to negative perceptions, to make necessary interventions to change these perceptions and to properly design the learning environment.[16] The analysis of the relationship between the descriptive characteristics of nursing students and their attitudes towards distance education in various studies revealed the importance of gender.[34,41,42] This study, which aimed to determine the opinions and attitudes of nursing students towards distance education during the COVID-19 pandemic, found that the mean age of the participants was 21.62±1.90 years and most of them were female. Our findings were consistent with the findings of other studies on nursing students.[8,40,43] Unlike these studies, gender in our study was not an important factor which influenced the attitudes and opinions of the participants. Regarding gender, we only found that female nursing students considered distance education as being more instructive. Additionally, we found that increasing age correlated with more positive opinions and attitudes towards distance education. Similarly, the students in the study of Düşünceli et al.[26] generally considered distance education as being a more effective learning method as their ages increased. In our study, older students considered distance education as personally more suitable, effective and instructive.

Sustaining effective distance education without interruption depends on the computer skills of the students and their access to the internet. In our study, those participants without internet access problems had more positive opinions on and attitudes towards distance education. These students also considered distance education as a personally suitable and effective learning method. On the other hand, those participants with internet access problems considered distance education as an instructive and familiar method. The study of Barış[44] found that university students without internet access problems had positive attitudes towards distance education. We also found that participants with advanced computer skills had more positive attitudes towards distance education and considered it as an effective learning method. Additionally, those participants with high and moderate levels of computer skills believed that distance education was instructive. Ateş and Altun[45] evaluated the effects of various factors on the attitudes towards distance education and found that experience of computer use and perceived computer skills significantly influenced attitudes towards distance education. Therefore, before starting distance education, students might receive courses to improve their computer skills regarding how to use the software for distance education. These courses may contribute to positive opinions and attitudes towards distance education.

Study Limitations

The main limitation of this study was related with the fact that it was conducted on nursing students from a single university. Therefore, our findings may not reflect the opinions and attitudes of all nursing students towards distance education.

CONCLUSION

The opinions and attitudes of nursing students towards distance education in our study was generally negative. These opinions and attitudes improved as the age of students increased, their internet access

Table 2. Opinions on distance education scale and attitude scale towards distance education mean scores of the participants

	Mean (SD)	Min.-max. points
Opinion scale for distance education		
Personal suitability	14.59±0.51	1-30
Effectiveness	10.16±0.41	1-25
Instructiveness	15.07±0.36	1-20
Familiarity	5.70±0.21	1-15
Total score average	45.50±0.77	5-90
Attitude scale towards distance education		
Total score average	95.74±2.15	35-175

SD: Standard deviation, min: Minimum, max: Maximum

Table 3. Relationship between opinions on distance education scale scores and descriptive characteristics of the participants					
	Scale score averages				
	Personal suitability	Effectiveness	Teaching	Predisposition	Total score average
Age					
20 years and below	12.29±5.37	8.04±3.75	16.80±4.18	6.25±3.14	43.38±8.63
21-22 years	13.61±6.80	9.32±5.18	15.25±4.86	5.30±2.82	46.49±10.62
23 years and above	19.29±8.39	14.37±7.12	12.43±5.76	5.57±2.95	51.67±11.98
X^2	21.98	27.89	20.69	4.64	19.65
p	0.001	0.001	0.001	0.098	0.001
Gender					
Male	15.03±8.08	10.92±6.62	13.86±5.56	5.96±3.24	45.76±12.53
Female	14.36±6.91	9.75±5.40	15.71±4.81	5.55±2.83	45.37±9.99
Z	-0.056	-0.825	-2.310	-0.779	-0.243
p	0.955	0.409	0.021	0.436	0.808
Class					
2nd class	13.61±7.09	9.41±5.35	15.82±4.82	5.80±3.44	44.64±9.22
3rd class	13.83±6.68	9.54±5.44	15.44±5.07	5.91±2.87	44.72±11.90
4th class	14.60±8.10	12.04±6.76	13.56±5.43	5.22±2.41	44.78±11.36
X^2	5.81	5.05	5.73	1.58	3.59
p	0.055	0.080	0.057	0.453	0.166
Owning a computer					
Yes	14.85±7.36	10.18±5.78	14.87±5.20	5.58±2.77	45.48±10.94
No	14.17±7.30	10.12±6.06	15.40±5.07	5.87±3.30	45.56±10.96
Z	-0.704	-0.495	-0.654	-0.083	-0.286
p	0.481	0.621	0.513	0.934	0.775
Device used in distance education					
Desktop computer	17.07±8.77	12.36±7.23	14.64±5.60	5.71±3.56	49.79±12.93
Laptop	14.62±6.80	9.96±5.56	14.88±5.02	5.46±2.63	44.92±10.68
Mobile phone	14.27±7.49	10.02±5.90	15.25±5.21	5.84±3.14	45.37±10.80
X^2	1.826	1.815	0.402	0.600	1.612
p	0.401	0.403	0.818	0.741	0.447
Computer skills					
Low level	14.08±7.77	10.08±6.04	14.20±5.61	6.40±3.51	44.75±13.49
Moderate level	14.17±7.04	9.52±5.55	15.49±5.00	5.77±2.85	44.94±10.21
High level	15.04±7.15	10.60±5.74	15.73±4.52	5.23±2.85	46.60±10.23
Advanced level	20.13±8.87	16.38±7.27	9.88±5.62	3.88±1.46	50.25±10.22
X^2	4.121	8.767	8.199	7.184	2.497
p	0.249	0.033	0.042	0.066	0.476
Internet use skill					
Low level	13.47±7.28	9.33±5.52	12.73±5.81	6.33±3.24	41.87±16.90
Moderate level	14.56±7.24	9.95±5.68	15.39±5.02	6.16±3.17	46.06±10.20
High level	14.67±7.31	10.18±5.99	15.55±5.00	5.14±2.63	45.54±10.64
Advanced level	15.69±8.78	12.54±6.70	12.46±5.22	4.69±2.66	45.38±3.79
X^2	0.405	2.487	7.546	7.197	1.310
p	0.939	0.478	0.056	0.066	0.727
Daily internet usage time					
3 hours or below	13.75±6.56	9.06±4.02	14.38±4.45	5.17±2.30	42.35±9.86
4-6 hours	15.77±7.94	11.14±6.30	14.63±5.12	5.86±3.31	47.41±10.73
7 hours or above	13.96±7.10	9.87±5.84	15.91±4.93	5.85±3.00	45.59±11.39
X^2	2.057	3.040	3.519	0.441	5.079
p	0.358	0.219	0.172	0.802	0.079
Internet access status					
Hassle-free	16.98±7.95	12.69±6.41	13.27±5.75	4.65±2.31	47.60±10.42
Problematic	13.77±6.94	9.28±5.42	15.69±4.78	6.05±3.10	44.79±11.26
Z	-2.626	-3.749	-2.694	-2.928	-2.271
p	0.009	0.001	0.007	0.003	0.023
Z: Mann-Whitney U test, X^2:Kruskal-Wallis H test					

Table 4. Correlation between mean opinions on distance education scale and attitude scale towards distance education scores of the nursing students

		Opinion scale for distance education average	Attitude towards distance education scale average score
Opinion scale for distance education average	r	-	0.491(*)
	p		0.001
Attitude towards distance education scale average score	r	0.491(**)	-
	p	0.001	

*Correlation is significant at the 0.05 level (2-tailed), **Correlation is significant at the 0.01 level (2-tailed).

problems decreased and they had higher computer skills. Consequently, in case of the possible extension of distance education in universities due to the COVID-19 pandemic, universities or the government might provide unlimited internet access to university students and initiate programs to increase their computer skills. Asynchronous lectures might be used as an alternative to synchronous lectures in case of internet access problems. In such cases, flipped classroom, lecture videos or discussion platforms may be used to gain access to resources prior to the course. Additionally, the content of applied courses might be enriched and learning methods, such as, virtual reality platforms, digital game-based learning or video-based learning could be used to improve the skills of the students. Given that the course and the length of pandemic is currently unknown, universities might prepare emergency plans in order to cope with possible developments. Curriculum or course plans might be revised in order to compensate for the lab courses and clinical practices postponed due to COVID-19 pandemic, with face-to-face courses once the universities have reopened.

