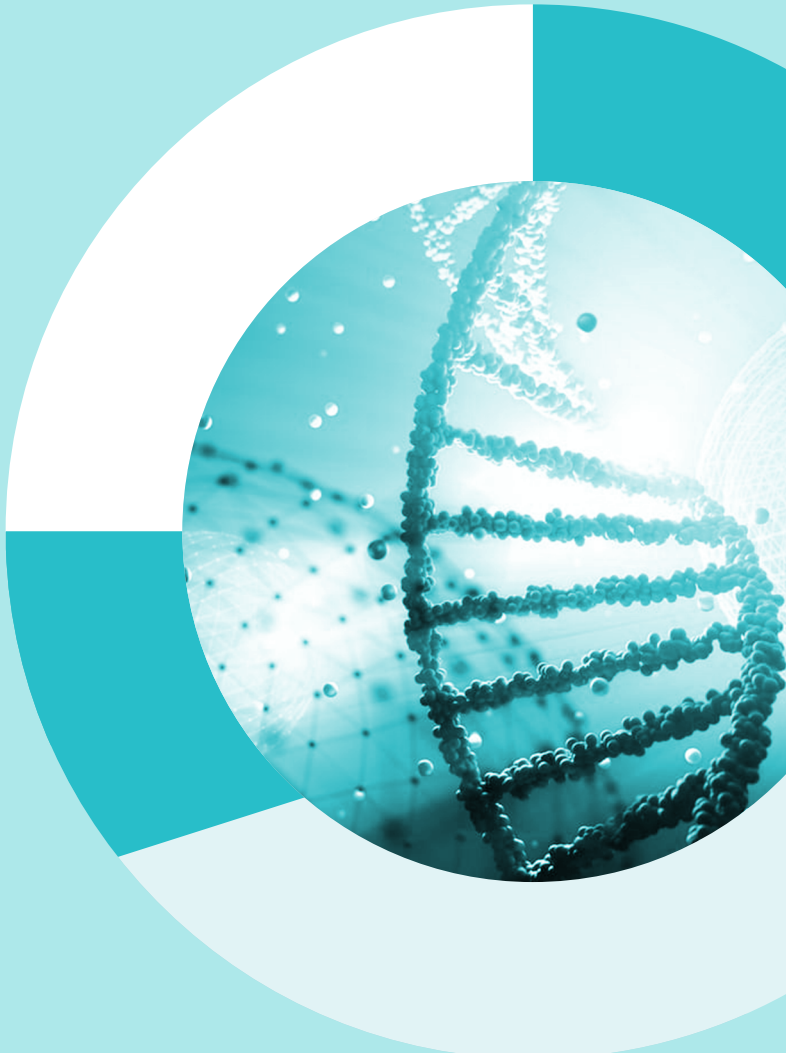
ISSN:2149-7893 E-ISSN: 2536-507X

CYPRUS

JOURNAL OF MEDICAL SCIENCES

Indexed in the Web of Science

Volume: 8 Issue: 3 June 2023



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: 3 | June 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: Haziran 2023/June 2023
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: 3 | June 2023

EDITORIAL BOARD

CYPRUS
JOURNAL OF MEDICAL SCIENCES
Indexed in Web of Science
Volume: 8 | Issue: 3 | June 2023

EDITORIAL BOARD

Biostatistical Editors

National Advisory Board

CYPRUS
JOURNAL OF MEDICAL SCIENCES
Indexed in Web of Science
Volume: 8 | Issue: 3 | June 2023

EDITORIAL BOARD

CYPRUS
JOURNAL OF MEDICAL SCIENCES
Indexed in Web of Science
Volume: 8 | Issue: 3 | June 2023

EDITORIAL BOARD

International Advisory Board

CYPRUS
JOURNAL OF MEDICAL SCIENCES
Indexed in Web of Science
Volume: 8 | Issue: 3 | June 2023

EDITORIAL BOARD

CYPRUS
JOURNAL OF MEDICAL SCIENCES
Indexed in Web of Science
Volume: 8 | Issue: 3 | June 2023

EDITORIAL BOARD

CYPRUS
JOURNAL OF MEDICAL SCIENCES
Indexed in Web of Science
Volume: 8 | Issue: 3 | June 2023

EDITORIAL BOARD

CYPRUS
JOURNAL OF MEDICAL SCIENCES
Indexed in Web of Science
Volume: 8 | Issue: 3 | June 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: 3 | June 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: 3 | June 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: 3 | June 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: 3 | June 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: 3 | June 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: 3 | June 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: 3 | June 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: 3 | June 2023

CONTENTS

REVIEW

CYPRUS
JOURNAL OF MEDICAL SCIENCES

DOI: 10.4274/cjms.2023.2022-60
Cyprus J Med Sci 2023;8(3):166-172

The Reciprocal Relationship between Inflammation and Diabetes: Importance of Medical Nutrition Therapy

Mustafa Hoca

Department of Nutrition and Dietetics, Near East University Faculty of Health Sciences, Nicosia, North Cyprus

Abstract

Diabetes, which can be defined as an inflammatory disease, is a common condition. While the presence of inflammation can cause diabetes, in reverse it can also be caused by diabetes. Inflammation-related mechanisms can deteriorate the effect of insulin, causing hyperglycemia and insulin resistance. Various factors such as nutrition, physical activity, medication, and insulin are involved in the management of diabetes. The potential mechanisms regarding diabetes, inflammation, and nutrition were examined using the PubMed, ScienceDirect, Medline, EBSCO, Google Scholar, Wiley Online Library, BioMed Central, SpringerLink, Taylor & Francis, and Web of Science databases. In particular, anti-hyperglycemic and anti-inflammatory dietary recommendations are important in terms of maintaining homeostasis in the body. In this way, in addition to body weight control, improvements in biochemical indicators related to diabetes and inflammation are attained. Furthermore, with the improvement in inflammation, future diabetic complications can be prevented by providing glycemic control. Therefore, in this review, the mechanisms underlying diabetes and inflammation are explained and the potential effects of nutritional components on diabetes and inflammation are discussed.

Keywords: Diabetes, insulin, inflammation, cytokine, medical nutrition therapy

INTRODUCTION

Inflammation is a natural reaction of the immune system against injury, infection, and fear. In other words, inflammation is a complex biological response of vascular tissue to harmful stimuli (irritants etc.) to eliminate them. In response of the immune system to physiological and metabolic stress, pro-inflammatory molecules (such as adipokines and cytokines) are produced. These cell signaling components take part in cell-to-cell interaction, allowing cells to move to the site of inflammation in the case of infection and injury. That is why the immune system and inflammation are closely linked. Inflammation can be local or systemic and acute or chronic.[1]

In diabetes, certain changes occur in immune system components (such as macrophages and T-cells). The most prominent changes occur in leukocytes, which take part in the adipose tissue, liver, pancreas, vascular system, and circulation. These immunological changes affect the levels of certain cytokines. Thus, inflammation is contained in diabetes pathogenesis.[2]

Diabetes is a chronic disease that causes low-grade inflammation. In conditions such as heart diseases and metabolic syndrome, which are closely related to diabetes, concentrations of cytokines in the circulation increase. Inflammatory cytokines are generated by various types of cells and released into the circulatory system. Cytokines have regulatory functions by acting locally, centrally or peripherally in various tissues such as adipose and muscle tissues. In the case of low-grade systemic inflammation, there is a 2-to 3-fold increase in systemic plasma tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and C-reactive protein (CRP) levels. These cytokines play important roles in the development of diabetes.[3]

To cite this article: Hoca M. The Reciprocal Relationship between Inflammation and Diabetes: Importance of Medical Nutrition Therapy. Cyprus J Med Sci 2023;8(3):166-172

ORCID ID of the author: M.H. 0000-0003-3609-5868.

Address for Correspondence: Mustafa Hoca
E-mail: mustafa.h.91@hotmail.com
ORCID ID: orcid.org/0000-0003-3609-5868

Received: 06.12.2022
Accepted: 27.03.2023

Nutrition plays an essential role in diabetes in which inflammation occurs. Omega-3 (n-3) polyunsaturated fatty acids with anti-inflammatory properties, antioxidant vitamins in vegetables and fruits, and whole grain or low glycemic index foods are among the nutritional strategies against diabetes. In addition, it should be aimed to reach and provide a healthy body weight by decreasing the saturated fat intake and enhancing the intake of complex carbohydrates.[4]

Adipose tissue is an important endocrine organ that exhibits pro- or anti-inflammatory effects and releases the bioactive substances known as adipokines. Disruption of the balance between these anti- and pro-inflammatory adipokines causes dysfunction in adipose tissue and is critical in the pathogenesis of metabolic diseases (diabetes etc.).[5]

In this review, the mechanisms underlying diabetes and inflammation are explained and the potential effects of nutritional components on diabetes and inflammation are discussed.

MATERIALS AND METHODS

Literature research was carried out using selected websites containing PubMed, ScienceDirect, Medline, EBSCO, Google Scholar, Wiley Online Library, BioMed Central, SpringerLink, Taylor & Francis, and Web of Science. The information in the article was obtained using keywords such as "inflammation, diabetes, cytokine, pro-inflammatory, anti-inflammatory, adipokine, insulin resistance, hyperglycemia, nutrition, inflammation and diabetes, inflammation and nutrition, diabetes and nutrition." In studies on diabetes, inflammation, and nutrition, animal studies and clinical human studies were reviewed. In addition, review, randomized controlled, and meta-analysis studies were also examined.

Relationship of Pro- and Anti-inflammatory Adipokines with Diabetes

TNF-α: It is an inflammatory indicator greatly related with diabetes. TNF-α, which is also related with obesity and insulin resistance, is produced from adipose tissue and secreted from macrophages, T-cells, and natural killer cells. Chronically increased TNF-α has deleterious effects on glucose metabolism. TNF-α can vary the sensitivity of insulin in various ways, such as reducing insulin receptor (IR) signal pathways, reducing glucose transporter 4 (GLUT 4) function in adipocytes and repressing adiponectin.[3] In a study conducted with diabetic individuals; it was stated that TNF-α negatively affect the functioning of intracellular GLUT and IR.[6]

IL-6: IL-6 is a pro-inflammatory cytokine that can cause disruption of the insulin-mediated phosphorylation process by acting on insulin receptor substrate-1 and -2 (IRS-1 and -2) in liver and skeletal muscles. Therefore, IL-6 can inhibit the action of insulin.[7] Atlı et al.[8] reported that IL-6 concentrations increased in individuals with diabetic and non-diabetic retinopathy.

Adiponectin: Adiponectin, another cytokine related to insulin sensitivity and produced by adipose tissue, also has an important place in relation to diabetes. High adiponectin levels are related to increased insulin sensitivity and lower cardiovascular disease risk. Adiponectin can reduce glucose production (in liver) by suppressing gluconeogenic enzymes such as phosphoenolpyruvate carboxykinase and glucose-6 phosphatase. Therefore, it can improve glycemic control and insulin sensitivity. In addition, TNF-α and IL-6 can diminish the adiponectin expression. Furthermore, adiponectin exerts an anti-inflammatory effect by repressing TNF-α-dependent nuclear factor kappa B (NF-κB) stimulation.[3] In the study conducted in 154 newly diagnosed type 2 diabetic and 1077 newly diagnosed pre-diabetic individuals; adiponectin concentrations were detected to be negatively related with pre-diabetes and diabetes.[9]

Visfatin: It is a cytokine responsible for regulating insulin sensitivity. In addition, insulin resistance has a significant function in inflammation and diabetes pathogenesis. Visfatin binds to the IR. It can also cause a decrease in blood glucose by diminishing the release of glucose by liver and stimulating the glucose utilization in adipose tissue and muscles.[10] According to Mir et al.[11]; visfatin concentrations were detected to be considerably higher in type 2 diabetic individuals.

Resistin: It is a hormone with the potential to cause insulin resistance. It functions in glucose tolerance and glucose homeostasis. In a study involving 124 individuals, serum resistin concentrations were detected to be significantly higher in overweight or obese people with impaired glucose tolerance compared with individuals with normal body weight.[12] Azab et al.[13] Showed that resistin levels were detected to be higher in diabetic people than in those who were not. In addition, a relationship was determined between resistin levels and diabetes-related risk factors (insulin resistance and obesity, etc.). Furthermore, resistin levels were found to be higher in individuals with retinopathy, a complication of diabetes.[13]

IL-1β: It is a cytokine that can disrupt insulin secretion and induce β-cell apoptosis. In addition to affecting β-cell function, it has an effect on type 2 diabetes development by causing a decline in the mass of β-cell.[14] Mojtaba et al.[15] Conducted a study in individuals with diabetes (30 people) and without diabetes (36 people). In this study, insulin resistance, fasting blood glucose, and serum IL-1β concentrations were detected to be significantly greater in diabetic individuals; serum insulin levels and β-cell function were detected to be significantly lower. In individuals with diabetes, serum IL-1β concentrations were negatively correlated with insulin levels and β-cell function, while positively correlated to fasting blood glucose. Additionally, it was concluded that IL-1β (inflammatory cytokine) has a crucial function in insulin secretion by β-cells.[15]

IL-10: It is one of the anti-inflammatory cytokines secreted from macrophages and lymphocytes. It also acts as a role in reducing inflammation induced by TNF-α, IL-6, and IL-1. Additionally, IL-10 has a potential effect to increase insulin sensitivity.[16,17] In a study conducted in Indian population, the association between IL-10 gene promoter polymorphisms and type 2 diabetes risk was assessed and it was concluded that the type 2 diabetes risk increased in those with IL-10 gene promoter polymorphisms.[18]

CRP: CRP, a cytokine produced by the liver, is an indicator responsible for the development of inflammation. CRP concentration increases in situations such as diabetes, obesity, coronary heart diseases, sedentary lifestyle, and smoking. While there is an increase in CRP production from the liver with the release of TNF-α and IL-6, CRP can also be released from mature adipocytes via lipopolysaccharide, resistin, and TNF-α.[3] The cut-off point of 3 mg/L for the CRP level is used to identify high- and low-risk groups.[19] According to an 11-year prospective study; individuals with a CRP value of ≥3 mg/L developed diabetes. Individuals with high serum CRP levels were shown to have a higher risk of developing type 2 diabetes within 5 years.[20] Regarding the CRP level as a cardiovascular risk marker in individuals with diabetes; it is considered that CRP concentrations 0 - <1 mg/L indicate low risk, 1-3 mg/L indicate

moderate risk, >3-10 mg/L indicate high risk, and >10 mg/L indicate an indeterminate increase.[21]

The relationship of pro- and anti-inflammatory factors with diabetes is shown in Table 1.[3,7,10,12,14,16,17,22]

Adipose Tissue and Insulin Resistance

The function of adipose tissue under normal conditions is to store circulating free fatty acids as triglycerides in adipocytes. Adipose tissue suppresses the negative effects of lipids in the insulin pathway. As a result, glucose utilization by cells (especially skeletal muscle cells) increases and lipolysis of stored triglycerides is inhibited. Having enough intracellular glucose in peripheral tissues inhibits glucose output from the liver. In the case of obesity; insulin resistance, chronic inflammation, and dysfunction in adipose tissue occur. Disturbance in adipokine secretion also occurs. As a result, lipolysis and the release of free fatty acids by adipose tissue increase. The function of the insulin pathway also decreases. However, the glucose output from liver is stimulated.[23]

The increased reactive oxygen species (ROS) formation in adipose tissue leads to an increase in oxidative stress in the blood. In the case of increased oxidative stress, an enhancement in nicotinamide adenine dinucleotide phosphate oxidase and a reduction in antioxidant enzymes occur. Consequently, the production of adipokines (in adipose tissue) is impaired. Plasminogen activator inhibitor-1, TNF-α, and monocyte chemotactic protein-1 levels increase, whereas adiponectin levels decrease. Therefore, decrease in insulin sensitivity can occur and insulin resistance can develop.[24]

Mitochondrial dysfunction in white adipose tissue in humans adversely affects insulin sensitivity in the liver and skeletal muscles. Because of this dysfunction, there is a decline in lipogenesis and an enhancement in lipolysis in white adipose tissue. Therefore, depending on the increase in glycerol and fatty acids and the release of IL-1β and TNF-α, glucose uptake in skeletal muscles and liver decreases and ectopic lipid accumulation occurs.[25]

Table 1. The relationship of pro- and anti-inflammatory factors with diabetes[3,7,10,12,14,16,17,22]

Factors	Potential effects
TNF-α	Insulin resistance ↑ Insulin receptor signal ↓ GLUT 4 ↓ Adiponectin ↓
IL-6	Insulin resistance ↑ Insulin action ↓ Adiponectin ↓
Adiponectin	Insulin sensitivity ↑ Glucose production ↓ Gluconeogenic enzymes ↓ TNF-α-mediated NF-κB ↓
Visfatin	Blood glucose ↓ Glucose utilization ↑
Resistin	Insulin resistance ↑
IL-1β	Insulin secretion ↓ Beta cell mass ↓
IL-10	Insulin sensitivity ↑

GLUT 4: Glucose transporter 4, IL-10: Interleukin-10, IL-1β: Interleukin-1β, IL-6: Interleukin-6, NF-κB: Nuclear factor-kappa B, TNF-α: Tumor necrosis factor-alpha.

The Pathogenesis of Diabetes

Hyperglycemia can cause oxidative stress and endoplasmic reticulum stress. Oxidative stress can stimulate the formation of numerous pro-inflammatory mediators that can lead to inflammation in pancreatic islet cells and peripheral tissues. Insulin resistance can develop in peripheral tissues due to inflammation. With inflammation in pancreatic islet cells, the normal function of beta cells is disrupted and diabetes can occur. Some metabolic pathways causing insulin resistance can cause inflammation and affect stress-induced kinases [JUN N-terminal kinase and IKB kinase-β (IKK-β)]. These kinases are involved in diabetes pathogenesis. In particular, IKK-β can stimulate the release of IL-1β and TNF-α in the liver and adipose tissue by activating NF-κB. These cytokines can also lead to insulin resistance.[26]

The disruption of the balance between insulin action and secretion plays a crucial role in the pathophysiology of diabetes. The disruption of the balance results in hyperglycemia. Furthermore, when there is β-cell dysfunction, insulin secretion is decreased. In this case, the capacity to maintain glucose levels in the body is limited. In addition, insulin resistance can lead to increased hepatic glucose production and reduced glucose uptake in muscles, liver, and adipose tissue. Therefore, hyperglycemia (due to insulin resistance and β-cell dysfunction) has a critical place in diabetes progression.[27]

Hyperglycemia, Insulin Resistance and Inflammation

Chronically elevated TNF-α and IL-6 levels are seen in metabolic diseases. TNF-α and IL-6 can change the sensitivity of insulin through activating various steps of the insulin signal pathway. These pro-inflammatory components induce serine phosphorylation instead of tyrosine in IRS-1. Therefore, insulin resistance can occur by inhibiting insulin signal activation.[3]

Various stimuli such as hyperglycemia, increased free fatty acid levels, and cytokines can cause oxidative stress by increasing the formation of ROS. Oxidative stress and ROS activate the serine/threonine kinase signaling cascades. These activated kinases target IRS proteins and IR in the insulin signaling pathway. IRS-1 and IRS-2 enhance phosphorylation of serine and reduce tyrosine phosphorylation. With the decrease in the activity of signal molecules such as phosphatidylinositol-3-kinase, the effect of insulin decreases and insulin resistance can develop. In addition, serine kinase triggers inflammation by affecting the NF-κB pathway and thus the effect of insulin can be inhibited.[28] Furthermore, hyperglycemia is associated with inflammation by stimulating IL-6 production from endothelium and macrophages.[3]

High circulating glucose concentrations (hyperglycemia) activate protein kinase C (PKC) by increasing the formation of diacylglycerol. The increased PKC pathway causes the secretion of pro-inflammatory components through increasing NF-κB.[29] Thus, it was emphasized that there is a close connection between hyperglycemia and inflammation.