MAIN POINTS

- The opinions and attitudes of nursing students towards distance education in our study was generally negative.
- These opinions and attitudes improved as the age of students increased, their internet access problems decreased and when they had higher computer skills.
- Curriculum or course plans might be revised in order to compensate for lab courses and clinical practices postponed due to the COVID-19 pandemic with face-to-face courses once the universities have reopened.

ETHICS

Ethics Committee Approval: We obtained permission from the Ethical Commission of the Eastern Mediterranean University where this study was conducted (approval number: ETK 00-2020-0245) and institutional permission from the head of the department of nursing.

Informed Consent: All participants were informed before the study and their consent was obtained.

Peer-review: Externally peer-reviewed

Authorship Contributions

Concept: S.C., S.T., S.S.D., Design: S.C., S.T., S.S.D., Data Collection and/or Processing: S.C., S.T., S.S.D., Literature Search: S.C., S.T., S.S.D., Writing: S.C., S.T., S.S.D.

DISCLOSURES

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

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

REFERENCES

1. Er AG, Ünal S. 2019 Coronavirus Pandemic in Türkiye and Across the World. Flora J Infect Dis Clin Microbiol. 2020; 25(1): 1-8.
2. Viner MR, Russell JS, Croker H, Packer J, Ward J, Stansfield C, et al. School Closure and Management Practices During Coronavirus Outbreaks Including COVID-19: A Rapid Systematic Review. Lancet. 2020; 397-404.
3. Wang C, Cheng Z, Yue X-G, McAleer M. Risk Management of COVID-19 by Universities in China. J Risk Financ Manag. 2020; 13(2): 36.
4. KKTC MEB. KKTC Milli Eğitim Ve Kültür Bakanlığı Kararı [Internet]. 2020 [cited 2021 Jan 29]. Available from: http://www.mebnet.net/?q=content/milli-eğitim-ve-kültür-bakanlığı'nın-koronovirüs-ile-ilgili-ivedi-olarak-aldığı-tedbirler
5. YÖKa. Yüksek Öğretim Kurumu [Internet]. 2020 [cited 2021 Jan 29]. Available from: https://www.yok.gov.tr/Sayfalar/Haberler/2020/YKS Ertelenmesi Basın Açıklaması.aspx
6. Domenico L Di, Pullano G, Coletti P, Hens N, Colizza V. Expected Impact of School Closure and Telework to Mitigate COVID-19 Epidemic in France. epicx-lab.com [Internet]. 2020; 1-15.
7. YÖKb. Yüksek Öğretim Kurumu [Internet]. 2020 [cited 2021 Jan 29]. Available from: https://www.yok.gov.tr/Sayfalar/Haberler/2020/coronavirus_bilgilendirme_1.aspx
8. Kürtüncü M, Kurt A. COVID-19 Pandemisi Döneminde Hemşirelik Öğrencilerinin Uzaktan Eğitim Konusunda Yaşadıkları Sorunları. Avrasya Sos ve Ekon Araştırmaları Derg [Internet]. 2020;7(5):66–77. Available from: https://dergipark.org.tr/tr/download/article-file/1128112
9. Sakarya G, Zahal O. The Student Opinions on Distant Violin Education During Covid-19 Epidemic Gül Sakarya - Onur Zahal. Turkish Stud. 2020; 15(6).
10. Kışla T. Development of a Attitude Scale towards Disance LearningEge Eğitim Derg. 2016; 17(1): 258.
11. Alonso F, López G, Manrique D, Viñes JM. An instructional model for web-based e-learning education with a blended learning process approach. Br J Educ Technol. 2005; 36(2): 217-35.
12. Bahar A. An innavation in nursing basic skills education: Web based education. Anadolu Hemşirelik ve Sağlık Bilim Derg. 2015; 18(4): 304-11.
13. Kızıltepe Keskin S, Kurtgöz A. Hemşirelik Öğrencilerinin Covid-19 Pandemisi Sürecinde Aldıkları Uzaktan Eğitime Yönelik Tutum ve Görüşlerinin Belirlenmesi. J Int Soc Res. 2020; 13(74-8): 558- 66.
14. Gülbahar Y. E- Öğrenme. 5. Baskı. Ankara: Pagem Akademi; 2019.p.25-8.
15. Kahraman ME. COVID-19 Salgınının Uygulamalı Derslere Etkisi ve Bu Derslerin Uzaktan Eğitimle Yürütülmesi: Temel Tasarım Dersi Örneği.

Medeni Sanat Derg [Internet]. 2020; 6(1): 44-56. Available from: https://dergipark.org.tr/tr/doi/10.46641/medeniyetsanat.741737

16. Fidan M. Distance Education Students' Attitudes Towards Distance Education and Their Epistemological Beliefs. Hacettepe Egit Derg. 2016; 31(3): 536-50.
17. Alqurashi E. Predicting Student Satisfaction and Perceived Learning within Online Learning Environments. Distance Educ [Internet]. 2019; 40(1): 133-48. Available from: https://doi.org/10.1080/01587919.2018.1553562
18. Gerdprasert S, Pruksacheva T, Panijpan B, Ruenwongsa P. Development of a web-based learning medium on mechanism of labour for nursing students. Nurse Educ Today [Internet]. 2010; 30(5): 464-9.
19. Koch J, Andrew S, Salamonson Y, Everett B, Davidson PM. Nursing students' perception of a web-based intervention to support learning. Nurse Educ Today [Internet]. 2010; 30(6): 584-90.
20. Öztürk D, Dinç L. Effect of web-based education on nursing students' urinary catheterization knowledge and skills. Nurse Educ Today [Internet]. 2014; 34(5): 802-8.
21. Horiuchi S, Yaju Y, Koyo M, Sakyo Y, Nakayama K. Evaluation of a web-based graduate continuing nursing education program in Japan: A randomized controlled trial. Nurse Educ Today [Internet]. 2009; 29(2): 140-9.
22. Suliman WA, Abu-Moghli FA, Khalaf I, Zumot AF, Nabolsi M. Experiences of nursing students under the unprecedented abrupt online learning format forced by the national curfew due to COVID-19: A qualitative research study. Nurse Educ Today [Internet]. 2021; 100(July 2020): 104829.
23. Li W, Gillies R, He M, Wu C, Liu S, Gong Z, et al. Barriers and facilitators to online medical and nursing education during the COVID-19 pandemic: perspectives from international students from low- and middle-income countries and their teaching staff. Hum Resour Health. 2021; 19(1): 1-15.
24. Abbasi S, Ayoob T, Malik A, Memon SI. Perceptions of Students Regarding e-Learning During Covid-19 at a Private Medical College. Pakistan J Med Sci. 2020; 36(COVID19-S4): S57-61.
25. Yıldırım S, Yıldırım G, Çelik E, Karaman S. perception of distance education students about distance education: a scale development study. Eğitim ve Öğretim Araştırmaları Derg. 2014; 3(3): 365-70.
26. Düşünceli F, Arı Ö, Evren M, Kavak O. Covid-19 Sürecinde Mardin Artuklu Üniversitesi Uzaktan Eğitim Sistemi, Eğitim- Öğretim ile Araştırma Faaliyetlerinin Değerlendirilmesi. Mardin Artuklu Üniversitesi Eğitim Araştırma Faaliyetlerinin Değerlendirilmesi Raporu. 2020; (Haziran): 1-63.
27. Zan N, Umut B. Koronavirüs ile Acil Durumda Eğitim: Türkiye' nin Farklı Bölgelerinden Uzaktan Eğitim Sistemine Dahil Olan Edebiyat Fakültesi Öğrencilerine Genel Bakış Education in Emergency at Coronavirus : Overview of Faculty of Letters Students Included to Distance. Turkish Stud. 2020.
28. YÖKc. Yüksek Öğretim Kurumu [Internet]. 2020. Available from: https://www.yok.gov.tr/Sayfalar/Haberler/2020/ogrencilere-egitime-destek-kotasi.aspx
29. Kozan EH, Colak M, Demirhan BS. Distance Education in the COVID-19 Pandemic: Reflections on Nursing Education. J Educ Res Nurs. 2021; 18(Supp1): S60-4.
30. Keskin M, Özer Kaya D. COVID-19 Sürecinde Öğrencilerin Web Tabanlı Uzaktan Eğitime Yönelik Geri Bildirimlerinin Değerlendirilmesi. İzmir Katip Çelebi Üniversitesi Sağlık Bilim Fakültesi Derg [Internet]. 2020; 5(2): 59-67.
31. Armstrong-Mensah E, Ramsey-White K, Yankey B, Self-Brown S. COVID-19 and Distance Learning: Effects on Georgia State University School of Public Health Students. Front Public Heal. 2020; 8(September): 1-10.
32. Dinçer S, Şahinkayası Y. A Cross- Cultural Study of Ict Competency, Attıtude And Satısfactıon of Turkısh, Polish and Czech Unıversity Students. TOJET Turkish Online J Educ Technol. 2011; 10(4): 31-8.
33. Usta E, Korkmaz Ö. Pre-Service Teachers Computer Competencies, Perception of Technology Use and Attitudes toward Teaching Career. Int J Hum Sci. 2010; 7(1): 1335-49.
34. Yıldız S. The Attitudes Of The Students Having Pedagogical Formation Training Towards Distance Education. AİBÜ Sos Bilim Enstitüsü Derg. 2016; 16(1): 301-29.
35. Karakuş N, Ucuzsatar N, Karacaoğlu MÖ, Esendemir N, Bayraktar D. Turkish teacher candidates' views on distance education. Rumeli J Lang Lit Stud. 2020; 19: 220-41.
36. Iyer P, Aziz K, Ojcius DM. Impact of COVID-19 on Dental Education in the United States. J Dent Educ. 2020; 84(6): 718-22.
37. Altuntaş Yılmaz N. Investigation Of Students' Attitudes Towards Applied Distance Education In The Covid-19 Pandemic Process In Higher Education Institutions: Example Of Physiotherapy And Rehabilitation Department. Necmettin Erbakan Üniversitesi Sağlık Bilim Fakültesi Derg. 2020; 3(1): 15-20.
38. Ali N, Jamil B, Sethi A, Ali S. Attitude of Nursing Student Towards e- learning. Adv Heal Prof Educ. 2016; 2(1): 24-9.
39. Balaman F. Web Tabanlı Uzaktan Eğitimin Meslek Yüksekokulu Öğrencilerinin İnternet Programcılığı 2 Dersindeki Akademik Başarılarına Etkisi. Dicle Üniversitesi; 2014.
40. Kahyaoğlu Süt H, Küçükkaya B. The Views of Nursing Students on Distance Education. Hemşirelikte Eğitim ve Araştırma Derg. 2016; 13(3): 235-43.
41. Baek Y, Zhang H, Yun S. Teachers' Attitudes toward Mobile Learning in Korea. Turkish Online J Educ Technol. 2017; 16(1): 154-63.
42. Bayram M, Peker AT, Aka ST, Vural M. Examination of Attitudes of University Students to wards Distance Learning. Gaziantep Üniversitesi Spor Bilim Derg. 2019; (September): 330-45.
43. Xiong P, Zhang J, Wang X, Wu TL, Hall BJ. Effects of a Mixed Media Education Intervention Program on Increasing Knowledge, Attitude, and Compliance with Standard precautions among nursing Students: A Randomized Controlled Trial. Am J Infect Control [Internet]. 2017; 45(4): 389-95.
44. Barış MF. Analyzing the University Students' Attitudes Towards Distance Education: Namık Kemal University Case Study. Sak Univ J Educ. 2015; 5(2): 36.
45. Ateş A, Altun E. Investigating Preservice Computer Teachers' Attitudes Towards Distance Learning Regarding Various Variables. Gazi Eğitim Fakültesi Derg. 2008; 3(54): 125-45.

RESEARCH ARTICLE

CYPRUS
JOURNAL OF MEDICAL SCIENCES

DOI: 10.4274/cjms.2022.2021-31
Cyprus J Med Sci 2023;8(1):74-82

How Much do We Know About Pulse Oximeters Used in Every Field from Home Care to Critical Care? A Descriptive Study

Emine Pınar Keti[1], Nigar Ünlüsoy Dinçer[2]

[1]Kırıkkale University, Vocational School of Health Services, Kırıkkale, Türkiye
[2]Ankara Yıldırım Beyazıt University, Faculty of Health Science, Ankara, Türkiye

Abstract

BACKGROUND/AIMS: Aim of this study was to evaluate the knowledge and actions of nurses regarding the use of pulse oximeters. The measurement of oxygen saturation is considered as an additional indicator for measuring vital signs. The measurement of oxygen saturation by pulse oximeters is a part of nursing care in terms of the rapid evaluation of patients. The literature suggests that errors made in taking measurements influence the reliability of these measurement even though it is easy to use pulse oximeters.

MATERIALS AND METHODS: Sample selection was not carried out in this descriptive study, all of the nurses (n=588) in the population were included in this study; however, the sample of this study consisted of 393 nurses who agreed to participate. This study was conducted with nurses working at a university hospital in Ankara province in Türkiye. In order to assess the knowledge and attitudes of nurses about the use of pulse oximetry, research data and nursing identifying characteristics were collected through a questionnaire developed according to the literature.

RESULTS: In order to evaluate the data, the pulse oximeter knowledge mean scores of nurses were calculated from the answers given to 28 statements. The knowledge levels of the nurses working in both clinical fields and services on the use of pulse oximeter were observed to not be at the desired level.

CONCLUSION: The fact that nurses know how to correctly use of all technological devices in the units where they work is essential for patient outcomes to be reliable and for patients to receive efficient care. The fact that hospital management regulates in-service training intermittently for the nurses employed is considered to be effective in eliminating these deficiencies.