In hyperglycemia, the advanced glycation end products (AGEs) production increases.[3] AGEs are heterogeneous compounds resulting from the non-enzymatic reaction between the nitrogenous groups of proteins, lipoproteins, and/or nucleic acids with the carbonyl groups

of reducing sugars. Protein glycation begins when the carbonyl group of the monosaccharides and the free amino group of the amino acids form a Schiff base. While Schiff base is formed in hours, this structure turns into Amadori products within days. Amadori products convert to dicarbonyl compounds and then to AGEs within weeks. One of the mechanisms in AGE formation is the polyol pathway. Hyperglycemia caused by diabetes in the polyol pathway, some of the abundant glucose is first converted to sorbitol and then to 3-deoxy-glucosone, an AGE intermediate and produced in the creation of AGE.[30] The AGE formed bind to relevant receptors, causing pro-inflammatory response. Therefore, increased AGE can cause an increase in plasma CRP and TNF-α concentrations.[3] Furthermore, the interplay between AGEs and specific receptors accelerates oxidative stress, resulting in an increased inflammatory signal. Activation of NF-κB and formation of ROS are effective in increasing this inflammatory signal.[31]

Diabetes, Inflammation and Medical Nutrition Therapy

The most studied nutritional component in relation to diabetes and inflammation is omega-3 fatty acids. Furthermore, there is also evidence for whole grains, foods with low glycemic index, antioxidant vitamins, and polyphenolic compounds.[4,32]

Eicosanoids consisting of n-3 (prostaglandin-3 and leukotriene-5) have a weaker inflammatory effect than those consisting of n-6 (prostaglandin-2, and leukotriene-4). Therefore, fish oil containing n-3 has an inflammation-reducing effect and n-3 can show its anti-inflammatory effect by directly affecting the cytokine production. Fish oil can achieve this effect by suppressing NF-κB activation.[4] Koopmans et al.[33] conducted a study on diabetic pigs and found that CRP concentrations were significantly greater in the group fed rich in saturated fat and cholesterol, while it was importantly lesser in the group fed rich in unsaturated fat. It was concluded that the diet rich in saturated fat and cholesterol stimulated inflammation by showing pro-inflammatory effect. According to a meta-analysis study; type 2 diabetic patients who took omega-3 supplements showed that triglyceride levels significantly declined, while there were no significant differences in hemoglobin A1c (HbA1c), total cholesterol, fasting and postprandial plasma glucose levels. However, high (>1.5) eicosapentaenoic acid/docosahexaenoic acid intake was found to cause a tendency in plasma insulin, HbA1c, total cholesterol and triglyceride levels to decrease.[34]

Consumption of fruits and vegetables has been related to inflammation.[4] A study related to diabetes found that vegetable and fruit consumption was adversely correlated to oxidative stress.[35] According to a meta-analysis study; vegetable and fruit consumption was found to reduce CRP and TNF-α levels. They attributed that consumption of fruits and vegetables can reduce pro-inflammatory markers due to their nutritional components such as antioxidant vitamins (A-C-E), soluble fiber, and flavonoids.[36]

According to 28 randomized controlled studies; no relationship was found among IL-6, TNF-α and CRP levels with the glycemic index or load.[37] However, there is also evidence in the literature showing that there is a relationship between inflammation and glycemic index. It was found that lower serum CRP concentrations in individuals on low-glycemic diet.[38] For this reason, care should be taken to provide that the glycemic index or load is not too high and that the amount of carbohydrates to be taken at meals is balanced. Wolever et al.[39] carried out a study in individuals with diabetes and examined the relationship between glycemic index and inflammation. They reported that CRP levels were significantly lower by 30% in those who followed a low glycemic index nutritional therapy in comparison with those who followed a high glycemic index nutritional therapy. It was stated that hyperglycemia, which is seen as a result of high glycemic index nutritional therapy, stimulates the release of inflammatory cytokines from monocytes.[39]

Dietary AGE proteins can cause inflammation in individuals with diabetes. Animal-based foods such as red meat, cheese, and egg yolk contain high levels of protein and fat. Consuming large amounts of these foods can cause high levels of AGE intake.[4] In an intervention study on diabetics; it was observed that the TNF-α, CRP, and intercellular adhesion molecule-1 levels increased in those fed a diet containing high AGE (16.3 ± 3.7 x10^6 AGE units per day) compared to those fed a diet containing low AGE (3.67 ± 1.2 x10^6 AGE units per day).[40] By preventing the accumulation of AGEs, the progression of diabetes-associated atherosclerosis can be slowed. Methylglyoxal, formed via a non-enzymatic reaction, is the precursor of most AGEs formed in diabetes. It was also shown in mice with diabetes that increased plasma methylglyoxal levels cause endothelial inflammation.[41]

Magnesium is an essential cofactor for more than 300 enzymes in carbohydrate metabolism (including all enzymes of glycolysis).[42,43] Magnesium is involved in the regulation of insulin signaling, in the insulin-mediated glucose utilization and in the phosphorylation of IR kinase.[43] The lack of dietary magnesium can cause impaired IR signaling and insulin resistance. Additionally, there was a reverse relation between magnesium intake and fasting insulin levels. In the study conducted in a Japanese population for the incidence of diabetes; increased magnesium intake was found to be a significant protective element, especially in those with insulin resistance and low-grade inflammation.[42]

Vitamin B_{12} shows potential antioxidant properties because it can trigger the methionine synthase activity. In addition, it directly reacts with reactive oxygen and/or nitrogen species and can reduce oxidative stress by affecting signal molecules. At the same time, vitamin B_{12} shows potential anti-inflammatory properties by inhibiting NF-κB. Vitamin B_{12} is also involved in the use of carbohydrates. Low levels of vitamin B_{12} can cause hyperglycemia.[44] Furthermore, a relationship among vitamin B_{12} deficiency with glucose intolerance and insulin resistance was reported by Madhu.[45] Lee et al.[44] carried out a study in individuals with diabetes and reported that blood glucose and CRP concentrations were significantly greater in individuals with low vitamin B_{12} levels. In addition, it was stated that the risk of vitamin B_{12} deficiency and inflammation was higher in diabetic vegetarians.[44]

The Mediterranean diet is a diet rich-olive oil, whole grains, vegetables, fruits, legumes, and nuts with moderate consumption-poultry and fish, low consumption-whole-fat dairy products and red meat and moderate wine consumption.[46] Esposito et al.[47] reported that the Mediterranean diet is a suitable diet for diabetes management.[47] Maiorino et al.[48] carried out a randomized controlled study and detected that diabetic patients with a higher score (6-9 points) on adherence to the Mediterranean diet were detected to have lower CRP and higher adiponectin levels than those who scored <3.[48] In order to improve glycemic control in diabetic individuals; vegetarian diets, the Dietary Approaches to Stop Hypertension diet, the German Food Pyramid Index, and the Alternative Healthy Eating Index can be used as alternatives besides the Mediterranean diet.[46,49]

In adults with diabetes, the percentages of total energy from macronutrients should be 45-60% for carbohydrates, 15-20% for proteins, 20-35% for fats, and each should be evaluated individually. In addition, the percentage of total daily energy from saturated fats should not exceed 7% and trans-fat intake should be kept to a minimum. To adequately control blood glucose, the percentage of energy from added sucrose or fructose should not exceed 10% of total daily energy.[49] The recommended amount for fiber intake should be a minimum of 14 g/1000 kcal. Furthermore, the average daily protein intake for diabetics (without kidney diseases) should be 1-1.5 g/kg body weight/day. At the same time, it is recommended for individuals with diabetes to consume 1 portion of fish at least 2 times a week for omega-3 fatty acid intake due to its anti-inflammatory properties. In addition, while 5% weight loss is recommended for clinical benefit in individuals with diabetes, 7-10% weight loss should be targeted to inhibit development to type 2 diabetes in people with pre-diabetes.[50]

The importance of nutrition in the reciprocal relationship between inflammation and diabetes is shown in Figure 1. Furthermore, the effects of cytokines and the factors affecting the levels of cytokines are shown in Figure 2.

CONCLUSION

Since diabetes is a disease characterized by chronic hyperglycemia and low-grade inflammation, anti-hyperglycemic and anti-inflammatory strategies in diabetes should be included in national and international dietary guidelines. Therefore, in order to keep blood glucose and inflammatory markers levels within normal ranges, achieving a healthy body weight should be targeted and body weight maintenance should be ensured. Energy, macro- and micronutrient intakes should be taken at recommended levels. Intakes of saturated fats, trans fats, and refined carbohydrates should be reduced. The consumption of fruits and vegetables should be increased, paying attention to the low glycemic index or load. Meal times and the numbers of macronutrients (especially carbohydrates) in the meal should be adjusted in accordance with the medicine or insulin therapy used. It should not be forgotten that medical nutrition therapy should be planned specifically for the individual to provide homeostasis in metabolism. Thus, complications that may occur due to diabetes and inflammation will be prevented.

MAIN POINTS

- Diabetes is a disease characterized by chronic hyperglycemia and low-grade inflammation.

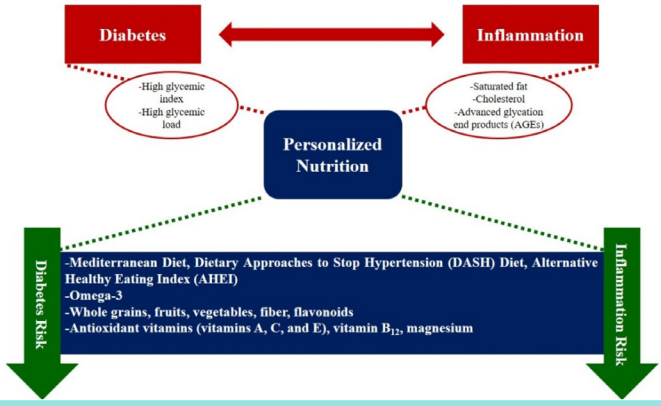


Figure 1. The importance of nutrition in the reciprocal relationship between inflammation and diabetes. Providing adequate and balanced individual nutrition within the scope of anti-hyperglycemic and anti-inflammatory nutritional strategies is effective in reducing the risk of diabetes and inflammation.

AGEs: Advanced glycation end products, AHEI: Alternative Healthy Eating Index, DASH: Dietary Approaches to Stop Hypertension.

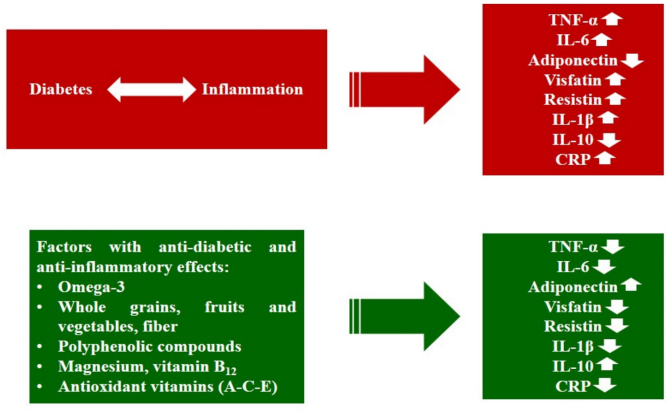


Figure 2. Effects of cytokines and factors affecting the levels of cytokines.

CRP: C-reactive protein, IL-10: Interleukin-10, IL-1β: Interleukin-1β, IL-6: Interleukin-6, TNF-α: Tumor necrosis factor-alpha.

- Inflammation-related mechanisms can cause hyperglycemia, insulin resistance, and diabetes.
- Diabetes-related mechanisms can cause inflammation.
- Anti-hyperglycemic and anti-inflammatory dietary recommendations are important for maintaining homeostasis.
- Medical nutrition therapy should be planned specifically for the person to provide homeostasis in metabolism.

Acknowledgment: The author thanks to Prof. Dr. Emel Özer for valuable contributions.

Peer-review: Externally peer-reviewed.

DISCLOSURES

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

REFERENCES

1. Mahan LK, Raymond JL. Krause's food & the nutrition care process. 14th ed. Canada: Elsevier; 2017.
2. Donath MY, Shoelson SE. Type 2 diabetes as an inflammatory disease. Nat Rev Immunol. 2011; 11(2): 98-107.
3. Calle MC, Fernandez ML. Inflammation and type 2 diabetes. Diabetes Metab. 2012; 38(3): 183-91.
4. Browning LM, Jebb SA. Nutritional influences on inflammation and type 2 diabetes risk. Diabetes Technol Ther. 2006; 8(1): 45-54.
5. Ouchi N, Parker JL, Lugus JJ, Walsh K. Adipokines in inflammation and metabolic disease. Nat Rev Immunol. 2011; 11(2): 85-97.
6. Alisherovna KM, Rustamovich TD, Baxtiyorovich UJ, Sobirovna SM. Diabetes mellitus and hyperglycemia in patients with rheumatoid arthritis. Texas Journal of Medical Science. 2022; 13: 99-103.
7. Schmidt-Arras D, Rose-John S. IL-6 pathway in the liver: From physiopathology to therapy. J Hepatol. 2016; 64(6): 1403-15.
8. Atlı H, Onalan E, Yakar B, Duzenci D, Dönder E. Predictive value of inflammatory and hematological data in diabetic and non-diabetic retinopathy. Eur Rev Med Pharmacol Sci. 2022; 26(1): 76-83.
9. Hong X, Zhang X, You L, Li F, Lian H, Wang J, et al. Association between adiponectin and newly diagnosed type 2 diabetes in population with the clustering of obesity, dyslipidaemia and hypertension: a cross-sectional study. BMJ Open. 2023; 13(2): e060377.
10. El-Shafey EM, El-Naggar GF, Al-Bedewy MM, El-Sorogy H. Is there a relationship between visfatin level and type 2 diabetes mellitus in obese and non obese patients. J Diabetes Metab. 2012; 11(2): 1-5.
11. Mir MM, Mir R, Alghamdi MAA, Wani JI, Sabah ZU, Jeelani M, et al. Differential Association of Selected Adipocytokines, Adiponectin, Leptin, Resistin, Visfatin and Chemerin, with the Pathogenesis and Progression of Type 2 Diabetes Mellitus (T2DM) in the Asir Region of Saudi Arabia: A Case Control Study. J Pers Med. 2022; 12(5): 735.
12. Luo R, Li X, Jiang R, Gao X, Lü Z, Hua W. Serum concentrations of resistin and adiponectin and their relationship to insulin resistance in subjects with impaired glucose tolerance. J Int Med Res. 2012; 40(2): 621-30.
13. Azab N, Abdel-Aziz T, Ahmed A, El-deen IM. Correlation of serum resistin level with insulin resistance and severity of retinopathy in type 2 diabetes mellitus. J Saudi Chem Soc. 2016; 20(3): 272-7.
14. Banerjee M, Saxena M. Interleukin-1 (IL-1) family of cytokines: role in type 2 diabetes. Clin Chim Acta. 2012; 413(15-16): 1163-70.
15. Mojtaba E, Mahdi K, Mehdi KJR, Amir S. Serum interleukin-1 beta plays an important role in insulin secretion in type II diabetic. Int J Biosci. 2011; 1(3): 93-9.
16. Charles BA, Doumatey A, Huang H, Zhou J, Chen G, Shriner D, et al. The roles of IL-6, IL-10, and IL-1RA in obesity and insulin resistance in African-Americans. J Clin Endocrinol Metab. 2011; 96(12): E2018-22.
17. de Luca C, Olefsky JM. Inflammation and insulin resistance. FEBS Lett. 2008; 582(1): 97-105.
18. Chikoti S, Najiya U, Sumanlatha G, Jahan P. Cytokine gene variants of TNF-☒ and IL-10 in the propensity of type 2 diabetes in south Indian population. J Diabetes Complications. 2022; 36(10): 108304.
19. Hermans MP, Ahn SA, Rousseau MF. Increased CRP: An extended biomarker of microvascular risk in men with type 2 diabetes. J Diabetes Complications. 2019; 33(11): 107413.
20. Rubio-Martín E, Soriguer F, Gutiérrez-Repiso C, Garrido-Sánchez L, de Adana MS, García-Fuentes E, et al. C-reactive protein and incidence of type 2 diabetes in the Pizarra study. Eur J Clin Invest. 2013; 43(2): 159-67.
21. Pfützner A, Forst T. High-sensitivity C-reactive protein as cardiovascular risk marker in patients with diabetes mellitus. Diabetes Technol Ther. 2006; 8(1): 28-36.
22. Wellen KE, Hotamisligil GS. Inflammation, stress, and diabetes. J Clin Invest. 2005; 115(5): 1111-9.
23. Rezaee F, Dashty M. Role of adipose tissue in metabolic system disorders adipose tissue is the initiator of metabolic diseases. J Diabetes Metab. 2013; 13(2): 1-9.
24. Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, et al. Increased oxidative stress in obesity and its impact on metabolic syndrome. J Clin Invest. 2004; 114(12): 1752-61.
25. Bódis K, Roden M. Energy metabolism of white adipose tissue and insulin resistance in humans. Eur J Clin Invest. 2018; 48(11): e13017.
26. Akash MS, Rehman K, Chen S. Role of inflammatory mechanisms in pathogenesis of type 2 diabetes mellitus. J Cell Biochem. 2013; 114(3): 525-31.
27. Galicia-Garcia U, Benito-Vicente A, Jebari S, Larrea-Sebal A, Siddiqi H, Uribe KB, et al. Pathophysiology of Type 2 Diabetes Mellitus. Int J Mol Sci. 2020; 21(17): 6275.
28. Evans JL, Goldfine ID, Maddux BA, Grodsky GM. Are oxidative stress-activated signaling pathways mediators of insulin resistance and beta-cell dysfunction? Diabetes. 2003; 52(1): 1-8.
29. Singh A, Kukreti R, Saso L, Kukreti S. Mechanistic Insight into Oxidative Stress-Triggered Signaling Pathways and Type 2 Diabetes. Molecules. 2022; 27(3): 950.
30. Parmaksız İ. Advanced glycation end-products in complications of diabetes mellitus. Marmara Med J. 2011; 24(3): 141-8.
31. Chang SC, Yang WV. Hyperglycemia, tumorigenesis, and chronic inflammation. Crit Rev Oncol Hematol. 2016; 108: 146-53.
32. Hoca M, Becer E, Vatansever HS. The role of resveratrol in diabetes and obesity associated with insulin resistance. Arch Physiol Biochem. 2023; 129(2): 555-561.
33. Koopmans SJ, Dekker R, Ackermans MT, Sauerwein HP, Serlie MJ, van Beusekom HM, et al. Dietary saturated fat/cholesterol, but not unsaturated fat or starch, induces C-reactive protein associated early atherosclerosis and ectopic fat deposition in diabetic pigs. Cardiovasc Diabetol. 2011; 10: 64.