Keywords: Knowledge, nursing, oximetry

INTRODUCTION

Respiration, the most basic physiological human need, is based on oxygen (O_2) reaching the body's cells and the removal of carbon dioxide from cells.[1] Since it cannot meet the metabolic requirements, O_2 binds to hemoglobin (Hb) and enters circulation as oxyhemoglobin. The binding rate of O_2 to Hb is defined as "O_2 saturation" and expressed as a percentage. While arterial O_2 saturation is shown as "SaO_2", peripheral O_2 saturation is shown as "SpO_2".[2,3] SaO_2 refers to the O_2 saturation of Hb in erythrocytes. The normal O_2 saturation value is between 90-100%.[4-6]

Monitoring the SpO_2 levels of patients in the assessment of the respiratory process and the vital functions of the patient and for the early detection and intervention of hypoxic conditions is one of the fundamental duties of the nurse. This level is measured and evaluated

To cite this article: Keti EP, Ünlüsoy Dinçer N. How Much do We Know About Pulse Oximeters Used in Every Field from Home Care to Critical Care? A Descriptive Study. Cyprus J Med Sci 2023;8(1):74-82

ORCID IDs of the authors: E.P.K. 0000-0003-0120-4796; N.Ü.D. 0000-0002-9578-5669.

Address for Correspondence: Emine Pınar Keti
E-mail: pinar-eminemartli@hotmail.com **ORCID ID:** orcid.org/0000-0003-0120-4796

Received: 06.02.2021
Accepted: 13.02.2022

by nurses through analysis of an invasive arterial blood gas sample or by using a pulse oximeter device non-invasively.[7]

Pulse oximeters entering into the health care scene for the first time in 1850 with Sechenov's experiments[8] and have now reached the ability to follow up patients from home by becoming android-based mobile devices enabling them to be used easily in non-clinical environments with today's technology.[9] Pulse oximeters consist of a probe and a monitor.[10] There is a photo-transmitter with light emitting diodes emitting infrared rays (at 940 nm wave width) and red light (at 660 nm wave width) on one side of the pulse oximeter probe and a photodetector receiving these light wave lengths on the other side. In the monitor section, the absorption degree of these wavelengths is determined and the O_2 saturation value and the heart rate are displayed on the screen as a wave sample.[11] The types of pulse oximeter devices are desktop, finger, portable and those which can be connected to bed-side monitors.[12] There are forehead, ear, nose, and finger probes which can be connected to pulse oximeter devices. These probes are either detachable clip-on or adhere to the skin.[6]

Pulse oximeters are easy-to-use and painless[10,11] devices which have the capability to measure arterial blood O_2 saturation, do not require calibration, have a high accuracy rate and widespread usage in the medical world. Pulse oximeters are used in patients with impaired respiratory and circulatory system, operating rooms, intensive care units, emergency departments, clinics, when transferring patients and during patient follow-up at home.[6] In addition, pulse oximeters have a great importance in determining silent hypoxia during the coronavirus disease 19 pandemic period in the current situation.[13] The use of pulse oximeters which provide information on the patient's immediate status quickly and with low cost is advantageous since they are portable and rechargeable. Since the measurements are performed with a non-invasive method, they do not have any complications such as damaging veins. However, burns arising from not replacing the pulse oximeter probes at proper intervals, allergies to sticky probes, and tissue ischemia risk are some of the disadvantages in their use.[6,14,15]

The reliability of the SpO_2 value is affected by having a clean and dry area, skin color, nail varnish and henna, perfusion insufficiency, excessive movement, ambient light, proper placement of the probe and appropriate probe selection. The fact that nurses know the working mechanism of pulse oximeters, how to use them and what affects O_2 saturation reduces measurement errors.[7,16] Knowing the factors affecting the use of pulse oximeters and the reliability of the SpO_2 values measured in pulse oximetry and following up the patient's oxygenation are important in terms of medical treatment and nursing care. The fact that nurses evaluate SpO_2 correctly and prepare a nursing care plan concerning any problems in this regard increases the efficiency of the care given to the patients.

Studies have revealed that nurses have a lack of knowledge about the use of pulse oximeter. For example, in their study, Kiekkas et al.[17] stated that the knowledge mean scores of the nurses on a total of 21 information evaluation questions were $\overline{x}=12.8\pm3.2$. In this study, it was seen that the lack of knowledge was mostly related to the working principles of pulse oximeters. In a study which was similar to the present study in terms of methodology conducted by Yıldız et al.[18] on intensive care nurses in a university hospital in Türkiye, it was observed that the knowledge mean score was 14.52 out of a total of 26 statements for the use and the principles of pulse oximeters. The results of this research show that nurses do not have enough knowledge about first aid for hypoxic patients. Apart from these, from the late 90s to the present day, we have come across several studies investigating the level of nurses' knowledge about the use pulse oximeters in many different countries.[19] Studies have shown that there was a lack of knowledge about the use of pulse oximeters, and at this point, training should be organized regarding both the use of pulse oximeters and their working principles. The study of Stathoulis et al.[20] showed the effect of training on the level of knowledge of health care personnel.

Pulse oximeters, which are known to be widely used in critical areas, have become tools which can be used in daily life from home care to critical areas, and even in mobile devices. In terms of patient care and safety, it is very important that nurses, responsible for caregiving and patient education, have sufficient knowledge and experience about pulse oximetry. For these reasons, our research was implemented in all units of a university hospital in Türkiye in order to assess the theoretical information and the behavior of the nurses regarding pulse oximetry.

MATERIALS AND METHODS

Study Design and Participants

This descriptive study was conducted in a university hospital located within the boundaries of Ankara province. The population of the study consisted of all the nurses (n=588) working in the university hospital located within the boundaries of Ankara province. This research was conducted with a total of 393 nurses who volunteered to participate in this study and were not off duty or on sick leave at the time of this study. The rate of participation in this research was 66.8%.

Data Collection

The data were collected by using a questionnaire developed by the researcher. The questionnaire consisted of three parts. In the first part, there were 15 questions about the descriptive characteristics of nurses and the use of pulse oximeters. The second part included an information test which identifies the pulse oximeter device and consists of correct or incorrect statements (a total of 28 statements) about which situations can affect the value read in pulse oximeter devices. The second part was developed specifically for this study because no instrument which captured the relevant content with measured reliability and validity was available after a literature review. There are 12 statements which assess the possible situations where nurses use pulse oximeters in the last part of the questionnaire. The nurses were asked to mark the appropriate answers from the options of "always", "occasionally" or "never" for the frequency of use of pulse oximeters.

In order to ensure the content validity of the questionnaire in this study, the opinions of three experts in the nursing field and an expert in the field of assessment and evaluation in education were obtained. Whether or not each item in the questionnaire was appropriate for the assessment instrument was evaluated by these experts at the content validity phase. The necessary modifications were made in line with the suggestions received from the experts. A preliminary application was carried out by obtaining the written consent of 20 nurses using a face-to-face interview technique through pre-scheduled appointments. Those nurses who were interviewed in the pre-practice were excluded from the final sample. The questionnaire was evaluated in accordance with the feedback received from the preliminary application and it was reassessed and arranged in terms of its flow order, function, and quality.

This study was conducted between the 15th of March and the 15th of June, 2014. The nurses who signed the informed consent form were asked to respond to demographic questions and to mark as "correct" or "incorrect" options for the knowledge expressions and to mark as "always", "occasionally" or "never" for the behavior expressions. It took approximately 20 minutes to complete the questionnaire.