34. Chen C, Yu X, Shao S. Effects of Omega-3 Fatty Acid Supplementation on Glucose Control and Lipid Levels in Type 2 Diabetes: A Meta-Analysis. PLoS One. 2015; 10(10): e0139565.

35. Wronka M, Krzemińska J, Młynarska E, Rysz J, Franczyk B. The Influence of Lifestyle and Treatment on Oxidative Stress and Inflammation in Diabetes. Int J Mol Sci. 2022; 23(24): 15743.

36. Hosseini B, Berthon BS, Saedisomeolia A, Starkey MR, Collison A, Wark PAB, et al. Effects of fruit and vegetable consumption on inflammatory biomarkers and immune cell populations: a systematic literature review and meta-analysis. Am J Clin Nutr. 2018; 108(1): 136-55.

37. Milajerdi A, Saneei P, Larijani B, Esmaillzadeh A. The effect of dietary glycemic index and glycemic load on inflammatory biomarkers: a systematic review and meta-analysis of randomized clinical trials. Am J Clin Nutr. 2018; 107(4): 593-606.

38. Navarro SL, Tarkhan A, Shojaie A, Randolph TW, Gu H, Djukovic D, et al. Plasma metabolomics profiles suggest beneficial effects of a low-glycemic load dietary pattern on inflammation and energy metabolism. Am J Clin Nutr. 2019;110(4):984-92.

39. Wolever TM, Gibbs AL, Mehling C, Chiasson JL, Connelly PW, Josse RG, et al. The Canadian Trial of Carbohydrates in Diabetes (CCD), a 1-y controlled trial of low-glycemic-index dietary carbohydrate in type 2 diabetes: no effect on glycated hemoglobin but reduction in C-reactive protein. Am J Clin Nutr. 2008; 87(1): 114-25.

40. Vlassara H, Cai W, Crandall J, Goldberg T, Oberstein R, Dardaine V, et al. Inflammatory mediators are induced by dietary glycotoxins, a major risk factor for diabetic angiopathy. Proc Natl Acad Sci U S A. 2002; 99(24): 15596-601. Erratum in: Proc Natl Acad Sci U S A. 2003; 100(2): 763.

41. Yuan T, Yang T, Chen H, Fu D, Hu Y, Wang J, et al. New insights into oxidative stress and inflammation during diabetes mellitus-accelerated atherosclerosis. Redox Biol. 2019; 20: 247-60.

42. Hata A, Doi Y, Ninomiya T, Mukai N, Hirakawa Y, Hata J, et al. Magnesium intake decreases Type 2 diabetes risk through the improvement of insulin resistance and inflammation: the Hisayama Study. Diabet Med. 2013; 30(12): 1487-94.

43. Barbagallo M, Dominguez LJ. Magnesium and type 2 diabetes. World J Diabetes. 2015; 6(10): 1152-7.

44. Lee YJ, Wang MY, Lin MC, Lin PT. Associations between Vitamin B-12 Status and Oxidative Stress and Inflammation in Diabetic Vegetarians and Omnivores. Nutrients. 2016; 8(3): 118.

45. Madhu SV. Vitamin B 12 and diabetes risk-myth or reality. Int J Diabetes Dev Ctries. 2020; 40(1): 1-3.

46. Georgoulis M, Kontogianni MD, Yiannakouris N. Mediterranean diet and diabetes: prevention and treatment. Nutrients. 2014; 6(4): 1406-23.

47. Esposito K, Maiorino MI, Bellastella G, Chiodini P, Panagiotakos D, Giugliano D. A journey into a Mediterranean diet and type 2 diabetes: a systematic review with meta-analyses. BMJ Open. 2015; 5(8): e008222.

48. Maiorino MI, Bellastella G, Petrizzo M, Scappaticcio L, Giugliano D, Esposito K. Mediterranean diet cools down the inflammatory milieu in type 2 diabetes: the MÉDITA randomized controlled trial. Endocrine. 2016; 54(3): 634-41.

49. Canadian Diabetes Association Clinical Practice Guidelines Expert Committee; Dworatzek PD, Arcudi K, Gougeon R, Husein N, Sievenpiper JL, Williams SL. Nutrition therapy. Can J Diabetes. 2013; 37 Suppl 1: S45-55.

50. Evert AB, Dennison M, Gardner CD, Garvey WT, Lau KHK, MacLeod J, et al. Nutrition Therapy for Adults With Diabetes or Prediabetes: A Consensus Report. Diabetes Care. 2019; 42(5): 731-54.

RESEARCH ARTICLE

CYPRUS
JOURNAL OF MEDICAL SCIENCES

DOI: 10.4274/cjms.2023.2022-49
Cyprus J Med Sci 2023;8(3):173-183

Effects of Irinotecan (CPT-11), Ellagic Acid and Combination of both on HeLa Cells in 2D and 3D Culture

Gülistan Sanem Sarıbaş[1], Gamze Turna Saltoğlu[2], Serap Yalçın Azarkan[3], Özen Akarca Dizakar[4], Seda Yalçınkaya[5]

[1]Department of Histology and Embryology, Kırşehir Ahi Evran University Faculty of Medicine, Kırşehir, Türkiye
[2]Department of Biochemistry, Kırşehir Ahi Evran University Faculty of Medicine, Kırşehir, Türkiye
[3]Department of Pharmacology, Kırşehir Ahi Evran University Faculty of Medicine, Kırşehir, Türkiye
[4]Department of Histology and Embryology, İzmir Bakırçay University Faculty of Medicine, İzmir, Türkiye
[5]Department of Food Engineering, Süleyman Demirel University, Institute of Science and Technology, Isparta, Türkiye

Abstract

BACKGROUND/AIMS: Investigation of new potential treatments is important today to increase the power of cervical carcinoma treatment. Although chemotherapeutic drugs are widely used for clinical tumor treatment, their severe toxicity limits their therapeutic efficacy. Irinotecan (CPT-11) is an agent with therapeutic activity against various cancers including cervical cancer. Ellagic acid (EA) is a phenolic natural substance that can be found in various fruits and has chemopreventive and chemotherapeutic effects. In this work, we looked into EA as an alternative agent to improve the therapeutic effect of CPT-11 *in vitro*.

MATERIALS AND METHODS: The effects of the two agents and their combination on 2 and 3D HeLa cultures at different time periods were evaluated by real-time-polymerase chain reaction and intraclass correlation coefficient analyses. In cultures, MMP-2 and -9 were used to determine the basis of cancer cell metastasis, HIF1α and TGF-β1 were used to evaluate the mechanism of angiogenesis and epithelial-mesenchymal change, and Bax and Bcl-2 were used to evaluate the apoptotic mechanism.

RESULTS: In comparison to the DMSO (Dimethyl Sulfoxide) group, HeLa cells treated with EA and its combination showed considerable suppression of MMPs, TGF-β1, and HIF1α. In addition, the increase in proapoptotic Bax level with treatment and the suppression of antiapoptotic Bcl-2 expression were revealed as another important finding of EA and combined treatment.

CONCLUSION: We propose that EA would be a good candidate for additional preclinical studies for the management of human cervical cancer.

Keywords: Cervical cancer, CPT-11, ellagic acid, HeLa

INTRODUCTION

With a 13.3% incidence rate, cervical cancer is the third most prevalent disease in women worldwide and a significant public health issue. In terms of mortality, it ranks third with a rate of 73%.[1] Although postoperative radiation therapy and chemotherapy are used for treating cervical cancer, chemotherapeutic agents have many side effects. Therefore, research for new drugs with less side effects that selectively show greater cytotoxic effects on cancer cells at low doses continues.[2,3]

A semi-synthetic plant alkaloid derived from camptothecin is called irinotecan (CPT-11). Clinical trials in the 1990s and 2000s revealed that CPT-11 is therapeutically effective against several malignancies, including cervical carcinoma.[4] Toxic effects are seen in a significant

To cite this article: Sarıbaş GS, Turna Saltoğlu G, Yalçın Azarkan S, Akarca Dizakar ÖA, Yalçınkaya S. Effects of Irinotecan (CPT-11), Ellagic Acid and Combination of both on HeLa Cells in 2D and 3D Culture. Cyprus J Med Sci 2023;8(3):173-183

ORCID IDs of the authors: G.S.S. 0000-0001-7582-6235; G.T.S. 0000-0002-7847-2898; S.Y.A. 0000-0002-9584-266X; Ö.A.D. 0000-0002-4358-6510; S.Y. 0000-0003-0947-8505.

Address for Correspondence: Gülistan Sanem Sarıbaş
E-mail: sanemarik@gmail.com
ORCID ID: orcid.org/0000-0001-7582-6235

Received: 05.11.2022
Accepted: 18.03.2023

number of patients using the CPT-11 drug, which is used to treat cancer patients. This makes it difficult for this drug to be used in chemotherapy.[5]

A naturally occurring phenolic substance called ellagic acid (EA) is present in berries such as red and black currants, red and black raspberries, blackberries, blueberries, and strawberries. Studies both *in vivo* and *in vitro* have established that it has chemopreventive and chemotherapeutic effects by inhibiting the proliferation of different cancer cells at low doses.[6] EA reduces tumor development, angiogenesis, and metastasis, although the exact biochemical pathways by which it does so are still poorly known.

Based on the aboveobservations, it is hypothesized in this study that human cervical cancer cells can be inhibited by EA and CPT-11, and the inhibition mechanism may play a role in the control of angiogenetic, proliferative, and metastatic signals. In this study, we developed an alternative treatment for cervical cancer. For this purpose, we used 2 and 3D HeLa cell cultures exposed to CPT-11, EA and its combination at different times in our study. In the study, MMP-2 and MMP-9 to reveal metastatic findings with real-time PCR and immunocytochemical analyzes, TGF-β1 and HIF1α to determine angiogenesis, and *Bax* and *Bcl-2* genes and proteins to evaluate apoptosis were used as targets.

MATERIALS AND METHODS

2D Cell Culture

Cervical cancer cell lines (HeLa) were purchased from ATCC (USA). A consent form was not required for this study as the cells were purchased commercially. Cervical cancer (HeLa) cells were cultured in RPMI-1640 media with 10% fetal bovine serum, 1% gentamicin, and 1% penicillin antibiotic added in 75 cm^2 flasks at 37 °C and 5% CO_2. Cells were passaged using trypsin as 80% of the flask surface was covered by cells.

3D Cell Culture

Cell lines were propagated spherically using the three-dimensional hanging drop method. For this, 96-well cell seeding plates without scaffolds were used. Suspended cells were dropped into the wells with the help of pipette into a volume of 40-45 µL. Then, cells were grown at 37 °C and in an incubator with 5% carbon dioxide. To the wells, 4 µL of fresh medium was introduced every day to ensure effective growth of cell lines globally in the hanging drop method. Cell groups were expected to take a spherical shape and reach a size of 300-500 µL at the end of approximately 4 days.

Cytotoxicity Analysis

The cytotoxic impact of metabolites on HeLa cells was assessed using XTT assay kit (Biological Industries, USA). For the analysis of 2D cultures, $8x10^3$ cells were seeded in each well of a 96-well plate. The cells were given drugs after incubation for 24 h. For 3D cultures, the drug was applied after the 3D structure had already been created. The solutions from XTT kit were applied to the cells after 48 h of incubation. Then, using a microplate reader (BIOTEK ELX808, USA), cell viability was evaluated at a wavelength of 450 nm. Briefly, IC_{50} values were determined by applying certain doses of CPT-11 (Irinocam-Irinotecan Hydrochloride Trihydrate, Kocak, Türkiye) and EA (EA, Cat. No: sc-202598A, Santa Cruz Biotech, Dallas, ABD) to HeLa cells. Real-time PCR and immunocytochemical experiments were performed at these concentrations.

Total RNA Isolation and qRT-PCR

Total RNA isolation was carried out using the WizPrep Total RNA Mini Kit (WizBio, Korea) in accordance with the instructions using cells that had been grown in 2D and 3D. RNA concentrations were determined using a Genova Nano Micro-volume Spectrophotometer (Jenway, UK). cDNA synthesis was performed using the HyperScript™ First strand Synthesis cDNA Synthesis Kit (GeneAll, Korea) for real-time PCR. A thermal cycler was used to perform the reaction. Real-time quantitative polymerase chain reaction (RT-qPCR) was then used to assess the change in gene expression. The Applied Biosystems™ 7500 Fast Real-Time PCR apparatus was used to perform the real-time qPCR reaction. The internal reference control and housekeeping gene beta-actin was used. The gene-specific primers used in the study are shown in Table 1. The formula $2^{-\Delta\Delta Ct}$ was used to determine the mRNA expression fold change.

Immunocytochemical Method

Samples that were previously seeded on glass coverslips coated with poly-D-lysine in 24-well dishes were used. The samples were divided into groups with different durations. When the cells in the culture medium reached a sufficient density and after the agent applications, the cells were fixed in the well with 3% paraformaldehyde. TBS + 2.5% triton X solution was filled to the wells. Then, with cold PBS, cells were cleaned and incubated with block solution (PBST +1% BSA). The block solution was removed from the wells and rabbit polyclonal primary antibodies MMP-2 (bs-41146R, Bioss), MMP-9 (bs-41146R, Bioss), TGF-β1 (bs-20411R, Bioss), HIF1α (bs-20399R, Bioss), Bax (PA5-86062, Thermo Fisher) and Bcl-2 (PA5-27094, Thermo Fisher) were used for immunolabeling of cells. Primary antibodies were added to different wells at 1:200 dilution and incubated at 4 °C overnight. The secondary antibody with biotin was applied to them for 10 min. After, the streptavidin peroxidase enzyme complex was activated for 10 min. Finally chromogen containing aminoethyl carbazole was added and visible immune reaction was achieved. Mayer's hematoxylin was used as a background marker. The stained samples were evaluated with a computer assisted imaging system. While the blue-violet parts in the images express the cell nuclei; red-pink areas mark immune-positive areas.

Statistical Analysis

The Shapiro-Wilk test was used to assess the distribution of the data. Post-hoc paired ANOVA test was used to evaluate normally distributed data. Independent data that did not show a normal distribution were evaluated with the Kruskal-Wallis test. If there was a statistically

Table 1. RT-PCR primer sequences

Gene	Primer sequences	
	Forward	Reverse
Beta actin	5'-CACCAACTGGGACGACAT-3'	5'-ACAGCCTGGATAGCAACG-3'
HIF1α	5'-AGACCTTCCTTAGCCGTCAC-3'	5'-GTCTCCACCCACACAAAACC-3'
TGF-β1	5'-TTGAGACTTTTCCGTTGCCG-3'	5'-CGAGGTCTGGGGAAAAGTCT-3'
MMP-2	5'-AATCCCACCAACCCTCAGAG-3'	5'-GTGCCCTCTTGAGACAGTCT-3'
MMP-9	5'-GAGTTCCCGGAGTGAGTTGA-3'	5'-AAAGGTGAGAAGAGAGGGCC-3'
Bax	5'-CATCATGGGCTGGACATTGG-3'	5'-CCTCAGCCCATCTTCTTCCA-3'
Bcl-2	5'-CTCCTTCATCGTCCCCTCTC-3'	5'-CGGCGGCAGATGAATTACAA-3'

RT-PCR: Real-time-polymerase chain reaction.

significant distinction between the groups, Bonferroni correction post-hoc pairwise comparisons were used to identify the different groups. Statistical analyzes were carried out using IBM SPSS Statistics 21 (IBM Corp. Armonk, NY). Statistics were considered significant at $p<0.05$.

RESULTS

Cytotoxicity Effects of CPT-11, Ellagic Acid and Combination of both (CPT-11 + EA) on HeLa Cells in 2D and 3D Culture

Because of XTT analysis, it was found that the cell viability in HeLa cell lines decreased as the concentration of CPT-11 and EA increased. The IC_{50} for CPT-11 in 2D cultures was 57.8 µM; the IC_{50} value for EA was determined as 45.4 µM (Figure 1A, B). In 3D cultures, the IC_{50} value for CPT-11 is 94 µM; the IC_{50} value for EA was determined as 120 µM (Figure 2A, B). When the two agents were applied in combination with IC_{50} doses, it was determined that the viability of the 2D and 3D cultures was statistically significantly decreased ($p<0.05$) (Figure 1C, 2C).

Alteration of MMP Expression on HeLa Cells in 2D and 3D Culture

When *MMP-2* gene expressions were evaluated in the 2D culture experiment, it was determined that the expression levels of EA, CPT-11 and EA + CPT-11 groups were statistically significantly decreased compared to the DMSO (Dimethyl Sulfoxide) group after 12 and 48 h of application (for 12 hours: p=0.021; 0.017; 0.031, respectively, for 48 hours: p<0.001; <0.001; <0.001, respectively). This decrease in the 24-hour application was insignificant ($p>0.05$) (Figure 3A). When *MMP-9* gene expressions were evaluated in the 2D culture experiment at 12, 24 and 48 hours, it was determined that the expression levels of EA, CPT-11 and EA + CPT-11 groups decreased compared to the DMSO group after 12, 24 and 48 h of applications. This decrease was found to be statistically significant in the 24-h and 48-h EA + CPT-11 groups (p=0.014; 0.005, respectively). This decrease was not statistically significant in the 12-hour application ($p>0.05$) (Figure 3B). Statistical data for all groups are presented in Table 2.

When *MMP-2* gene expressions were evaluated in 12, 24 and 48 hours applications in 3D culture experiment, it was seen that the expression levels of all groups decreased compared to the DMSO group. According to our findings, the expression levels of the EA, CPT-11, and EA + CPT-11 groups were found to be statistically significantly decreased compared with the DMSO group after 12 h of administration (p=0.002; 0.016; <0.001, respectively). Expression levels of the EA and EA + CPT-11 groups were statistically significantly decreased compared to the DMSO group in 24-hour application ($p<0.001$; <0.001, respectively). In the 48-hour application, only the expression level of the EA + CPT-11 group was found to be statistically significantly decreased compared with the DMSO group (p=0.010) (Figure 4A).

When *MMP-9* gene expressions were evaluated in 12, 24 and 48 hours applications in 3D culture experiment, it was seen that the expression levels of all groups decreased compared to the DMSO group. According to our findings, it was determined that the expression level of the EA + CPT-11 group was statistically significantly decreased compared with the DMSO group in only 12 h of application (p=0.003) (Figure 4B). Statistical data for all groups are presented in Table 3.

When protein expressions of MMPs in 2D and 3D cultures were evaluated by immunocytochemical analysis, it was seen that they were compatible with gene expression levels (Figure 5, 6). Briefly, MMP-2 and -9 levels were found to be highest in the DMSO group at 12, 24 and 48 h of treatment. In addition, it was determined that the expression of MMPs, which are metastasis markers, decreased in the treatment groups (EA and Irino) and especially in the combined group (EA + CPT-11) compared to the DMSO group.

Alteration of TGF-β1 Expression on HeLa Cells in 2D and 3D Cultures

When *TGF-β1* gene expressions were evaluated in the 2D culture experiment, it was seen that the expression levels of all groups decreased compared to the DMSO group. Our data indicate that after, and 48 h of treatment, TGF-β1 expression levels in the EA, CPT-11, and EA + CPT-11 groups were statistically substantially lower than in the

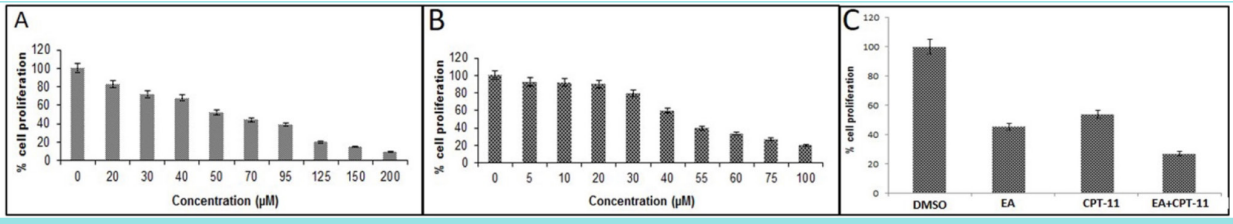


Figure 1. Cytotoxicity findings in 2D HeLa cultures, (A) CPT-11 (IC_{50}: 57.8 µM); (B) ellagic acid (IC_{50}: 45.4 µM); (C) toxicity of agents alone or in combination.

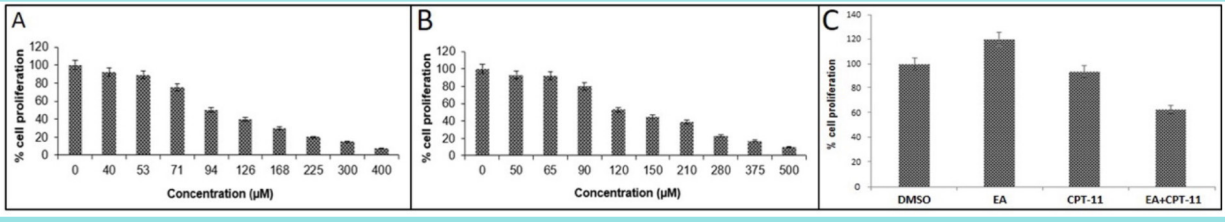


Figure 2. Cytotoxicity findings in 3D HeLa cultures, (A) CPT-11 (IC_{50}: 94 µM); (B) ellagic acid (IC_{50}: 120 µM); (C) toxicity of agents alone or in combination.

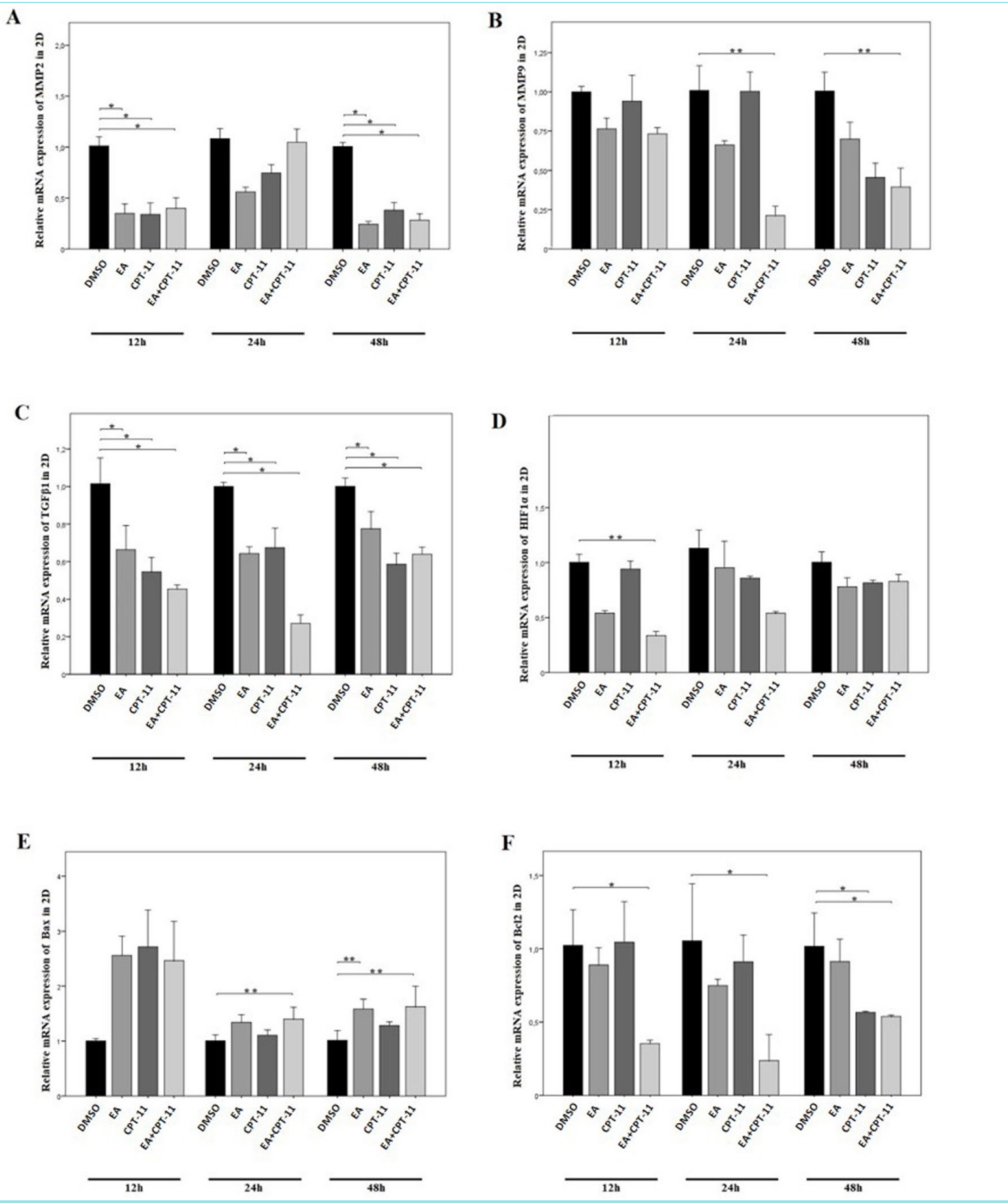


Figure 3. Relative gene expression of *MMP-2* (A), *MMP*-9 (B), *TGF-β*1 (C), *HIF1α* (D), *Bax* (E), and *Bcl-2* (F) of all groups at different times in 2D planar HeLa cell cultures. Data ($2^{-\Delta\Delta Ct}$ ratio to beta-actin mRNA) are expressed as the mean relative to the DMSO group (mean: 1). *: $p<0.05$, vs. The DMSO group (One-Way ANOVA with Bonferroni correction). **: $p<0.05$, vs. The DMSO group (Kruskal-Wallis test with Bonferroni correction pos-hoc test). All experiments were conducted four times.

DMSO group (for 12 hours: p=0.039; 0.005; 0.001, respectively, for 24 hours: p<0.001; <0.001; <0.001, respectively, for 48 hours: p=0.001; <0.001; <0.001, respectively) (Figure 3C). Statistical data for all groups are presented in Table 2.

When *TGF-β1* gene expressions were evaluated at 12, 24 and 48 h in the 3D culture experiment, it was seen that the expression levels of all groups decreased compared to the DMSO group. According to our data, the expression level of the EA + CPT-11 group was found to be statistically significantly decreased compared to the DMSO group in the 12 and 24 h applications (p=0.002; 0.045, respectively). In the 48-hour application, this reduction was statistically insignificant (p>0.05) (Figure 4C). Statistical data for all groups are presented in Table 3.

TGF-β1 protein expressions in 2D and 3D cultures was also found to be compatible with gene expression levels when evaluated by immunocytochemical analysis (Figure 7). Briefly, TGF-β1 expression levels were found to be highest in the DMSO group at 12, 24 and 48 h of administration. In addition, it was determined that TGF-β1 expressions, which are markers of angiogenesis and epithelial mesenchymal transition (EMT), were decreased in the treatment groups and especially in the combined group (EA + CPT-11) compared to the DMSO group.

Alteration of HIF1α Expressions on HeLa Cells in 2D and 3D Cultures

When *HIF1α* gene expressions were evaluated in the 2D culture experiment, it was seen that the expression levels of all groups decreased compared to the DMSO group. It was determined that the expression level of the EA + CPT-11 group was statistically significantly decreased compared with the DMSO group in only 12 h of application (p=0.008) (Figure 3D). Statistical data for all groups are presented in Table 2.

When *HIF1α* gene expressions were evaluated in the 3D culture experiment, it was seen that the expression levels of all groups decreased compared to the DMSO group. It was determined that the expression level of HIF1α in the EA + CPT-11 group was statistically lowered compared to the DMSO group in all applications at different times (p=0.031; 0.001; 0.001, respectively) (Figure 4D). Statistical data for all groups are presented in Table 3.

When HIF1α protein expressions in 2D and 3D cultures were evaluated by immunocytochemical analysis, it was found to be compatible with gene expression levels (Figure 8). Briefly, HIF1α expression levels were found to be decreased in all treatment groups compared to the DMSO group in applications at different times. This decrease in HIF1α expression, which promotes angiogenesis, was more pronounced in the combination group than in the DMSO group, especially in 3D cells.

Elevated Expression of Bax and Decreased Expression of Bcl-2 in Response to EA and CPT-11 + EA Treatment on HeLa Cells in 2D and 3D Culture

When the expression levels of the proapoptotic gene *Bax* were analyzed in 2D culture experiments, it was seen that the Bax levels of all groups increased compared to the DMSO group. In the 24 and 48 h applications, it was found that the Bax level in the EA + CPT-11 group was statistically important compared to the DMSO group (p=0.036; 0.045, respectively). In addition, it was determined that the expression level of the lean EA group increased statistically significantly compared with the DMSO group in the 48-hour application (p=0.023) (Figure 3E). Statistical data for all groups are presented in Table 2.

Table 2. Post-hoc pairwise comparisons for 2D cell culture

	Pairwise groups	Adjusted p-value					
		MMP-2*	MMP-9**	TGF-β1*	HIF1α**	Bax**	Bcl-2*
12 h	DMSO vs. EA	**0.021**	0.321	**0.039**	0.270	0.105	1.000
	DMSO vs. CPT-11	**0.017**	1.000	**0.005**	1.000	0.069	1.000
	DMSO vs. EA + CPT-11	**0.031**	0.069	**0.001**	**0.008**	0.155	**0.002**
24 h	DMSO vs. EA	0.087	0.380	**<0.001**	1.000	0.069	0.535
	DMSO vs. CPT-11	0.528	1.000	**<0.001**	1.000	1.000	1.000
	DMSO vs. EA + CPT-11	1.000	**0.014**	**<0.001**	0.105	**0.036**	**0.002**
48 h	DMSO vs. EA	**<0.001**	1.000	**0.001**	0.084	**0.023**	1.000
	DMSO vs. CPT-11	**<0.001**	0.056	**<0.001**	0.084	1.000	**0.003**
	DMSO vs. EA + CPT-11	**<0.001**	**0.005**	**<0.001**	0.322	**0.045**	**0.002**
DMSO	12 h vs. 24 h	1.000	1.000	1.000	1.000	1.000	1.000
	12 h vs. 48 h	1.000	1.000	1.000	1.000	1.000	1.000
	24 h vs. 48 h	1.000	1.000	1.000	1.000	1.000	1.000
EA	12 h vs. 24 h	0.689	0.084	1.000	**0.018**	**0.013**	0.352
	12 h vs. 48 h	1.000	0.992	0.809	0.150	0.187	1.000
	24 h vs. 48 h	0.225	1.000	0.589	1.000	0.980	0.219
CPT-11	12 h vs. 24 h	**0.047**	1.000	0.159	0.596	**0.007**	1.000
	12 h vs. 48 h	1.000	0.118	1.000	0.332	0.287	**0.019**
	24 h vs. 48 h	0.076	**0.024**	0.482	0.332	0.509	0.092
EA + CPT-11	12 h vs. 24 h	**<0.001**	**0.013**	**<0.001**	0.350	**0.032**	0.454
	12 h vs. 48 h	0.471	0.187	**<0.001**	**0.005**	0.350	0.097
	24 h vs. 48 h	**<0.001**	0.980	**<0.001**	0.350	0.980	**0.008**

*ANOVA test, **Mann-Whitney U test, bolded values remain significant after correction for multiple comparisons (p<0.05)

When Bax expression levels were analyzed in 3D culture experiments, it was seen that the expression levels of all groups increased compared to the DMSO group. In the 12- and 48-h applications, it was determined that the EA + CPT-11 group expression level was statistically higher than that of the DMSO group (p=0.006; 0.010, respectively). In addition, it was determined that the expression level of the lean CPT-11 group increased statistically significantly compared with the DMSO group in the 12-hour application (p=0.043) (Figure 4E). Statistical data for all groups are presented in Table 3.

When the expression levels of anti-apoptotic gene *Bcl-2* were analyzed in 2D culture experiments, it was seen that the expression levels of all

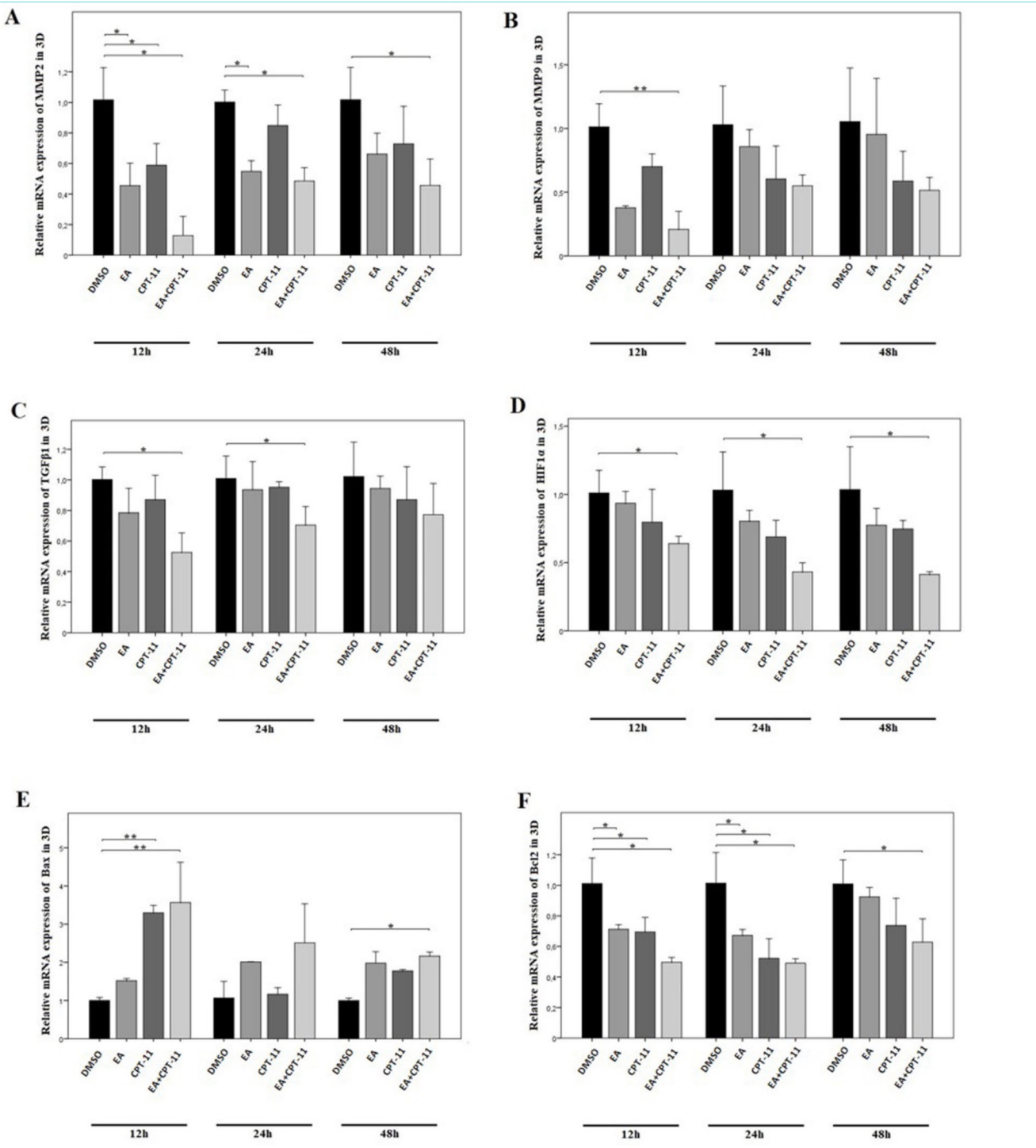


Figure 4. Relative gene expression of *MMP-2* (A), *MMP-9* (B), *TGF-β1* (C), *HIF1α* (D), *Bax* (E), and *Bcl-2* (F) of all groups at different times in 3D HeLa cell cultures. Data ($2^{-\Delta\Delta Ct}$ ratio to beta actin mRNA) are expressed as the mean relative to the DMSO group (mean: 1). *: p<0.05, vs. The DMSO group (One-Way ANOVA with Bonferroni correction). **: p<0.05, vs. The DMSO group (Kruskal-Wallis test with Bonferroni correction post-hoc test). All experiments were conducted four times.

Table 3. Post-hoc pairwise comparisons for 3D cell culture

	Pairwise groups	Adjusted p-value					
		MMP-2*	MMP-9**	TGF-β1*	HIF1α*	Bax**	Bcl-2*
12 h	DMSO vs. EA	**0.002**	0.128	0.259	1.000	1.000	**0.006**
	DMSO vs. CPT-11	**0.016**	1.000	1.000	0.440	**0.043**	**0.004**
	DMSO vs. EA + CPT-11	**<0.001**	**0.003**	**0.002**	**0.031**	**0.006**	**<0.001**
24 h	DMSO vs. EA	**<0.001**	1.000	1.000	0.413	0.101	**0.011**
	DMSO vs. CPT-11	0.254	0.270	1.000	0.066	1.000	**0.001**
	DMSO vs. EA + CPT-11	**<0.001**	0.128	**0.045**	**0.001**	0.101	**<0.001**
48 h	DMSO vs. EA	0.149	1.000	1.000	0.315	0.067	1.000
	DMSO vs. CPT-11	0.355	0.332	1.000	0.208	0.813	0.125
	DMSO vs. EA + CPT-11	**0.010**	0.084	0.535	**0.001**	**0.010**	**0.017**
DMSO	12 h vs. 24 h	1.000	1.000	1.000	1.000	1.000	1.000
	12 h vs. 48 h	1.000	1.000	1.000	1.000	1.000	1.000
	24 h vs. 48 h	1.000	1.000	1.000	1.000	1.000	1.000
EA	12 h vs. 24 h	0.931	0.072	0.548	0.272	0.225	0.743
	12 h vs. 48 h	0.122	**0.043**	0.484	0.139	0.053	**<0.001**
	24 h vs. 48 h	0.664	1.000	1.000	1.000	1.000	**<0.001**
CPT-11	12 h vs. 24 h	0.222	1.000	1.000	1.000	**0.005**	0.329
	12 h vs. 48 h	0.919	1.000	1.000	1.000	0.338	1.000
	24 h vs. 48 h	1.000	1.000	1.000	1.000	0.338	0.166
EA + CPT-11	12 h vs. 24 h	**0.013**	**0.043**	0.413	**0.001**	0.234	1.000
	12 h vs. 48 h	**0.021**	0.072	0.154	**<0.001**	0.720	0.217
	24 h vs. 48 h	1.000	1.000	1.000	1.000	1.000	0.186

*ANOVA test, **Mann-Whitney U test, bolded values remain significant after correction for multiple comparisons ($p<0.05$).