Statistical Analysis

Statistical Package for the Social Sciences (SPSS) version 21.0 (IBM SPSS Corp.; Armonk, NY, USA) was used for statistical analyses. The demographic data were analyzed in percentages. We eliminated 3 questionnaire forms as they were not completely filled out. Thus, the data obtained from 393 questionnaires were analyzed. One point was given for each correct answer of the respondents to the statements, and then, the total score was calculated. Mean, standard deviation, minimum, and maximum values were used to indicate the knowledge scores of the nurses. While the normality of numerical variables was examined by the Shapiro-Wilk test, the homogeneity of variances were investigated by Levene's test. Since the parametric test conditions were not met, the Mann-Whitney U test was used to examine whether or not there was a difference between the knowledge mean scores and status of using pulse oximeter of the nurses. Kruskal-Wallis and Siegel-Castellan tests were used for the multiple group comparisons. Statistical significance was set at $p<0.05$.

Ethics Committee Approval

The study protocol was officially approved and ethics committee approval was obtained from Ankara University Ethics Committee (approval number: 160/961, date: 19.12.2013). We obtained written permission from the medical directors of the hospitals and written informed consent from the nurses who agreed to participate in this study.

RESULTS

Table 1 shows the descriptive characteristics of the nurses participating in this study. The average age of the nurses was 33.15±7.39 years and 39.7% were in the age group of 28-36 years. It was observed that the nurses participating in this study received different nursing education, and more than half of the nurses (58.5%) had a bachelor's degree in nursing, and 4.6% had postgraduate education in nursing. One out of five nurses participating in this study (20.4%) stated that they had been working as a nurse for 1-5 years; and another one out of five (20.9%) stated they had been working as a nurse for 6-10 years. 33.6% of the nurses were working in critical units, 22.4% were working in surgical units, and 21.9% were working in internal medicine units.

More than half of the nurses (62.6%) followed up their patients by using a pulse oximeter. While patient follow-up pulse oximeter use was highest for those cases requiring emergency intervention (43.3%), the rate of pulse oximeter use was 21.1% in routine/all patient follow-up. When the distribution of the body parts measured by the pulse oximeter was examined, on the finger was highest with a rate of 73.4% and on the toe was in second place with a rate of 15.7%; whereas, only one person was taking measurements from the nose (0.3%) (Table 1).

It was determined that 49.5% of the nurses received their information about pulse oximeter at school during their nursing education and 39.2% received this information in the hospital via in-service training. The rate of receiving information from journals and articles was only 4.9% among the information sources regarding pulse oximeters (Table 1).

Table 2 shows the distribution of responses given by the nurses to the pulse oximeter related statements. The "Failure to properly place the pulse oximeter probe affects the pulse oximeter value" statement was given correct answers at the highest rate (83.5%) from all the statements. The nurses gave correct answers with the highest rate (81.7%) for the statement about the measurement made on the finger among the statements about the correct placement of the light source of the pulse oximeter probe. The most incorrectly answered statements about the placement of the light source of the pulse oximeter probe were those statements about the correct measurement parts of the pulse oximeter on the forehead (89.1%), nose (78.4%) and ears (76.1%). 74.6% of the nurses gave the correct answer to the statement about the limit of the value read from the pulse oximeter and 69.2% gave the correct answer to the statement on what the pulse oximeter measures.

The nurses gave correct answers for the statements about the factors affecting the value read from pulse oximeter mostly for the proper placement of the pulse oximeter probe (83.5%), for shivering and tremors (73.5%), and for the humidity of the measurement area (71.8%). 75.6% of the nurses responded incorrectly to the effect of surgical lights on the values given by pulse oximeters (Table 2).

The knowledge mean score of the nurses to the total of 28 statements was $\overline{x}$ =15.06±6.0. This result indicated that 53.8% of the statements were known correctly by the nurses. There was no nurse who responded to all of the statements correctly.

There was no statistically significant difference between the average ages of the nurses and their knowledge mean scores ($p>0.05$). The difference between the knowledge mean scores and educational levels, working periods and the units of the nurses was found to be significant ($p<0.05$). When their educational levels were examined, it was observed that the knowledge mean scores of those nurses with a bachelor's degree were significantly higher than the knowledge mean scores of those nurses with an associate's degree in nursing and vocational health school education in advanced statistical analysis. In the pairwise comparison related to the working period, it was determined that the difference between the groups was between those nurses working for 20 years or more and those nurses working for 1-5 years and the knowledge mean scores of those nurses having a working period of 20 years or more were significantly lower. The knowledge mean scores of the nurses working in the critical units were observed to be higher than that of those working in the other units ($p<0.05$). The difference between the nurses' status of using pulse oximeter and their knowledge mean scores was determined to be significant ($p<0.05$) (Table 3).

Table 4 examines the frequency of nurses' actions of using pulse oximeters in certain possible situations. It was determined that the nurses "always" used pulse oximeter for following up patients with respiratory problems (79.4%), for following up patients using mechanical ventilators (75.1%) and for following up patients after a surgical operation (67.4%).

DISCUSSION

Pulse oximeters are very important devices for detecting hypoxic events which are considered as an additional indicator in assessing vital signs which are not visible in various situations.[21] Nurses are responsible

for knowing how patients' O_2 saturation values are measured and the correct measurement techniques in order to follow-up the patient's saturation. Therefore, the nurses' knowledge about pulse oximeters is very important in terms of the correct evaluation of the patient.

In our study, the mean score for the knowledge of nurses regarding the total of 28 statements including pulse oximeter knowledge statements was 15.06±6.0. In this study, it was observed that the knowledge mean score of the nurses was not at the expected level when considering that more than half of the nurses participating in the present study used pulse oximeter in their units and about half of the nurses were working in the critical units. This situation revealed that the nurses were conducting measurements without knowing the details of the technological device they were using. When similar studies were

Table 1. Descriptive characteristics of the nurses (n=393)

Descriptive characteristics	**n**	**%**
Age groups		
19-27	111	28.2
28-36	156	39.7
37-45	101	25.7
46+	25	6.4
Average age mean score (± SD): 33.15±7.39, minimum: 19, maximum: 53		
Educational level/graduation		
Vocational health school	77	19.6
Associate degree	68	17.3
Bachelor's degree	230	58.5
Postgraduate degree	18	4.6
Clinical experience (in years)		
<1	43	10.8
1-5	80	20.4
6-10	82	20.9
11-15	58	14.8
16-20	53	13.5
>20	77	19.6
Department		
Critical units	132	33.6
Surgical units	88	22.4
Internal units	86	21.9
Outpatient clinic	56	14.2
Mixed units	14	3.6
Special branch nursing	12	3.1
Management	5	1.2
Nurses' use of pulse oximeter in the units (n=393)		
Using	246	62.6
Not using	147	37.4
Body parts measured by pulse oximeter (n=331)†		
Finger	246	74.3
Toe	53	16.0
Earlobe	31	9.4
Nose	1	0.3
Place where the training/information on pulse oximeter was received (n=416)†		
At school during nursing education	163	49.5
In the hospital with in-service training	129	39.2
Experience, learning from other occupational members	68	17.3
The internet	37	11.2
Related journals and articles	16	4.9
Conferences, courses, seminars	3	0.8

†Since the nurses responded to more than one option, "n" is multiplied, SD: Standard deviation, min: Minimum, max: Maximum

examined,[17,18,22-24] we came across several studies, the results of which are compatible with the results of our research. In the study of Yıldız et al.[18] that was conducted with nurses of an intensive care unit at a university hospital in Türkiye, it was observed that nurses had relatively low averages on their knowledge scores for the use of pulse oximeters. In their study, the insufficiency of the knowledge score average was explained by the fact that most of the participants gained their first experience on pulse oximeter use during their clinical practices and they did not receive sufficient formal education on pulse oximetry. In the study of Nimbalkar et al.[22] from India, it was noted that the average knowledge score for the use of pulse oximeter was below expectations and the average knowledge scores of the nurses with formal education were higher for theoretical knowledge questions. The study of Milutinovic et al.[23] from Serbia stated that the average knowledge scores were low mainly for those questions regarding the principles of pulse oximeter use and an increase in the level of knowledge for this is a situation which can be achieved with formal education, not through experience. On the other hand, Kiekkas et al.[17] stated that knowledge is mostly obtained with clinical experience rather than formal education with regards to low levels of pulse oximeter knowledge score averages. Seeley et al.[24] stated that there were considerable deficiencies regarding the theoretical factors affecting the practice and interpretation of pulse oximeters in their study with fresh graduates who had some clinical experience. The findings of their study are similar to the knowledge score average in our study. In our study, half of the participants had received information about pulse oximeter at school during their formal education, and nearly half of them had received in-service training. The low number of those who had received formal education and those who had received in-service training after graduation may explain the result that the knowledge point average was not at the expected level. In addition, the research conducted by Elliot and Williamson[25] in 2020 offers a new perspective on the lack of knowledge of nurses on the use of pulse oximeters. Elliot and Williamson[25] examined the lecture notes about pulse oximetry in 32 current nursing books used by undergraduate students and concluded that superficial and variable information may be one of the factors associated with this lack of

Table 2. Distribution of nurses' responses to statements about pulse oximetry (n=393)

No	Statements	Those who gave the correct answers	
		n	%
1.	Pulse oximeters are an invasive assessment tool	269	68.4
2.	The adhesive pulse oximetry sensors are disposable†	165	42.0
3.	Heart rate is assessed by pulse oximeters†	245	62.3
4.	Respiratory rate is measured by pulse oximeters	245	62.3
5.	Partial oxygen pressure is measured by pulse oximeters	100	25.4
6.	Pulse oximeters measure the amount of oxygen carried by hemoglobin†	272	69.2
7.	When measuring from the finger, the light source of the pulse oximeter should be on the nail†	321	81.7
8.	The location of the pulse oximeter probe should be changed every 8 hours	80	20.4
9.	Pulse oximeter probes may cause burns if they remain in the same area for a long time†	101	25.7
10.	In the forehead measurement, the light source of pulse oximeter should be placed just above the eyebrows by centering the iris†	43	10.9
11.	If the value read from the pulse oximeter is 80, then the patient does not need oxygen support	293	74.6
12.	The region in which the pulse oximeter probe is placed should be evaluated for allergic reactions†	196	49.9
13.	In measurements on the ears, the light source of the pulse oximeter should be on the tragus	94	23.9
14.	In measurements on the nose, the light source of the pulse oximeter should be on the nose wing†	85	21.6
15.	The pulse oximeter value is affected by the humidity of the measurement area of pulse oximeter†	282	71.8
16.	Dried liquid or blood on the pulse oximeter probe does not affect the pulse oximeter value	266	67.7
17.	Failure to place the pulse oximeter probe properly affects the pulse oximeter value†	328	83.5
18.	Surgical lights on the pulse oximeter probe do not affect the pulse oximeter value	96	24.4
19.	Hypothermic state affects the reading time of the pulse oximeter device†	275	70.0
20.	Carbon monoxide poisoning does not affect pulse oximeter values	252	64.1
21.	Dark nail polishes affects pulse oximeter values†	265	67.4
22.	Fake nails affect pulse oximeter values†	267	67.9
23.	The presence of anemia does not affect pulse oximeter values	201	51.1
24.	Excessive movement of the probe-inserted extremity affects shivering and tremor pulse oximeter value†	289	73.5
25.	Hypotension affects pulse oximeter values†	184	46.8
26.	The probe attached to the extremity where the patient follow-up instruments are located does not affect pulse oximeter values	244	62.1
27.	Peripheral vascular disease affects pulse oximeter values†	271	69.0
28.	A tight connection of the pulse oximeter probe does not affect pulse oximeter values	191	48.6

†Correct statements

knowledge. When the contents of the books were examined, it was determined that there was not enough information on how the pulse oximeter works, what the pulse oximeter measures and how O_2 enters the tissues, and there was no correlation between those resources in terms of conveying the information completely.[25]

Unlike these studies, it was seen in the study of Raza et al.,[26] (USA, 2019) that the knowledge score average of the participants was higher than ours and the other studies we discussed above. It is thought that this difference arises from the differences in the nursing curriculums between countries.[26] As in the countries where these studies were conducted,[17,18,22-24] there is no examination for this field after undergraduate graduation in Türkiye. However, U.S. nursing candidates must pass the national NCLEX exam upon successful completion of their nursing school to be eligible to work as a Registered Nurse.[26] This exam covers a variety of basics, including information on the respiratory

Table 3. Distribution of the knowledge mean scores on pulse oximetry according to the descriptive characteristics of the nurses (n=393)

Demographical variables	Knowledge mean score (for 28 statements)				
	Number	Mean ± SD	Min.	Max.	Pair-wise comparison
Age groups					
19-27	111	16.51±4.2	2	25	
28-36	156	15.47±5.5	0	25	
37-45	101	13.35±7.2	0	26	-
46+	25	13.00±8.5	0	24	
Evaluation†	**KW=7.623; p>0.05**				
Educational level/graduation					
1. Vocational health school	77	13.66±6.3	0	24	2-3
2. Associate degree	68	10.43±8.0	0	22	2-4
3. Bachelor's degree	230	16.73±4.1	1	26	1-3
4. Postgraduate degree	18	17.33±5.7	6	25	-
Evaluation‡	**KW=39.636; p<0.001**				
Clinical experience (in years)					
1. <1	43	15.37±4.1	2	22	
2. 1-5	80	16.94±3.7	7	25	
3. 6-10	82	15.82±5.6	0	25	
4. 11-15	58	15.07±5.3	0	23	2-6
5. 16-20	53	14.72±6.7	0	25	
6. >20	77	12.38±7.9	0	26	
Evaluation‡	**KW=13.308; p<0.05**				
Unit					
1. Critical units	132	16.70±4.5	2	25	
2. Surgical units	88	15.10±5.3	0	22	
3. Internal units	86	15.49±5.2	0	23	
4. Outpatient clinic	56	10.82±8.7	0	24	1-4
5. Mixed units	14	15.29±5.4	0	21	
6. Special branch nursing	12	13.00±8.25	1	26	
7. Management	5	15.90±3.9	11	21	
Evaluation‡	**KW=17.837; p<0.05**				
Use of pulse oximeters in units by nurses					
Using	246	16.98±4.0	0	25	
Not using	147	11.86±7.2	0	26	-
Total	393	15.06±6.0	0	26	
Evaluation†	**Z=-6.920; p<0.001**				
In-service training on the use of pulse oximeter					
Trained	129	16.86±4.1	0	25	
Not trained	200	16.20±4.7	0	26	-
Total	329	16.46±4.5	0	26	
Evaluation†	**Z=-1.120 p>0.05**				