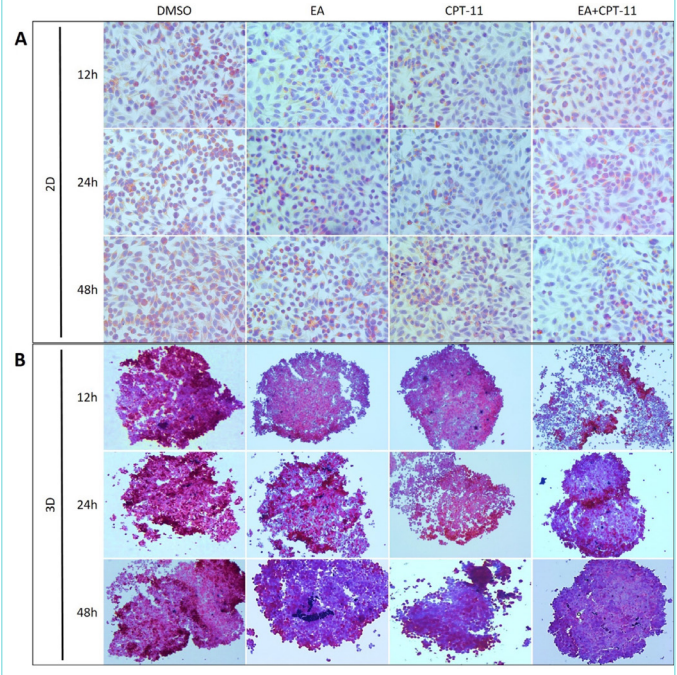


Figure 5. Representative images of MMP-2 immunoexpressions of HeLa cells in 2D planar culture (A) and 3D constructs (B) at 12, 24 and 48 h treatments (AEC & hematoxylin).

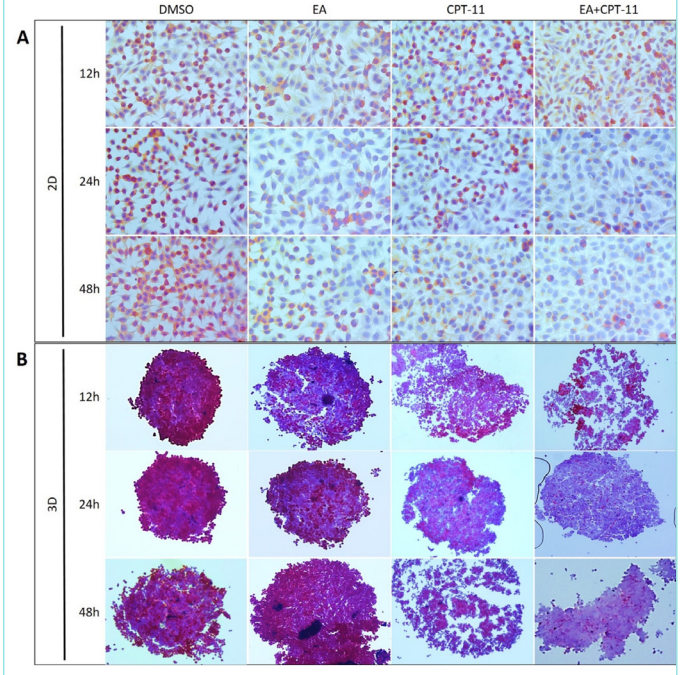


Figure 6. Representative images of MMP-9 immunoexpressions of HeLa cells in 2D planar culture (A) and 3D constructs (B) at 12, 24 and 48 h treatments (AEC & hematoxylin).

groups decreased compared to the DMSO group. The expression level of the EA + CPT-11 group was found to be statistically significant compared to the DMSO group in the 12, 24, and 48 h applications (p=0.002; 0.002; 0.002, respectively). In addition, it was determined that the expression level of the lean CPT-11 group was statistically significantly decreased compared with the DMSO group in the 48-hour application (p=0.003) (Figure 3F). Statistical data for all groups are presented in Table 2.

When Bcl-2 expression levels were analyzed in 3D culture experiments, it was seen that the expression levels of all groups decreased compared to the DMSO group. It was determined that the expression level of the EA, CPT-11, and EA + CPT-11 groups decreased statistically significantly in 12 and 24 h applications compared to the DMSO group (for 12 hours: p=0.006; 0.004; <0.001, respectively, for 24 hours: p=0.011; 0.001; <0.001, respectively). In addition, the expression level of the EA + CPT-11 group was found to be statistically significantly decreased compared with the DMSO group in the 48-hour application (p=0.017) (Figure 4F). Statistical data for all groups are presented in Table 3.

When Bax protein expressions in two- and three-dimensional cultures were evaluated by immunocytochemical analysis, they were found to be compatible with gene expression levels (Figure 9). Briefly, Bax expression levels were found to be increased in all groups compared with the DMSO group. Contrary to Bax protein, Bcl-2 protein expression was found to be highest in the DMSO group (Figure 10). Especially in the combined group (EA + CPT-11), the expression of anti-apoptotic gene *Bcl-2* was significantly decreased compared to the DMSO group.

DISCUSSION

Cancer is defined as a disease resulting from disruption of the balance of cell death and proliferation. Today, with the increase of carcinogenic agents, the incidence of cancer and the number of related deaths are also increasing.[7] The semi-synthetic camptothecin derivative CPT-11 is obtained from Camptotheca acuminate. This drug has a great antitumoral effect on many tumors such as lung, ovarian, colorectal, and cervical cancer. In addition, toxicology studies have shown that they have widespread toxic effects in the hematological system and lymphoid organs. Myelotoxicity, neutropenia, thrombocytopenia, and anemia are among the most serious toxic effects. Hematological side effects are dose-dependent. In a study investigating cell proliferation, cell migration, and cell cycle in HeLa cells, researchers found the lethal dose of CPT-11 to be 78.5 µg/mL.[8-10] In our study, we determined the IC_{50} dose of CPT-11 applied to 2D and 3D HeLa cells as 57.8 µM, and 94 µM, respectively.

Phytochemicals are useful in cancer prevention because they simultaneously target multiple pathways active in cancer progression. For improved cancer management and therapy, combining phytochemicals with anticancer medications may be a novel and very effective therapeutic approach. When taken alone, commercial medications can be more successful when combined with phytochemicals that are comparatively less harmful.[11,12] Previous research has shown that the phenolic component EA, which is generated from plants and is present in raspberries and other plant foods, has strong anticarcinogenic properties.[13,14] In a study, it was claimed that EA caused human cervical cancer Ca Ski cells to go into G0/G1 arrest and eventually die.[13] *In vivo* and *in vitro* studies, EA has been demonstrated to be a potent antioxidant with free radical scavenging ability, anti-aging, anti-proliferative, anti-fibrotic, anti-atherosclerotic, anti-cancer and anti-mutagenic properties.[15-17] In this study, we demonstrated the time-dependent effects of EA and its combination with irinotecan on HeLa cells. In a study examining the effect of different doses of EA (5, 25 and 50 µM) on the cell cycle, histogram findings demonstrated that EA mostly inhibited the cell cycle by dose-dependently arresting cells in

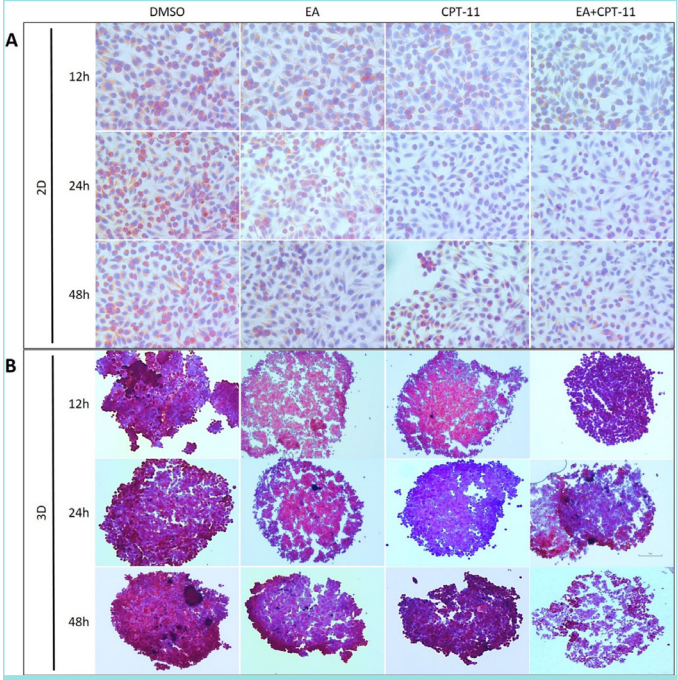


Figure 7. Representative images of TGF-β1 immunoexpressions of HeLa cells in 2D planar culture (A) and 3D constructs (B) at 12, 24 and 48 h treatments (AEC & hematoxylin).

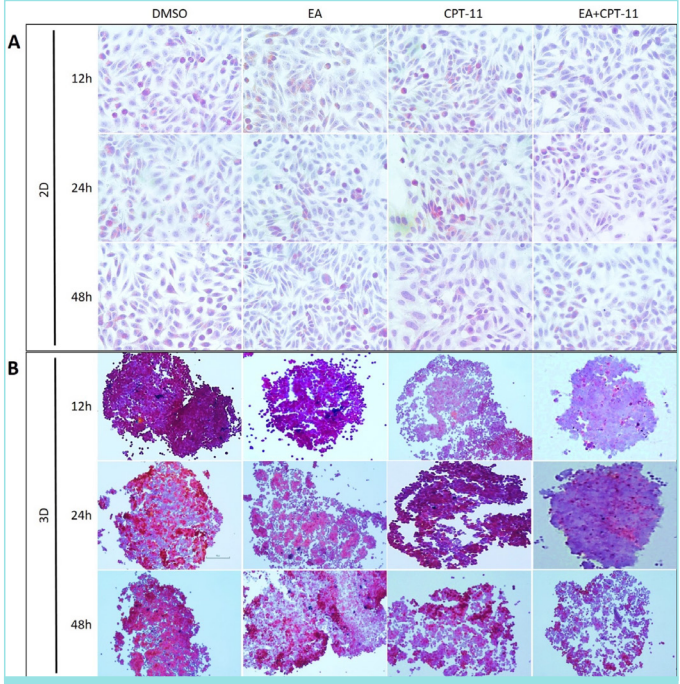


Figure 8. Representative images of HIF1α immunoexpressions of HeLa cells in 2D planar culture (A) and 3D constructs (B) at 12, 24 and 48 h treatments (AEC & hematoxylin).

the G0/G1 phase.[18] Li et al.[19] used a variety of human cervical cancer cell lines (HeLa, SiHa and C33A) in their study, they showed that EA applied to cell cultures at 10, 20 and 30 µM doses inhibited cell proliferation at a statistically significant level. In our study, we determined the IC_{50} dose of EA applied to 2-dimensional HeLa cells as 45.4 µM, and 120 µM, respectively.

Studies have demonstrated that EA suppresses angiogenesis-related markers in tumor tissues, including COX-2, HIF1α, VEGF, VEGFR, and IL-8, as well as inhibits the metastatic markers MMP-2 and MMP-9. In studies, it has been shown that tumor metastasis and development can be suppressed by the EMT mechanism in this way.[20] In a study, it was shown that EA significantly inhibited the migration of human ovarian carcinoma A2780 cells. In the same study, EA administered at doses of 10 and 15 µg/mL significantly downregulated the expression levels of MMP-2 and -9. In the *in vivo* part of the same study, it was stated that EA caused a significant decrease in the weight and volume of the tumor. The immunohistochemical findings of the study also showed that by reducing the expression of MMPs, EA had anticancer properties. In addition, the antitumor properties of EA were confirmed by serum ELISA analysis.[21]

A study with human and rat prostate cancer cell lines showed that EA treatment slightly reduced MMP-2 secretion but did not affect the MMP-9 expression.[22] In our study, it was determined that the levels of MMP-2 and -9 were highest in the DMSO group at 12, 24 and 48 h of treatment. In addition, it was determined that the expression of MMPs, which are markers of metastasis, decreased in the treatment groups and especially in the combined group (EA + CPT-11) compared to the DMSO group.

The characteristics of the tumor microenvironment, such as the synthesis of TGF-β, probably contribute to the induction of EMT even though it is a multistep process with complicated mechanisms.[23] *In vitro* results of a study revealed that partial inhibition of TGF-β1 must reduce the migration of cancer cells and induce apoptosis.[24] In a study, it was reported that EA induces cell cycle arrest in MCF-7 cells through TGF-β/Smad3 signaling.[25]

Recent findings have shown the role of HIF1α and TGF-β1 in the development in tumors.[23,26] Even though research suggests that HIF1α and TGF-β may cause EMT, further research is still needed to fully understand how HIF1α and TGF-β interact and how this interacts with EMT in cervical cancer. In our study, it was determined that TGF-β1 expression levels were highest in the DMSO group in 2D and 3D cultures in applications with varying durations. In addition, it was determined that TGF-β1 expressions, a marker of angiogenesis and EMT, was decreased in the treatment groups and especially in the combined group (EA + CPT-11) compared to the DMSO group.

Detecting intra-tumor oxygen tension, HIF1 promotes the activation of hypoxia-related reactions implicated in cancer progression, such as proliferation, metabolism, angiogenesis, invasion, metastasis, and treatment resistance.[23] The upregulation of HIF1α activity promotes tumor-associated angiogenesis and thus tumor cell survival and proliferation in solid tumors.[27,28] In a study, it was shown that EA decreased HIF1α in lung cancer cells and tumor tissues.[29] Numerous studies link elevated levels of HIF1α expression to the emergence and spread of various malignancies. Inhibiting HIF1α is therefore a promising anticancer therapy.[30,31] Since angiogenesis plays an important role in tumor growth,[32] we tried to determine the effects of EA, CPT-11, and their combination on angiogenesis by determining HIF1α expression levels in cancer cells by RT-PCR and intraclass correlation coefficient analyses. In our study, HIF1α expression levels were decreased in all treatment groups compared with the DMSO group in applications for varying durations. Although this decrease

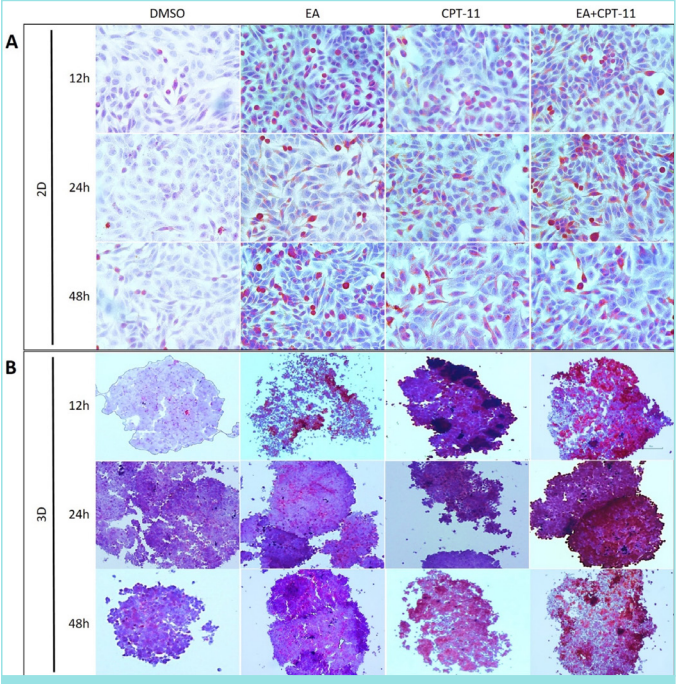


Figure 9. Representative images of Bax immunoexpressions of HeLa cells in 2D planar culture (A) and 3D constructs (B) at 12, 24 and 48 h treatments (AEC & hematoxylin).

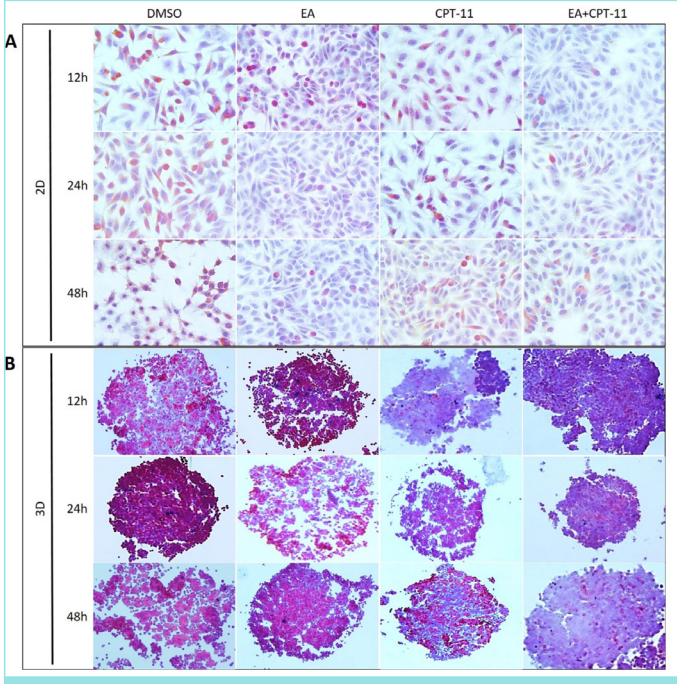


Figure 10. Representative images of Bcl-2 immunoexpressions of HeLa cells in 2D planar culture (A) and 3D constructs (B) at 12, 24 and 48 h treatments (AEC & hematoxylin).

was not significant in 2D cultures, this decrease in HIF1α expression level in 3D cultures was significantly higher in the combined group than in the DMSO group.

Promoting the synthesis of pro-apoptotic members of the Bcl-2 family and suppressing the expression of anti-apoptotic members are crucial in terms of increasing the effectiveness of chemotherapy and radiotherapy. It has been shown that Bax, one of the pro-apoptotic members of the Bcl-2 family, is suppressed in various cancers, and that promoting Bax expression also creates susceptibility to apoptosis in tumors.[33,34] According to studies, EA causes cancer tissue to express less antiapoptotic protein Bcl-2 and more proapoptotic protein Bax. Likewise, while EA increases the level of active caspase-3, it decreases the expression of proliferation markers such as Ki67 and PCNA. In this way, tumor development with apoptotic mechanisms can be suppressed by EA.[20] In the study of Li et al.[19], it was revealed by flow cytometric and cell cycle analyzes that EA stopped the cell cycle in G1 phase and induced cell apoptosis in HeLa cells. In one study, HeLa cells were treated with 25 μM dose of curcumin and EA for 24 hours. It has been demonstrated that the anticancer effects of this combination are superior to those of either medication alone. In the study, curcumin and EA restored p53, induce ROS generation and DNA damage. Immunocytochemical analysis in the study showed that EA and curcumin did not alter the expression of Bax. However, it was stated that the combination of curcumin and EA showed higher Bax expression in cells.[35] In our study, it was determined that Bax expression levels increased in all groups after 12, 24 and 48 h of treatment compared with the DMSO group. Contrary to Bax protein, Bcl-2 protein expression was found to be highest in the DMSO group. Especially in the combined group (E+ CPT-11), the expression of antiapoptotic gene Bcl-2 was significantly decreased compared to the DMSO group.

CONCLUSION

In the study, it was shown that the expressions of MMPs, TGF-β1 and HIF1α were significantly inhibited in HeLa cells treated with EA and treated with the combination of CPT-11. In addition, the increase in proapoptotic Bax level with the treatment and suppression of antiapoptotic Bcl-2 expression were revealed as another important finding of EA and combined treatment. From these results, we can conclude that EA can reduce the invasion of cervical cancer cell lines and increase apoptosis through modulation of MMP activity, TGF-β1 and HIF1α levels. Therefore, EA can be used as an adjuvant therapy for cervical cancer.

MAIN POINTS

- Cervical cancer is an important public health problem worldwide with a high incidence rate.
- Ellagic acid downregulated the expression of MMPs, TGF-β1 and HIF1α in HeLa cells.
- While ellagic acid increased the proapoptotic Bax expression level in HeLa cells, it suppressed the antiapoptotic Bcl-2 expression.
- These data indicate that ellagic acid would be a good candidate for additional preclinical studies for the treatment of human cervical cancer.

ETHICS

Ethics Committee Approval: Ethics committee form was not required.

Informed Consent: A consent form was not required for this study as the cells were purchased commercially.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

DISCLOSURES

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

Financial Disclosure: This work was supported by the Scientific Researches Project Unit at Kırşehir Ahi Evran University (grant number: TIP.A4.19.005).

REFERENCES

1. World Health Organization. The Global Cancer Observatory. [30.08.2022]; Available from: https://gco.iarc.fr/
2. Park SH, Kim M, Lee S, Jung W, Kim B. Therapeutic Potential of Natural Products in Treatment of Cervical Cancer: A Review. Nutrients. 2021; 13(1): 154.
3. Pectasides D, Kamposioras K, Papaxoinis G, Pectasides E. Chemotherapy for recurrent cervical cancer. Cancer Treat Rev. 2008; 34(7): 603-13.
4. Takeuchi S, Dobashi K, Fujimoto S, Tanaka K, Suzuki M, Terashima Y, et al. A late phase II study of CPT-11 on uterine cervical cancer and ovarian cancer. Research Groups of CPT-11 in Gynecologic Cancers. Gan To Kagaku Ryoho. 1991; 18(10): 1681-9.
5. Verschraegen CF, Levy T, Kudelka AP, Llerena E, Ende K, Freedman RS, et al. Phase II study of irinotecan in prior chemotherapy-treated squamous cell carcinoma of the cervix. J Clin Oncol. 1997; 15(2): 625-31.
6. Abe LT, Lajolo FM, Genovese MI. Potential dietary sources of ellagic acid and other antioxidants among fruits consumed in Brazil: jabuticaba (Myrciaria jaboticaba (Vell.) Berg). J Sci Food Agric. 2012; 92(8): 1679-87.
7. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell. 2000; 100(1): 57-70.
8. Kavanagh JJ, Verschraegen CF, Kudelka AP. Irinotecan in cervical cancer. Oncology (Williston Park). 1998; 12(8 Suppl 6): 94-8.
9. Fuchs C, Mitchell EP, Hoff PM. Irinotecan in the treatment of colorectal cancer. Cancer Treat Rev. 2006; 32(7): 491-503.
10. Lorusso D, Pietragalla A, Mainenti S, Masciullo V, Di Vagno G, Scambia G. Review role of topotecan in gynaecological cancers: current indications and perspectives. Crit Rev Oncol Hematol. 2010; 74(3): 163-74.
11. Yunos NM, Beale P, Yu JQ, Huq F. Synergism from sequenced combinations of curcumin and epigallocatechin-3-gallate with cisplatin in the killing of human ovarian cancer cells. Anticancer Res. 2011; 31(4): 1131-40.
12. Singh M, Bhatnagar P, Srivastava AK, Kumar P, Shukla Y, Gupta KC. Enhancement of cancer chemosensitization potential of cisplatin by tea polyphenols poly(lactide-co-glycolide) nanoparticles. J Biomed Nanotechnol. 2011; 7(1): 202.
13. Narayanan BA, Geoffroy O, Willingham MC, Re GG, Nixon DW. p53/p21(WAF1/CIP1) expression and its possible role in G1 arrest and apoptosis in ellagic acid treated cancer cells. Cancer Lett. 1999; 136(2): 215-21.

14. Perchellet JP, Gali HU, Perchellet EM, Klish DS, Armbrust AD. Antitumor-promoting activities of tannic acid, ellagic acid, and several gallic acid derivatives in mouse skin. Plant Polyphenols: Springer; 1992.p.783-801.

15. Suzuki N, Masamune A, Kikuta K, Watanabe T, Satoh K, Shimosegawa T. Ellagic acid inhibits pancreatic fibrosis in male Wistar Bonn/Kobori rats. Dig Dis Sci. 2009; 54(4): 802-10.

16. Kuo MY, Ou HC, Lee WJ, Kuo WW, Hwang LL, Song TY, et al. Ellagic acid inhibits oxidized low-density lipoprotein (OxLDL)-induced metalloproteinase (MMP) expression by modulating the protein kinase C-α/extracellular signal-regulated kinase/peroxisome proliferator-activated receptor γ/nuclear factor-κB (PKC-α/ERK/PPAR-γ/NF-κB) signaling pathway in endothelial cells. J Agric Food Chem. 2011; 59(9): 5100-8.

17. Polce SA, Burke C, França LM, Kramer B, de Andrade Paes AM, Carrillo-Sepulveda MA. Ellagic Acid Alleviates Hepatic Oxidative Stress and Insulin Resistance in Diabetic Female Rats. Nutrients. 2018; 10(5): 531.

18. Woo MS, Choi HS, Seo MJ, Jeon HJ, Lee BY. Ellagic acid suppresses lipid accumulation by suppressing early adipogenic events and cell cycle arrest. Phytother Res. 2015; 29(3): 398-406.

19. Li LW, Na C, Tian SY, Chen J, Ma R, Gao Y, et al. Ellagic acid induces HeLa cell apoptosis via regulating signal transducer and activator of transcription 3 signaling. Exp Ther Med. 2018; 16(1): 29-36.

20. Zhao M, Tang SN, Marsh JL, Shankar S, Srivastava RK. Ellagic acid inhibits human pancreatic cancer growth in Balb c nude mice. Cancer Lett. 2013; 337(2): 210-7.

21. Liu H, Zeng Z, Wang S, Li T, Mastriani E, Li QH, et al. Main components of pomegranate, ellagic acid and luteolin, inhibit metastasis of ovarian cancer by down-regulating MMP2 and MMP9. Cancer Biol Ther. 2017; 18(12): 990-9.

22. Pitchakarn P, Chewonarin T, Ogawa K, Suzuki S, Asamoto M, Takahashi S, et al. Ellagic acid inhibits migration and invasion by prostate cancer cell lines. Asian Pac J Cancer Prev. 2013; 14(5): 2859-63.

23. Su Q, Fan M, Wang J, Ullah A, Ghauri MA, Dai B, et al. Sanguinarine inhibits epithelial-mesenchymal transition via targeting HIF-1α/TGF-β feed-forward loop in hepatocellular carcinoma. Cell Death Dis. 2019; 10(12): 939.

24. Wang DG, Li TM, Liu X. RHCG suppresses cervical cancer progression through inhibiting migration and inducing apoptosis regulated by TGF-β1. Biochem Biophys Res Commun. 2018; 503(1): 86-93.

25. Chen HS, Bai MH, Zhang T, Li GD, Liu M. Ellagic acid induces cell cycle arrest and apoptosis through TGF-β/Smad3 signaling pathway in human breast cancer MCF-7 cells. Int J Oncol. 2015; 46(4): 1730-8.

26. Mallikarjuna P, Raviprakash TS, Aripaka K, Ljungberg B, Landström M. Interactions between TGF-β type I receptor and hypoxia-inducible factor-α mediates a synergistic crosstalk leading to poor prognosis for patients with clear cell renal cell carcinoma. Cell Cycle. 2019; 18(17): 2141-56.

27. Pugh CW, Ratcliffe PJ. Regulation of angiogenesis by hypoxia: role of the HIF system. Nat Med. 2003; 9(6): 677-84.

28. Zundel W, Schindler C, Haas-Kogan D, Koong A, Kaper F, Chen E, et al. Loss of PTEN facilitates HIF-1-mediated gene expression. Genes Dev. 2000; 14(4): 391-6.

29. Duan J, Li Y , Gao H , Yang D , He X , Fang Y , et al . Phenolic compound ellagic acid inhibits mitochondrial respiration and tumor growth in lung cancer. Food Funct. 2020; 11(7): 6332-9.

30. Peng J, Wang X, Ran L, Song J, Luo R, Wang Y. Hypoxia-Inducible Factor 1α Regulates the Transforming Growth Factor β1/SMAD Family Member 3 Pathway to Promote Breast Cancer Progression. J Breast Cancer. 2018; 21(3): 259-66.

31. Li G, Shan C, Liu L, Zhou T, Zhou J, Hu X, et al. Tanshinone IIA inhibits HIF-1α and VEGF expression in breast cancer cells via mTOR/p70S6K/RPS6/4E-BP1 signaling pathway. PLoS One. 2015; 10(2): e0117440.

32. Folkman J. Angiogenesis and proteins of the hemostatic system. J Thromb Haemost. 2003; 1(8): 1681-2.

33. Bargou RC, Wagener C, Bommert K, Mapara MY, Daniel PT, Arnold W, et al. Overexpression of the death-promoting gene bax-alpha which is downregulated in breast cancer restores sensitivity to different apoptotic stimuli and reduces tumor growth in SCID mice. J Clin Invest. 1996; 97(11): 2651-9.

34. Johnstone RW, Ruefli AA, Lowe SW. Apoptosis: a link between cancer genetics and chemotherapy. Cell. 2002; 108(2): 153-64.

35. Kumar D, Basu S, Parija L, Rout D, Manna S, Dandapat J, et al. Curcumin and Ellagic acid synergistically induce ROS generation, DNA damage, p53 accumulation and apoptosis in HeLa cervical carcinoma cells. Biomed Pharmacother. 2016; 81: 31-7.

RESEARCH ARTICLE

CYPRUS
JOURNAL OF MEDICAL SCIENCES

DOI: 10.4274/cjms.2022.2021-212
Cyprus J Med Sci 2023;8(3):184-189

Asymptomatic Group A Beta Hemolytic Streptococcal Pharyngeal Carriage in North Cyprus

Rüyam Kumsal[1,2], Selma Yılmaz[3], İlker Etikan[4], Eşref Çelik[1,2]

[1]Clinic of Infectious Diseases, Başkent Hospital, Nicosia, North Cyprus
[2]Department of Medical and Clinical Microbiology, Near East University Faculty of Medicine, Nicosia, North Cyprus
[3]Department of Medical Biology, Near East University Faculty of Medicine, Nicosia, North Cyprus
[4]Department of Biostatistics, Near East University Faculty of Medicine, Nicosia, North Cyprus

Abstract

BACKGROUND/AIMS: Group A beta-hemolytic streptococcus (GAS) *Streptococcus pyogenes* can cause benign to life-threatening infections such as pharyngitis, rheumatic fever, and acute glomerulonephritis. Asymptomatic carriers of GAS are a reservoir for transmission. The main aims of this study were to ascertain rates of asymptomatic carriage in adults in North Cyprus to prevent outbreaks and fatal complications and to gain insight into the risk factors for carriage in individuals with rheumatic and cardiovascular disease.

MATERIALS AND METHODS: This prospective study included 307 participants and was conducted from April 2019 to December 2019. The pharyngeal samples were collected from five districts of North Cyprus. Throat cultures were performed followed by rapid strep A antigen tests, catalase tests, gram staining, Lancefield latex agglutination, pyrrolidonyl arylamidase, bacitracin sensitivity, and trimethoprim-sulfamethoxazole resistance tests on subcultures.

RESULTS: In 307 participants, an asymptomatic prevalence rate of 4.9% was found. A higher and statistically significant ($p<0.05$) risk of GAS carriage was found in participants with rheumatoid and cardiovascular disease and in health care workers aged 18-29.

CONCLUSION: In North Cyprus, this is the first study to randomly sample a broad selection of the population for GAS pharyngeal carriage A higher risk of GAS carriage was found among participants with rheumatic and cardiovascular disease, in healthcare workers, and in the 18-29 years old age group, indicating the possibility of GAS hospital outbreaks and emphasizing the importance for outbreak prevention of screening of individuals with underlying diseases, and in school and healthcare settings.

Keywords: Streptococci, *S. pyogenes*, GAS, asymptomatic carriers

INTRODUCTION

Group A beta-hemolytic streptococcus (GAS) *(Streptococcus pyogenes)* colonizes the skin and oropharynx and can cause a significant illness in humans. It is a coccoid Gram-positive bacterium capable of causing illnesses ranging from self-limiting to life-threatening, making it an important organism for research. The annual rate of mortality from GAS-related infections is 517,000 and the rate of new cases is 1.78 million each year.[1] The transmission of GAS is exclusively human-to-human with respiratory droplets from the oropharynx largely being responsible.[2-4] Asymptomatic carriers are a reservoir for GAS transmission; therefore, it is crucial to identify carriers to prevent outbreaks and reduce the threat of serious infections acquired from these carriers.

The main virulence factor for GAS is the M protein, which is a major antigen and is therefore a target for vaccine development. However, it

To cite this article: Kumsal R, Yılmaz S, Etikan İ, Çelik E. Asymptomatic Group A Beta Hemolytic Streptococcal Pharyngeal Carriage in North Cyprus. Cyprus J Med Sci 2023;8(3):184-189

ORCID IDs of the authors: R.K. 0000-0003-2060-5544; S.Y. 0000-0002-5985-7629; İ.E. 0000-0001-9171-8269; E.Ç. 0000-0003-4962-9697.

Address for Correspondence: Eşref Çelik
E-mail: esref.celik@neu.edu.tr
ORCID ID: orcid.org/0000-0003-4962-9697

Received: 03.02.2022
Accepted: 13.12.2022

has a highly variable structure, which complicates antigenic targeting. The *emm* gene encodes M protein. The *emm* type is an important factor in what kind of disease is produced, and the *emm* type distribution varies by country according to geographical and economic position.[5,6] More than 200 *emm* types exist, which belong to two main divisions: Class 1 and class 2. M proteins belonging to class 1 are associated with acute rheumatic fever outbreaks. This is because antibodies targeting certain regions of M proteins can crossreact with cardiac myosin causing rheumatic heart disease (RHD). Specific M types are associated with serious complications including necrotizing fasciitis, sepsis, and rheumatic sequelae.[5,6]

M protein plays an important role in the inhibition of phagocytosis and opsonization of GAS. It can bind the Fc domain of antibodies and block the complement cascade through a variety of other mechanisms preventing complement C3b binding and formation of the membrane attack complex. M protein is also important in mediating the adhesion of GAS to the host cell.[5,6]

GAS causes suppurative and nonsuppurative infections. Pharyngitis, impetigo, scarlet fever, cellulitis, necrotizing fasciitis, toxic shock syndrome, and sepsis are suppurative infections. Non-suppurative post-sequelae include acute glomerulonephritis and rheumatic fever.[7]

Asymptomatic carriers were defined as having GAS in the posterior pharynx without illness, i.e., throat culture shows a positive result in the absence of symptoms. An additional criterion required in this study for a carrier to be considered asymptomatic is a negative test for the antibodies anti-deoxyribonuclease B, anti-streptolysin-O, and anti-hyaluronidase.[8] It is important to differentiate asymptomatic carriers from cases with symptomatic disease. This is crucial for correct treatment. For asymptomatic carriers, treatment may be necessary in special situations such as outbreaks in communities of GAS invasive disease, pharyngitis, rheumatic fever, or glomerulonephritis and in families with a history of rheumatic fever.

Several theories have been presented to explain the asymptomatic carriage of GAS, including internalization of GAS into epithelial cells and growth of GAS in biofilms.[9,10] All these theories suggest mechanisms by which GAS can evade antibiotic treatment.

A total of 307 volunteers were selected from the five main districts of North Cyprus for this study and analyzed for GAS pharyngeal carriage. The purpose of this research is to determine the rate of GAS asymptomatic pharyngeal carriage in North Cyprus to better understand the risk of outbreaks and to reduce the risk of serious complications.

MATERIALS AND METHODS

Ethical Approval

This study was approved by the Near East University Institution Ethics Evaluation Board (approval number: YDU/2019/67-768 date: 28.03.2019).

Participants and Study Design

This study is a prospective study conducted from April 2019 to December 2019. A total of 307 healthy adult participants were randomly selected from the five districts of North Cyprus: Nicosia, Famagusta, Kyrenia, Trigomo, and Lefke. Medical and non-medical salaried workers aged 18 to 65 years located in regional hospitals or medical faculties and long-term local residents of the five districts (regardless of occupation) were included in this study. Pharyngeal specimens were collected from all participants. Participants were presented with a questionnaire that asked for information including age, gender, nationality, area of residence, and occupation. Medical information was also required including whether they smoked, used alcohol, had any history of cardiovascular and rheumatic disease, had any respiratory or sleeping disorders, presence of tonsillectomy surgery, and had any usage of unprescribed antibiotics. All participants included in the study also signed a consent form.

Laboratory Studies

Swab samples were taken from the posterior pharynx and tonsils of the participants using sterile swabs. The collected specimens were immediately transferred to the Başkent Private Hospital microbiology laboratory for screening and analysis of GAS using several standard microbiological and serological methods for detection of GAS carriage. Collected specimens were inoculated into 5% defibrinated sheep blood agar (SBA) (Condalab, Madrid, Spain) and incubated for 24-48 hours at 37 °C and tested with Vesrapido, an *in vitro* rapid immunochromatographic strep A antigen test (Vesta medikal, İstanbul, Türkiye). The test was performed according to the manufacturer's instructions. The manufacturer claims a sensitivity of 94,3% and a specificity of 100% for group bacteria in throat samples. After 24 h, the plates were examined for beta hemolysis, and positive colonies were considered as potential GAS and subjected to further analysis. Suspected colonies were subjected to a catalase test and Gram-staining (Premed, İstanbul, Türkiye). The catalase test differentiates catalase-positive *Staphylococcaceae* and *Micrococcaceae* from catalase-negative *Streptococcaceae* and *Enterococcus* spp. GAS are catalase negative and their morphology is gram positive with cocci arranged in chains. Catalase-negative colonies confirmed by Gram-staining were subcultured into 5% SBA for 24 h at 37 °C. The cultures were tested for sensitivity to the antibiotic bacitracin to differentiate beta hemolytic group A streptococci (*Streptococcus pyogenes*-susceptible) and susceptible micrococci from beta hemolytic non-group A Streptococci. These were used together with a sulfamethoxazole/trimethoprim (SXT) antibiotic disc susceptibility test to detect group beta hemolytic streptococci, which are SXT resistant.

Bacitracin Susceptibility Test Discs (Bioanalyse, Ankara, Türkiye) and in conjunction with it, SXT Susceptibility Test Discs (Bioanalyse, Ankara, Türkiye) were placed on subcultures growing on SBA and incubated for 24 hours at 37 °C. Colonies that were both bacitracin sensitive and SXT resistant were subsequently analyzed with a **pyrrolidonyl aminopeptidase** (PYR) **test kit** (Bioanalyse, Ankara, Türkiye). PYR is used for the detection of PYR (also called PYR) activity in group A strep *Streptococcus pyogenes*), *Enterococcus* spp., some coagulase-negative *Staphylococci*, and some *Enterobacteriaceae*.