†Kruskal-Wallis test was conducted. ‡Kruskal-Wallis and Siegel Castellan tests were conducted. SD: Standard deviation, Min.: Minimum, Max.: Maximum

system, such as pulse oximetry. For this reason, students who are preparing for this exam are encouraged to have sufficient knowledge about pulse oximetry. Thus, the knowledge of the student remains up to date after graduation; the high knowledge scores of the nurses participating in the U.S. study can be explained by this situation.[26]

It was observed that the knowledge mean scores of those nurses who had higher education, received in-service training, had a working period of 1-5 years, and were working in critical units were higher. This can be explained by the fact that the education of newly graduated nurses is more up-to-date compared to those nurses who have been working for 6 years or more, and these new graduates are employed more often in critical units in our country and they use technology more effectively. The fact that technological devices are used more frequently in critical areas indicates that the knowledge levels of the nurses working in these units are higher. In addition, this situation reveals the effect of in-service training on the importance of updating information. Stathoulis et al.[20] emphasize the effectiveness and importance of training related to the use of pulse oximeters in their research which was carried out with 78 nurses in Greece. It was stated in their research that the average knowledge score increased significantly after training. Thus, it can be concluded that in-service training increases the knowledge levels of nurses. Unlike the results of our study, it was reported that the knowledge point averages of those nurses with a working period of more than 10 years were found to be higher in the study of Kiekkas et al.[17] In the results of Nimbalkar's[22] study conducted in a tertiary hospital in India, no significant difference was found among the pulse oximeter knowledge scores between those nurses working for less than one year and for those working for more than one year, and between those nurses working in the intensive care unit and those working in other units.

When the distribution of the responses given by the nurses to the statement "what pulse oximetry measures" was examined, it was observed that they generally did not have exact knowledge on what a pulse oximeter measures. It was observed that the nurses knew that O_2 saturation is measured by pulse oximeters but they did not know the connection between O_2 saturation and PaO_2. This makes us think that nurses only superficially use these medical devices in the clinical setting. When the studies conducted in different countries were examined,[18,22,27] their results were found to be compatible with the results of the present study. In the study conducted by Yıldız et al.[18] with 72 intensive care nurses in Türkiye, it was stated that "Pulse oximetry measures partial O_2 pressure" was the most frequently given wrong answer among the information propositions regarding pulse oximetry. In the study conducted by Bader in Saudi Arabia[27] which had similar features in terms of the method performed, it was stated that only 22% of the participants among 66 pediatric nurses gave the correct answer to "what the pulse oximeter measures". In Bader's study, it was stated that while 33% of the nurses knew the measurement value of the O_2 saturation read in the pulse oximeter, only 12% of them answered the measurement percentage of PaO_2 correctly. In the study of Milutinovic et al.[23] in Serbia, it was seen that there was a lack of knowledge on the meaning of pulse oximeter values and how to interpret them.

It can be asserted that there is a general lack of theoretical knowledge about the use of pulse oximeters by nurses, even when the education differences between countries were considered. In addition, these findings suggest that nurses access information from other professional members through a master-apprentice relationship without obtaining information from a guide or source on the subject and they gain experience without establishing a cause-effect relationship.

It was observed that more than half of the nurses gave the correct answer to the statement about the measurement made on the finger from the statements including the correct placement areas of the pulse oximeter probe on the body regions measured and they responded to the other statements at low rates. This is thought to be associated with the fact that measurements are performed mostly on the finger in the hospital where the study was conducted.

Correct answers were given mostly to those statements of proper placement of the pulse oximeter probe, and shivering and tremor among those factors affecting the value given by pulse oximeters. When considering that nearly half of the nurses participating in the present study were working as critical field nurses, the high level of response to this statement was a positive result. Conditions due to perfusion failure

Table 4. Nurses' status of evaluating the frequency of behavior related to the use of pulse oximeter in certain possible situations (n=393)

No	Behavior/action statements	Always		Sometimes		Never	
		No	(%)	No	(%)	No	(%)
1	I use a pulse oximeter during transfers between clinics.	100	25.4	189	48.1	104	26.5
2	I use a pulse oximeter in the follow-up of patients taking vasodilators and sedative drugs.	201	51.1	104	26.5	88	22.4
3	I use a pulse oximeter in the follow-up of patients taking inotropic drugs.	179	45.5	94	23.9	120	30.5
4	In addition to blood pressure, pulse rate, respiratory rate, and the body temperature, I measure and record the values read on the pulse oximeter on the first admission of patients to the clinic.	181	46.1	120	30.5	92	23.4
5	I use a pulse oximeter in the follow-up of patients connected to mechanical ventilators.	295	75.1	34	8.7	64	16.3
6	I use a pulse oximeter during the insertion of central catheter.	205	52.2	99	25.2	89	22.6
7	I use a pulse oximeter during invasive diagnostic methods related to chest diseases.	264	67.2	59	15.0	70	17.8
8	I use a pulse oximeter during invasive diagnostic procedures for the determination of cardiovascular diseases.	230	58.5	81	20.6	82	20.9
9	I use a pulse oximeter during the interventional procedures used in the diagnosis of gastrointestinal system diseases.	129	32.8	132	33.6	132	33.6
10	I use a pulse oximeter in patient follow-ups in the post-operative period.	265	67.4	61	15.5	67	17.0
11	I use a pulse oximeter in the follow-up of patients with respiratory problems.	312	79.4	35	8.9	46	11.7
12	I use a pulse oximeter in the follow-up of patients undergoing hemodialysis.	173	44.0	124	31.6	96	24.4

may cause the value read on the pulse oximeter to be misinterpreted. Accordingly, it was determined that the nurses participating in our study generally gave correct answers to the questions on this subject. In the study conducted by Yıldız et al.[18], it was stated that 70.8% of the nurses who frequently used pulse oximeters knew that a moving extremity had an effect on the measurement reliability of the pulse oximeter. These findings show similar results with our study. Similarly, most of the nurses correctly answered that pulse oximeters cause false readings in cases of perfusion failure and in cases of movement in the studies of Milutinovic et al.[23], Seeley et al.[24] and Giuliano and Liu.[28]

Since the pulse oximeter is designed to detect oxygenated and reduced Hb types, it causes incorrect SpO_2 reading in the presence of other Hb types such as methemoglobin and carboxyhemoglobin. More than half of the nurses participating in the present study knew that carbon monoxide poisoning has an effect on the value read on pulse oximeters. The high rate of correct answers to this statement was an important result for our study. At the same time, in our study, we saw that critical field nurses had high averages on their knowledge scores. Especially in emergency services, nurses who have high levels of knowledge in this field may play an active role in the treatment of patients with carbon monoxide poisoning. In the study conducted by Lee et al.[29] with 44 emergency workers through a similar method, most of the participants correctly knew that carbon monoxide poisoning has an effect on the pulse oximeter value, in parallel with our research results.

It is remarkable that the frequency of following up a patient's vital results and also their O_2 saturation value with pulse oximeter use in possible cases was given as "always" at a rate of only 46.1%. The fact that this behavior was not always carried out indicated that the nurses participating in the present study did not evaluate the O_2 saturation assessment as a vital result and pulse oximeters were not always used when necessary.

In our study, we saw that the frequency of using pulse oximeters in situations which required supplementary O_2 was quite high when the actions of the nurses regarding these possible situations was examined (Table 4). It was seen in the study of Milutinovic et al.[23] that most of the nurses stated that a pulse oximeter should be used when patients received O_2 support.