PYR-positive colonies were classified using the Lancefield latex agglutination test (Strep Test Kit, Plasmatec, Bridport, UK) to assign group A beta-hemolytic Streptococci to Lancefield groups.

Statistical Analysis

Statistical analysis was performed using the IBM Statistical Package for the Social Sciences software demo version 26.0 (SPSS Inc, Chicago, USA).

Potential risk factors for GAS carriage were identified using a questionnaire. Outcome measures were stratified using several

characteristics: participants age/s, gender, nationality, medical history of certain diseases such as cardiovascular and rheumatic diseases, occupational risk factors such as nature of work, factors of influence such as antibiotic usage, and the prevalence of asymptomatic GAS carriage alongside sociodemographic data. Outcome measures were expressed as summary point prevalence percentages (number of subjects having the diseases at a time point/total number of subjects in the population) with 95% confidence intervals (CIs). Chi-square test was used for statistical analysis (2 * 2 layouts). If any of the expected frequencies was less than 5, Fisher's exact chi-square test was used instead. Odds ratios related to variables were also calculated and given with 95% CIs.

RESULTS

This study included 307 healthy adult individuals. Sample collection and data analysis were performed from April 2019 to December 2019. Participants were randomly selected from the salaried personnel (medical and non-medical) in the regional hospitals or medical schools and from long-term local residents regardless of occupation living in the five main districts of North Cyprus and tested for GAS pharyngeal carriage. Participants under 18 years were excluded from the study, while hospital personnel were included only within the age range from 18 to 65 years. Pharyngeal specimens were collected from the participants and analyzed in the Baskent Private Hospital microbiology laboratory for GAS.

All participants completed a questionnaire. Potential risk factors for GAS carriage were identified, documented, and analyzed by the questionnaire which included basic demographic data as well as questions regarding medical history.

The overall rate of group A beta-hemolytic streptococci pharyngeal carriage was found to be 4.9% (307/15) (Table 1). Among the participants, 102 (33.2%) were males and 205 (66.8%) were females. 4.9% of males and 4.8% of females were GAS positive. There was no significant correlation between gender and the rate of GAS carriage $p>0.05$, t-test) (Table 2).

Participants were divided into the following age groups: 18-29 years old (63, 20.5%), 30-39 years old (50, 16.3%), 40-49 years old (52, 16.9%), 50-59 years old (69, 22.5%), and >60 years old (73, 23.8%). GAS carriage differences between age groups were not statistically significant ($p>0.05$, t-test) (the groups are included in Table 2).

The majority of participants were of Cypriot nationality (268, 87.3%). Other nationalities were also included (39, 12.7%). Row percentage of GAS carriage for Cypriots was 4.1% and for other nationalities was 10.26%. Statistical analysis using a chi-square test showed that GAS carriage did not differ significantly between nationalities (Cypriot vs. other) ($p>0.05$). In contrast, the odds ratio [odd ratio (OR): 2.67] showed that there was a higher risk of GAS carriage in other nationalities.

Participants were distributed between districts as follows: Nicosia (155, 50.5%) had the highest rate of GAS positivity, Famagusta and Trigomo (54, 17.6%), Kyrenia (47, 15.3%), Lefke and Omorfo (51, 16.6%). Chi-square test results showed no statistical significance between the distribution of GAS carriage between different districts ($p>0.05$) (Table 2).

222 (72.3%) participants used prescribed antibiotics, while 85 (27.7%) participants used unprescribed antibiotics. The difference in rates of GAS pharyngeal carriage between these groups was not statistically significant ($p>0.05$) (chi-square test) (Table 2).

The risk of GAS carriage in participants with rheumatic disease (row percent of GAS positivity was 21.4%) or cardiovascular disease (row percent of GAS positivity was 15%) was found to be higher relative to other participants and each one was individually statistically significant ($p<0.05$) (Table 2). Participants with rheumatic disease were found to have a 6 times higher risk (OR: 6.386) and with cardiovascular disease a 4 times risk (OR: 4.044) of GAS pharyngeal carriage than participants who had neither rheumatic nor cardiovascular disease (Table 2). There were no cases with the presence of both conditions in a single individual, so the combined risk of GAS carriage could not be measured.

Participants were divided by occupation into healthcare workers (79, 25.7%) and other occupational areas (228, 74.3%). There was no statistical significance observed by the use of the chi-square test between the occupational areas and GAS carriage of the participants over the entire study group ($p>0.05$) (Table 2). However, there was a statistical significance observed ($p<0.05$) between the occupational status of the participants within the age group 18-29 years old and GAS pharyngeal carriage (Table 3). The p-value was found to be 0.04 using the Fisher's exact chi-square test, a statistically significant result.

DISCUSSION

This study examined the rate of GAS asymptomatic carriage among 307 adult individuals living in North Cyprus. The overall carriage rate was found to be 4.9% (Table 1). To our knowledge, this is the first large-scale study conducted in North Cyprus to identify asymptomatic GAS carriers. One study of GAS carriage was previously conducted in North Cyprus. However, that study investigated the rate of GAS only among 140 pharmacy students from Iran, Syria, Iraq, and Nigeria in a particular university.[11] The carriage rate was found to be 4.6%, which was similar to the current study that sampled a more representative population.

In the existing literature, there are only a limited number of studies of GAS carriage in adults. In a meta-analysis study, membership status in the National Organization for Economic Cooperation and Development (OECD), which consists mostly of high-income countries, was used to classify populations by socioeconomic position.[4] The prevalence rate of GAS carriage among adults was compared at the country income level between OECD and non-OECD studies. In OECD studies, the adult prevalence of GAS was 2% and in non-OECD studies 4.6%.[4] This study

Table 1. Rate of GAS carriers identified in 307 participants

GAS pharyngeal carriage	Frequency, (n)	Percentage, (%)
Negative	292	95.1
Positive	15	4.9
Total	307	100.0

GAS: Group A beta-hemolytic streptococcus.

Table 2. Description of GAS pharyngeal carriage among participants

Variable	N_{total}=307, (n, %)	GAS positive, N_{GAS}=15, (n, %)[a]	p-value[b]	Odds ratio
Gender				
Female	205 (66.8)	10 (4.9)	0.993	1.0052
Male	102 (33.2)	5 (4.9)		
Age in years				
18-29	63 (20.5)	4 (6.3)	0.953	
30-39	50 (16.3)	3 (6.0)		
40-49	52 (16.9)	2 (3.8)		
50-59	69 (22.5)	3 (4.3)		
>60	73 (23.8)	3 (4.1)		
Nationality				
Cypriot	268 (87.3)	11 (4.1)	0.096	2.6701
Other nationality	39 (12.7)	4 (10.3)		
Region				
Nicosia	155 (50.5)	10 (6.45)	0.429	
Famagusta + trikomo	54 (17.6)	1 (1.85)		
Kyrenia	47 (15.3)	1 (2.13)		
Lefke + omorfo	51 (16.6)	3 (5.88)		
Rheumatoid disease	14 (4.6)	3 (21.4)	0.003	6.384
Cardiovascular disease	20 (6.5)	3 (15)	0.030	4.041
Occupation				
Healthcare	79 (25.7)	7 (8.86)	0.057	0.374
Other	228 (74.3)	8 (3.50)		
Antibiotic usage				
Prescribed	222 (72.3)	11 (4.95)	0.928	1.056
Unprescribed	85 (27.7)	4 (4.71)		

[a]Row percent, [b]Comparison of GAS positive against negative tested variables, GAS: Group A beta-hemolytic streptococcus.

Table 3. Description of GAS pharyngeal carriage among occupational areas within the age group 18-29

Age range 18-29					
Occupational area	**Frequency (n)**	**Percentage (%)**	**GAS frequency (n)**	**GAS percentage (%)**	**p/OR**
Healthcare	16	4.8	3	20.6	p<0.05
Other	47	1.6	1	73.0	OR: 3.119

Odds of observing $GAS_{Negative}$ in other/healthcare=2.674, GAS: Group A beta-hemolytic streptococcus.

showed similarity with the GAS prevalence rate in studies conducted in non-OECD countries.[4]

In this study, participants were consulted for the presence of rheumatoid disease, cardiovascular disease, sleeping disorders, and respiratory illnesses. We analyzed the risk factors for GAS carriage in individuals with rheumatoid and cardiovascular diseases. Higher risk and statistically significant GAS carriage was found among participants with rheumatoid and cardiovascular diseases (Table 2). GAS carriage is mainly known to cause pharyngitis.

Proper diagnosis and treatment of symptomatic pharyngitis caused by GAS is important for prevention of post sequelae complications including rheumatic fever and RHD. ARF causes damage to the heart and is still a problem in low- and middle-income countries worldwide.[1,6,12] GAS carriage becomes important when there is a risk of a carrier developing these or other nonsuppurative complications such as acute glomerulonephritis or when there is a risk of the carrier spreading GAS to other people. Out of 33.4 million patients with RHD worldwide, 275,000 cases are fatal, while 9 million cases result in disability, with most of these cases occurring in developing countries.[1] In the world, there are 33.4 million patients with RHD.[1]

Therefore, it is important to carry out *emm* typing[8] to understand *emm* prevalence in North Cyprus. A reduced number of participants with clinical outcomes may have caused the lower accuracy in statistical terms.

The skin and the nasopharyngeal mucosa are the primary reservoirs for asymptomatic maintenance and transmission of GAS. GAS persists in saliva for an extended period or can reside in the skin tissue of an infected person. GAS is therefore transmitted from an infected person or an asymptomatic carrier by respiratory droplets or skin-skin contact.[6] Many participants in our study were identified as healthcare

workers (79, 25.7%). The GAS carriage of healthcare workers within the age range of 18-29 (4.8%) was statistically significantly different from other occupations ($p<0.05$, OR: 3.119). Odds of observing GAS-negative cases in other occupational groups were 2,674 times higher than in the health care occupational group, suggesting that young healthcare workers are at high risk of GAS carriage. The healthcare workers, due to their occupation, were normally in close contact with patients. Family members or patients admitted to hospitals are in close contact with health workers in clinical settings, leading to an increased risk of subsequent infection. Infection of health workers, even if asymptomatic, would can drive hospital outbreaks.[6] Eradication of GAS carriage in healthcare settings is therefore needed to prevent transmission to patients.[13] More studies should be conducted in healthcare occupations as the risk of outbreak is high. The risk of GAS carriage is higher in individuals with rheumatoid or cardiovascular diseases requiring additional precautions in these groups.

CONCLUSION

We aim to gain insight into the high risk of GAS carriage in this community-level study of adult subjects. There are not many previous studies of adult asymptomatic GAS carriage. In contrast, many previous studies have investigated the asymptomatic carriage of GAS in children.[3] The asymptomatic carriage rate among adults provides crucial information needed to prevent outbreaks along with their potential for serious complications and for an epidemiologic understanding of GAS carriage.

GAS is a global health problem that can cause self-limiting to serious life-threatening complications. Mortality from GAS - related infections is at a critically important rate (about 517,000 annually) and 1.78 million new cases of GAS infection are reported each year.[1] More studies are needed, especially in healthcare settings and schools. To gain better insights into disease outcome and to understand the risk of GAS in North Cyprus, more studies including *emm* typing analysis should be conducted. The extension of such screening programs for GAS carriage to newcomers may serve as an effective preventive measure to avoid GAS transmission.

MAIN POINTS

- The first large-scale study conducted in North Cyprus for identification of adult asymptomatic carriers of GAS (4.9%).
- Showed the importance of screening especially for prevention of outbreaks and fatal complications related to GAS.
- Analyzed the risk factors for GAS carriage in individuals who had rheumatoid and cardiovascular disease.
- Our findings suggest that more studies are needed in North Cyprus, especially in health institutions and schools to understand the prevalence of asymptomatic GAS.

Acknowledgements: This paper is based in full on the Master's thesis of RK (neulibrary/6911201669) with the title "Asymptomatic group a beta-hemolytic streptococci pharyngeal carriage in North Cyprus".

Ethics

Ethics Committee Approval: This study was approved by the Near East University Institution Ethics Evaluation Board (approval number: YDU/2019/67-768, date: 28.03.2019).

Informed Consent: All participants included in the study also signed a consent form.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: R.K., E.Ç., Design: R.K., E.Ç., Supervision: R.K., E.Ç., Fundings: R.K., E.Ç., Materials: R.K., E.Ç., Data Collection or Processing: R.K., E.Ç., Analysis or Interpretation: R.K., S.Y., İ.E., Literature Search: R.K., S.Y., Writing: R.K., S.Y., İ.E., Critical Review: R.K., S.Y.

Conflict of Interest: The authors declared that they have no conflict of interest.

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

REFERENCES

1. Carapetis JR, Steer AC, Mulholland EK, Weber M. The global burden of group A streptococcal diseases. Lancet Infect Dis. 2005; 5(11): 685-94.
2. Ferretti JJ, Stevens DL, Fischetti VA, editors. Streptococcus pyogenes: Basic Biology to Clinical Manifestations [Internet]. Oklahoma City (OK): University of Oklahoma Health Sciences Center; 2016.
3. DeMuri GP, Wald ER. The Group A Streptococcal Carrier State Reviewed: Still an Enigma. J Pediatric Infect Dis Soc. 2014; 3(4): 336-42.
4. Oliver J, Malliya Wadu E, Pierse N, Moreland NJ, Williamson DA, Baker MG. Group A Streptococcus pharyngitis and pharyngeal carriage: A meta-analysis. PLoS Negl Trop Dis. 2018; 12(3): e0006335.
5. Gherardi G, Vitali LA, Creti R. Prevalent emm Types among Invasive GAS in Europe and North America since Year 2000. Front Public Health. 2018; 6: 59.
6. Walker MJ, Barnett TC, McArthur JD, Cole JN, Gillen CM, Henningham A, et al. Disease manifestations and pathogenic mechanisms of Group A Streptococcus. Clin Microbiol Rev. 2014; 27(2): 264-301.
7. Cunningham MW. Pathogenesis of group A streptococcal infections. Clin. Microbiol. Rev. 2000; 13: 470-511.
8. Spellerberg B, Brandt C. Laboratory Diagnosis of Streptococcus pyogenes (group A streptococci). 2016 Feb 10. In: Ferretti JJ, Stevens DL, Fischetti VA, editors. Streptococcus pyogenes: Basic Biology to Clinical Manifestations [Internet]. Oklahoma City (OK): University of Oklahoma Health Sciences Center; 2016.
9. Osterlund A, Popa R, Nikkilä T, Scheynius A, Engstrand L. Intracellular reservoir of Streptococcus pyogenes in vivo: a possible explanation for recurrent pharyngotonsillitis. Laryngoscope. 1997; 107(5): 640-7.
10. Ogawa T, Terao Y, Okuni H, Ninomiya K, Sakata H, Ikebe K, et al. Biofilm formation or internalization into epithelial cells enable Streptococcus pyogenes to evade antibiotic eradication in patients with pharyngitis. Microb Pathog. 2011; 51(1-2): 58-68.
11. Haddad S, Ilktac M, Ogmen S, Celik G. Investigation of Streptococcus pyogenes carriage among pharmacy students in North Cyprus. EMU Journal of Pharmaceutical Sciences. 2019; 2(1): 1-6.

12. Cunningham MW. Rheumatic fever, autoimmunity, and molecular mimicry: the streptococcal connection. Int Rev Immunol. 2014; 33(4): 314-29.

13. Steer AC, Lamagni T, Curtis N, Carapetis JR. Invasive group a streptococcal disease: epidemiology, pathogenesis and management. Drugs. 2012; 72(9): 1213-27.

RESEARCH ARTICLE

CYPRUS
JOURNAL OF MEDICAL SCIENCES

DOI: 10.4274/cjms.2022.2022-15
Cyprus J Med Sci 2023;8(3):190-196

Turkish Validity and Reliability Study of the Fear of Coronavirus Disease-2019 Scale: A Research on Nursing Students

Bilge Bal Özkaptan[1], Nurten Özen[2], Aylin Aydın Sayılan[3], Füsun Terzioğlu[4]

[1]Department of Nursing, Sinop University Faculty of Health Sciences, Sinop, Türkiye
[2]Demiroğlu Bilim University, Florence Nightingale Hospital School of Nursing, İstanbul, Türkiye
[3]Department of Surgical Nursing, Kırklareli University Faculty of Health Sciences, Kırklareli, Türkiye
[4]Kocaeli Health and Technology University, Kocaeli, Türkiye

Abstract

BACKGROUND/AIMS: The coronavirus disease-2019 (COVID-19) outbreak has taken its toll on individuals' mental health and physical health worldwide. Nurses, who play a key role in patient care, experience greater fear and anxiety due to their high-risk exposure to COVID-19 infection. This also leads to anxiety among undergraduate nursing students. This study aimed to determine the validity and reliability of the "Fear of COVID-19 Scale (FCV-19S)" for Turkish nursing students.

MATERIALS AND METHODS: The study was conducted with 351 nursing students from two different universities in Türkiye between May and June 2020. Participant characteristic form and the Turkish version of FCV-19S were utilized to gather the data. Psychometric tests including language validity, content validity, and reliability analyses were performed.

RESULTS: The item total test correlation of all items varies between 0.537 and 0.637. The Explanatory Factor Analysis made to detect the factor design of the scale found the threshold factor loading value as 0.40. The scale's 7 items and single sub-dimension were related to the scale structure to to the Confirmatory Factor Analysis. The scale Cronbach's alpha value was determined as 0.833. The test-retest results, performed to determine the time invariance, revealed a high level of correlation between the two measurements with the Pearson correlation coefficient ($r=0.734$; $p<0.05$).

CONCLUSION: A valid and reliable measurement tool was provided to Türkiye to assess nursing students' fears of COVID-19. The Turkish version of the scale can be utilized as a substantial and reliable estimation device in detecting the fear of coronavirus in nursing students.

Keywords: Coronavirus, COVID-19 fear, scale, nursing, validity and reliability

INTRODUCTION

The infection, first appearing in "Wuhan", "China" in December 2019 due to the new type of Coronavirus, was termed as coronavirus disease-2019 (COVID-19) and rapidly spread all over the world.[1] Türkiye announced its first COVID-19 case on March 10, 2020, one day before the announcement of the outbreak as a pandemic by the "World Health Organization".[1,2]

The speedy spread of the disease through respiratory droplets, mortality rate, uncertainty of the treatment process, social distance, and hygiene measures such as the use of surgical masks and handwashing have

To cite this article: Bal Özkaptan B, Özen N, Aydın Sayılan A, Terzioğlu F. Turkish Validity and Reliability Study of the Fear of Coronavirus Disease-2019 Scale: A Research on Nursing Students. Cyprus J Med Sci 2023;8(3):190-196

ORCID IDs of the authors: B.B.Ö. 0000-0001-9388-8333; N.Ö. 0000-0003-3988-0474; A.A.S. 0000- 0003-0576-8732; F.T. 0000-0002-8457-0048.