The generalizability of our findings is limited due to the fact that our study was a single-center study. Although this study was conducted in a medical faculty hospital in a large city in Türkiye, it revealed the conclusion that the nurses' knowledge about pulse oximetry was not at the desired level. Our results indicated a significant gap about pulse oximeters and their measurement and so we can conclude that continuous training is needed. The place of pulse oximeters in the evaluation of vital signs and also that the device is a quick source of information about the oxygenation of patient are supported by the literature. It is thought that the present study can help to determine the training needs of nurses regarding the evaluation of O^2 saturation, to create a protocol about the use of pulse oximeters by hospital management, to eliminate any deficiencies which may be experienced in practice, to enhance the quality of patient care and to contribute to the literature.

MAIN POINTS

- The measurement of oxygen saturation is considered as an additional indicator for measuring vital signs.
- The measurement of oxygen saturation by pulse oximeters is a part of nursing care allowing for rapid evaluation of patients.
- Training should be regulated to meet the knowledge gaps regarding pulse oximeters and their measurements.

ETHICS

Ethics Committee Approval: The study protocol was officially approved and ethics committee approval was obtained from Ankara University Ethics Committee (approval number: 160/961, date: 19.12.2013).

Informed Consent: We obtained written permission from the medical directors of the hospitals and written informed consent from the nurses who agreed to participate in this study.

Peer-review: Externally peer reviewed.

Authorship Contributions

Concept: E.P.K., N.Ü.D., Design: E.P.K., N.Ü.D., Data Collection and/or Processing: E.P.K., N.Ü.D., Literature Search: E.P.K., N.Ü.D., Writing: E.P.K., N.Ü.D.

DISCLOSURES

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

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

REFERENCES

1. Sungur G. Solunum fonksiyonları. Ovayolu N, Ovayolu Ö, editors. Patafizyoloji Pratik Bir Yaklaşım. Adana: Çukurova Nobel Tıp Kitapevi; 2016. p. 115-54.
2. Marino P. The ICU Book. Yorgancı K, İskit AT, editors. Ankara: Palme Publishing; 2009.
3. Widmaier EP, Raff H, Strang KT. Human Physiology. Demirgören S, editor. Vander's Human Physiology: the Mechanismas of Body Function. İzmir: Güven; 2010.
4. Dewit SC. Fundamentals Concepts and skills for nursing (3nd ed.). California: Saunders; 2009.
5. Nitzan M, Romem A, Koppel R. Pulse oximetry: fundamentals and technology update. Med Devices (Auckl). 2014; 7: 231-9.
6. Lynn PB. Wolters Kluwer Health, Lippincott Williams & Wilkins. Taylor's Clinical Nursing Skills - A Nursing Process Approach. Bektaş H, editor. Ankara: Nobel Akademik Yayıncılık; 2015.
7. Craven RF, Hirnle C, Jensen S. Fundamentals of nursing human health and function. N. Uysal, E. Çakırcalı, editors. Ankara: Palme Publishing; 2015.
8. Zislin B, Chistyakov A. The history of pulse oximetry. Biomedikal Engineering. 2006; 40(1): 53-6.
9. Eriş Ö, Korkmaz H, Toker K, Buldu A. PIC Microcontroller Based Wireless Pulse-Oximeter Measurement System Design for Patient Monitoring over WEB and LabVIEW Applications. Available from: URL: https://turkmia.net/kongre2010/cd/bildiriler/16-25%20Omer%20ERIS.pdf
10. Chan ED, Chan MM, Chan MM. Pulse oximetry: Understanding its basic principles facilitates appreciation of its limitations. Respir Med. 2013; 107(6): 789-99.
11. Booker R. Pulse oximetry. Nursing Standart. 2008; 22(30): 39-41.
12. Fetzer SJ. Vital Sign and Physical Assessment. Perry AG, Potter PA, Ostendorf WR, editors. Clinical nursing skills & techniques. Canada: Elsevier; 2013. pp. 101-2.

13. Starr N, Bitew S, Wilson I, Weiser TG, Rebollo D, Asemu YM. Pulse oximetry in low-resource settings during the COVID-19 pandemic. Lancet Glob Health. 2020; 8(9): e1121-2.

14. Kwak HY, Kim JI. Pulse oximetry-induced third-degree burn in recovery room. ANZ J Surg. 2009; 79(4): 307-8.

15. Murphy KG, Secunda JA, Rockoff MA. Severe burns from a pulse oximeter. Anesthesiology. 1990; 73: 350-2.

16. Martlı EP, Ünlüsoy Dinçer N. Doğru ve güvenli pulse oksimetre kullanımı. Journal of Human Sciences. 2020; 17(1): 369-79.

17. Kiekkas P, Alimoutsi A, Tseko F, Bakalis N, Stefanopoulos N, Fotis T, Konstantinou E. Knowledge of pulse oximetry: comparison among intensive care, anesthesiology and emergency nurses. J Clin Nurs. 2012; 22: 828-37.

18. Yıldız D, İyigün E, Fidancı BE. Knowledge levels of intensive care nurses regarding usage of pulse oximetry in a university hospital in Türkiye. Health Med. 2012; 6(3): 832-9.

19. Elliott M, Tate R, Page K. Do clinicians know how to use pulse oximetry? A literature review and clinical implications. Aust Crit Care. 2006; 19(4): 139-44.

20. Stathoulis J, Tsironi M, Konofaos N, Zyga S, Alikari V. Evaluation of pulse oximetry knowledge of Greek registered nurses. Adv Exp Med Biol. 2017; 988: 89-96.

21. Çakırcalı E. Vital Signs. AT. Aştı, A. Karadağ (Ed). Hemşirelik Esasları Bilgiden Uygulamaya: Kavramlar-İlkeler-Beceriler 1. İstanbul, Akademi Yayıncılık; 2016.p.603-10).

22. Nimbalkar S, Bansal SC, Patel CL, Patel DV, Patil KH, Nimbalkar AS. Clinical competency in pulse oximetry among medical professionals and nursing personnel in a tertiary care hospital. Journal of Clinical & Diagnostic Research. 2018; 12(9): 9-13.

23. Milutinovic D, Repic G, Arandelovic B. Clinical nurses' knowledge level on pulse oximetry: A descriptive multi-centre study. Intensive Crit Care Nurs. 2016; 37: 19-26.

24. Seeley MC, McKenna L, Hood K. Graduate nurses' knowledge of the functions and limitations of pulse oximetry. Journal of Clinical Nursing. 2015; 24(23-24): 3538-49.

25. Elliott M, Williamson R. Do nursing textbooks accurately describe pulse oximetry? An audit of current literature. Br J Nurs. 2020; 29(11): 594-600.

26. Raza O, Ayalew D, Quıgg A, Flowers H, Lamont K, Golden L, Murphy WP. Pulse oximetry understanding and application: a survey on nursing staff. Journal of Clinical & Diagnostic Research. 2020; 14(12): 10-4.

27. Bader RS. Basic knowledge of the clinical aplications of pulse oximetry technology among health care professionals in pediatrics. Journal of the Saudi Heart Association 2007; 19(3): 142-8.

28. Giuliano KK, Liu LM. Knowledge of pulse oximetry among critical care nurses. Dimens Crit Care Nurs. 2006; 25(1): 44-9.

29. Lee LLY, Yeung KL, Lo WYL, Chan JTS. Pulse oximetry: a survey of knowlwdge among staff of an emergency department. Hong Kong J Emerg Med. 2006; 13(4): 197-204.