Address for Correspondence: Bilge Bal Özkaptan
E-mail: bilgebal57@hotmail.com
ORCID ID: orcid.org/0000-0001-9388-8333

Received: 07.04.2022
Accepted: 20.09.2022

had significant impacts on social life. All these measures to control the pandemic have caused negative feelings like fear, anxiety, anger, boredom, disappointment, guilt, desperation, loneliness and irritability among individuals.[3] The publication of alarming images of COVID-19 patients (intensive care process, death cases, etc.) on social media and the fact that most people can easily access these images via their smartphones has caused Fear of COVID-19 in society.[4] The uncertainty of when the pandemic will end, isolation, negative economic impacts, change in daily living habits, and the prevalence and diversity of news about the disease on social media are among other factors leading to fear at the individual and social level.[2,3,5]

Too much fear may result in negative consequences for the people such as mental health issues and anxiety and social levels such as panic exchange or xenophobia, whereas too little fear can also be dangerous for the people and society (for example, failure to comply with national precautions to decelerate the dissemination of COVID-19 and neglect of risks). Moreover, fear triggers protective behaviors that can reduce specific threats (for example, washing dirty hands); however, paradoxically, it can also increase fear (e.g. contamination worries and health anxiety).[6]

Healthcare professionals have an increased risk of infection due to long working hours, increased workload, not getting enough rest, lack of motivation, anxiety, and stress.[7] This is also an important factor that triggers fear and anxiety among students studying in health departments. "Cao et al.[8]" found that 21.3% of the participants reported slight anxiety, 2.7% moderate anxiety, and 0.9% strong anxiety in medical school students in China. Another study reported that both nurses and nursing students experienced fear and anxiety due to COVID-19, although the fear experienced was higher among graduate/working nurses than among nursing students. However, it was stated that the students developed more negative coping strategies despite problems.[9] In a study of "Savitsky et al.[10]" revealed that 42.8% of the nursing students experienced moderate anxiety and 13.1% experienced severe anxiety. It is important for nursing students to adopt solution-oriented approaches in such crises before starting their professional life. To achieve this, it is essential to define the Fear of COVID-19 among nursing students with a suitable assessment tool. The COVID-19 fear scale was translated into Turkish by various studies. However, these studies included all individuals aged 18 years and older in the sample group to cover the general Turkish society. It is not specific to a particular group and these studies suggest repeating the study in specific groups.[2,11,12] It is significant to assess the Fear of COVID-19 in nurses and nurse candidates who are in the highest risk group in the COVID-19 pandemic. This situation reveals the need to assess the validity and reliability of this scale for this group. Therefore, this study was applied to assess the psychometric properties of Fear of COVID-19 Scale (FCV-19S) in Turkish nursing students.

MATERIALS

Sample and Participants

This study included 877 nursing students enrolled in two Turkish universities between May and June 2020. Inclusion criteria were as follows: (a) being a nursing student, (b) not having a communication problem, (c) voluntary participation, and (d) having the means to fill out the forms online (smart phone, internet, computer, etc.). The validity and reliability studies recommend including 5-10 times the number of scale items to calculate the sample size and 7-8 times the number of variables to form two data sets for factor analysis.[13] In this study, the sample included 351 students considering the number of scale items and the test-retest method was applied with 143 students to define the consistency of the scale over time.

The approval of the was obtained from Kırklareli University Research Ethics Board (Meeting Number: 54001588-199-E.7748, decision number: P0211R00).

Measures

Participant Characteristics Data Collection Form: It was developed by the researchers upon examining the appropriate literature including 20 questions that question the sociodemographic features (age, family type, gender, etc.) and about COVID-19 of nursing students.[2,7,11,14,15]

The Fear of COVID-19 Scale: It was developed by "Ahorsu et al.[16]". It is a one-dimensional 7-item 5-point Likert type. Higher scores indicate greater fear of COVID-19. The total score on the scale goes from a minimum of 7 to a maximum of 35. "Ahorsu et al.[16]" calculated the Cronbach's α value of the scale as 0.82. The Cronbach's alpha internal consistency value was calculated as 0.83 in this study.

Hospital Anxiety Depression (HAD) Scale: It was developed by "Zigmond and Snaith[17]" and the Turkish version of the scale was done by "Aydemir et al.[18]". Seven of the 14 items (even numbers) assess anxiety, whereas the remaining seven (odd numbers) assess the depression. The responses are graded on a four-point Likert scale ranging from 0 to 3. The scale consists of two subscales as anxiety and depression. The score that can be obtained from the scale is between 0 and 21.[18] In this study, it was calculated as 0.79 for the HAD-A sub-dimension and 0.75 for the HAD-D sub-dimension.

Health Anxiety Scale: The Turkish version of the scale was carried out by "Aydemir et al.[19]" which was before developed by "Salkovskis et al.[20]". The scale is a self-report scale consisting of 18 items. Responses for each item were evaluated between 0 and 3 points. The scale score ranges from 0 to 54. A high rating on the scale denotes a significant amount of health concern. The Cronbach's alpha of the scale was determined to be 0.91 by "Aydemir et al.[19]". Cronbach's alpha was calculated to be 0.80 in this study.

Data Collection

Written permission, the original version of the FCV-19S, and necessary information about the evaluation method were obtained from the authors who developed the scale via e-mail before its implementation on nursing students in Türkiye. The questionnaire prepared using Google Documents was forwarded to the students by the researchers responsible for each center, and data were collected online.

The forms took about 10 min to complete. Before filling in the data collection form, the participants were informed about the study to the first page. In addition, an informed consent form was attached informing the participants that no IP tracking would occur in the case of participation in the study. If they complied with the study, they were prompted to click the 'I approve' button on the screen with the online questionnaire.

The Translation of FCV-19S

Eight people, seven of whom were faculty members and one of whom was an authority on the English language, translated the scale into the Turkish. The researchers then evaluated eight translations, and after selecting suitable expressions, one translation was formed.[13] Then, the validity and reliability study of the Turkish form was started. Five specialists were applied to assess the content validity of the scale. The Davis[20] method was used to assess expert opinions.[21]

Psychometric Testing of FCV-19S

In this study, item analysis and Cronbach's alpha test were used to assess the reliability of the scale. Additionally, the test-retest method was applied. The scale was implemented to the participants twice with an interval of two weeks.[13] In the first stage, the scale was applied to 351 students. Two weeks after the initial implementation, the test was repeated with 143 students. The results of the FCV-19S were compared to those of the HAD Scale and the HAS to test concurrent and construct validity.

Statistical Analysis

The data obtained were analysed utilizing "SPSS for Windows 25.0" and "Amos 22.0" programs in the study. Data were stated within definitive statistics. Data reliability is the most important condition for the analysis of collected data with appropriate technique. Reliability analysis was applied to test the reliability of the scales, on the other hand, for testing the construct validity "Explanatory Factor Analysis (EFA)" and "Confirmatory Factor Analysis (CFA)" were performed. Also, the test-retest technique was used. Concurrent validity among the FCV-19S, HAD Scale, and HAS was examined via Pearson correlation. A statistically significant p-value of <0.05 was used.

RESULTS

Participant Features

The socio-demographic features of the students are given in Table 1. Seventy-two-point nine percent of the students were male and 46.7% were first year students. According to the income status, 64.4% of the participants had equal income and expenditures, and most of them did not smoke (85.5%) or drink alcohol (91.7%). Ninety-three point two percent of the participants reported not having any chronic disease, 98.3% were not diagnosed with COVID-19 infection, and 50.4% expressed that their knowledge about COVID-19 infection was "partially adequate".

Psychometric Features of FCV-19S

Results of the Content Validity of the Scale

The content validity rate and scale validity index for the scale items were calculated as 1.0 regarding the Davis Technique.

Results of the Item Analysis of the Scale

Table 2 shows 27% lower-upper independent group t-test results showing the discriminatory power of all items and item-total correlation. According to Table 2, the item total test correlation for all items varies between 0.537 and 0.637.

Table 1. Participants characteristics (n=351)

Characteristics		n	%
Gender	Female	95	27.1
	Male	256	72.9
Year	1	164	46.7
	2	92	26.2
	3	64	18.2
	4	31	8.8
Income status	Income exceeds expenditure	55	15.7
	Income equal to expenditure	226	64.4
	Income less than expenditure	70	19.9
Smoking	Yes	51	14.5
	No	300	85.5
Alcohol	Yes	29	8.3
	No	322	91.7
The status of employment	Employed	6	1.7
	Unemployed	345	98.3
Chronic disease	Yes	24	6.8
	No	327	93.2
COVID-19 diagnosis	Yes	6	1.7
	No	345	98.3
Adequate knowledge about COVID-19 infection	Partly	177	50.4
	Adequate	163	46.4
	Inadequate	11	3.1

COVID-19: Coronavirus disease-2019.

Results of the Construct Validity of the Scale

EFA was conducted to show the factor design of the scale. EFA of the scale are given in Table 3. The scale, which originally consisted of 7 items, was developed as a single subdimension. After the adaptation, it was determined that the scale was still unidimensional. No overlapping items were detected in the EFA. The EFA made to detect the factor design of the scale found the threshold factor loading value as 0.40 (Table 3). The analysis revealed a KMO value of 0.855 (Table 3). Moreover, the evaluation of the Bartlett sphericity test results exposed a chi-square value of 809.888; p<0.01. The Principal Components Analysis demonstrated that there was only one component for 7 items (Table 3).

The Scree Plot of the scale is revealed in Figure 1. The evaluation in the graph in Figure 1 with the number of factors on the horizontal axis and the eigenvalues on the vertical axis shows that the high accelerated decline decreases after the first point. After the first point, the contribution of every factor to the variance decreases and the contributions of the variances to be added are observed to be quite similar. A one-factor structure was deemed appropriate based on the eigenvalue and variance percentages as well as the data taken from the graph in line with the EFA (Table 3).

The confirmatory factor fit indices for the pre- and post-modification scales are given in Table 4. The scale's structural equation modeling results were important at the p=0.001 level, and the scale's 7 items and single sub-dimension had a relation with the scale structure, according to the CFA. The model underwent some improvements. Variables reducing consent were defined during the adjustments, and new covariances were formed for the ones with significant equivalence among residual values (e1-e5; e6-e7). Renewed calculations of fit indices are given in Table 4.

The t-statistics regarding the significance of the factors after the CFA shows that all factors were significant (p<0.05) (Table 5). The outcomes of the first-order CFA are revealed in Figure 2. As a result, the scale consists of seven elements, the lowest of which has a factor load of 0.56 and the greatest of which has a factor load of 0.77 (Figure 2). Also, the "composite reliability (CR)" value computed to test the reliability of the measurement model was 0.85.

Table 2. Results of the item analysis of the Fear of COVID-19 Scale

	Item number	Item-total score correlation	t (Lower 27%*-Upper 27%*)	p-value, (Lower 27%*-Upper 27%*)
The Fear of COVID-19 Scale	Item 1	0.637	18.094	0.001**
	Item 2	0.600	18.488	0.001**
	Item 3	0.612	16.069	0.001**
	Item 4	0.551	15.836	0.001**
	Item 5	0.602	17.551	0.001**
	Item 6	0.537	13.022	0.001**
	Item 7	0.591	18.238	0.013**

n=351, *n_1= n_2=95, **Significant values for p<0.05. COVID-19: Coronavirus disease-2019.

Table 3. Results of Explanatory Factor Analysis for the Fear of COVID-19 Scale

Items	Explained variance	Eigenvalue (Λ)	Factor load
F1			
Item 3	51.010	3.571	0.744
Item 1			0.743
Item 2			0.725
Item 7			0.720
Item 5			0.718
Item 6			0.677
Item 4			0.669

Kaiser-Meyer-Olkin: 0.855; χ^2= 809.888; Bartlett's test of Sphericity p=0.001. COVID-19: Coronavirus disease-2019.

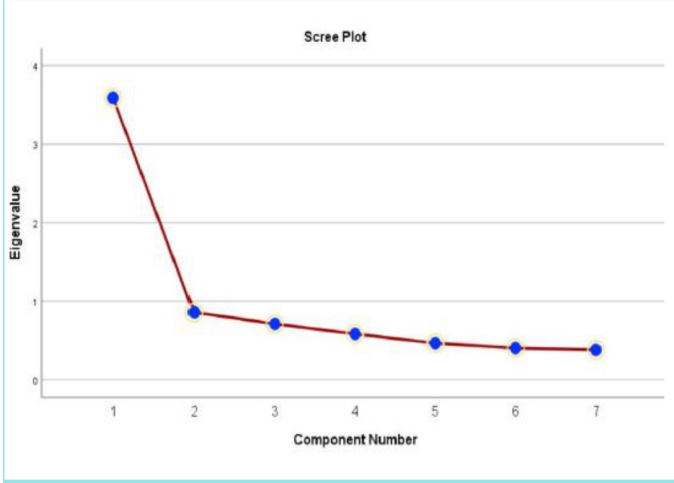


Figure 1. Scree Plot of the Fear of COVID-19 Scale.
COVID-19: Coronavirus disease-2019.

Table 4. Confirmatory factor fit indices for the pre- and post-modification Fear of COVID-19 Scale

RMSEA	NFI	CFI	IFI	GFI	TLI	AGFI	CMIN/df
0.084	0.795	0.840	0.845	0.960	0.760	0.919	3.491
0.075	**0.850**	**0.891**	**0.895**	**0.970**	**0.809**	**0.931**	**2.983**

RMSEA: Root-mean-square error of approximation, NFI: Normed Fit Index, CFI: Comparative fit index, IFI: Incremental Fit Index, GFI: Goodness of Fit Index, TLI: Tucker-Lewis Index, AGFI: Adjusted goodness of fit index, CMIN/df: chi-square/degree of freedom, COVID-19: Coronavirus disease-2019.

Table 5. Confirmatory Factor Analysis results of the Fear of COVID-19 Scale

Items	Composite reliability (t-statistics)	Factor load
Item 1		0.773
Item 2	12,382*	0.736
Item 3	10,873*	0.662
Item 4	11,031*	0.619
Item 5	12,808*	0.750
Item 6	9,323*	0.558
Item 7	10,423*	0.584

*p<0.05, COVID-19: Coronavirus disease-2019.

Results of the Concurrent Validity of the Scale

The correlation between the FCV-19S to be adapted and the other scales were determined to determine the reliability of parallel forms (Table 6). We can observe that there is a positive and weak correlation between FCV-19S and HAD-A in Table 6 ($r=0.396$; $p<0.01$). It was observed that a positive and very weak correlation between the FCV-19S were observed and the HAD-D ($r=0.196$; $p<0.01$). There was a positive and moderate correlation ($r=0.436$; $p<0.01$) between FCV-19S and HAS. There was a positive and moderate correlation between FCV-19S and hypersensitivity to physical symptoms and anxiety sub-dimension ($r=0.414$; $p<0.01$); a positive and weak correlation was observed between the negative outcomes of the disease sub-dimension ($r=0.288$; $p<0.01$) (Table 6).

Results of the Reliability of FCV-19S

Cronbach's alpha was determined as 0.833. The test-retest results, performed to determine the time invariance, revealed a high level of correlation between the two measurements with the Pearson correlation coefficient ($r=0.734$; $p<0.05$). This shows that the scale is consistent over time. In addition, the reliability of both measurements was sufficient and above 0.70 ($\alpha^2=0.819$, $\alpha1=0.844$).

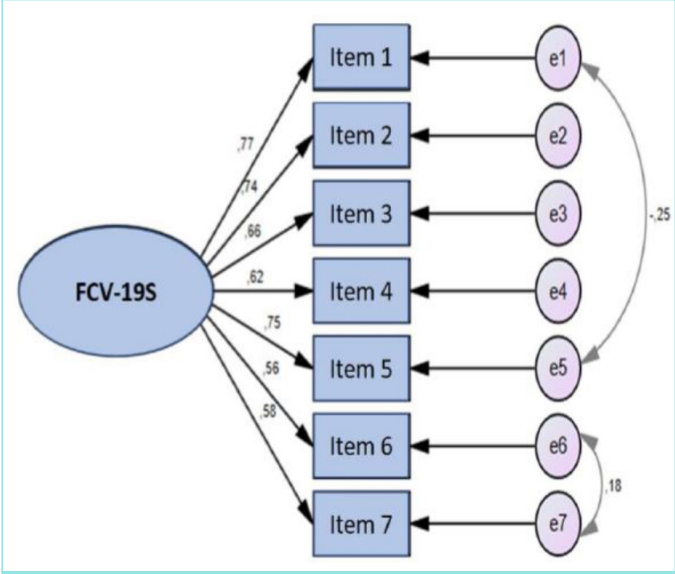


Figure 2. Results of the first-order Confirmatory Factor Analysis of the Scale.

FCV: Fear of COVID-19 Scale.

Table 6. Correlation analysis between scales

Scales	1	2	3	4	5	6
1 COVID-19 Fear Scale	1					
2 Hospital Anxiety Scale	0.396*	1				
3 Hospital Depression Scale	0.196*	0.574*	1			
4 Health Anxiety Scale	0.436*	0.418**	0.290*	1		
5 Sub-Dimension of Hypersensitivity to Physical Symptoms and Anxiety	0.414*	0.359*	0.172*	0.924*	1	
6 Sub-dimension of adverse consequences of the disease	0.288*	0.332*	0.39*	0.643*	0.344*	1

*p<0.01, COVID-19: Coronavirus disease-2019.

DISCUSSION

The scale was a viable and trustworthy measurement tool for Turkish nursing students in the study. Five experts evaluated the scale items depending on the Davis Technique to determine the content validity.[21] This technique involves the classification of expert opinions as "appropriate" (4 points), "appropriate but needs minor revision" (3 points), "appropriate but needs major revision (2 points)", and "not appropriate" (1 point). Instead of using a statistical criterion, the number of experts who state "suitable" and "appropriate but requires minor revision" is divided by the total number of experts to generate the "Content Validity Index (CVI)" for the item, which is then compared to the recognized value of 0.80.[13] The CVI value was calculated as 1.0. The result reveals that FCV-19S is suitable for Turkish society.

The validity and distinctiveness of the items on the COVID-19 scale were determined using item-total correlation and 27% upper-lower group comparisons. Item-total test correlation values of all items vary between 0.537 and 0.637 (Table 2). The minimum value for sufficient item total test is specified as 0.30.[22] The item-total test correlation values of the participants' responses to the questions were examined and no items below 0.30 were detected. According to the item total test correlation table, all remaining items were correlated with each other. The scale raw scores were sorted in descending order to measure item uniqueness, and the mean scores of the 27% lower-upper groups were analyzed using an independent group t-test. It was statistically significant that the means of the lower and upper group item scores differed. In this sense, the scale stands out in terms of determining the necessary level of quality (Table 2).[22]

The KMO value of 0.855 in the EFA managed to define the construct validity of FCV-19S, revealing that the factor analysis sample size was "perfectly sufficient".[23] Also, the evaluation for the test results of Bartlett's sphericity showed that the obtained chi-square value was significant [χ^2 (21)=809.888; $p<0.01$] (Table 3). As a result, it was presumed that the data was formed by a multivariate normal distribution. The Principal Components Analysis managed to examine the scale factor structure after accepting the fitness of the data for factor analysis, revealing only one component for 7 items. This component contributed 51.01% of the overall variance (Table 3). In a measurement tool, at least 40% explained variation is required for construct validity.[13] This indicates that the level of explained variation by the adapted scale is above the desired level of explained variation. The Scree Plot of the FCV-19S, which includes the number of factors on the horizontal axis and eigenvalues on the vertical axis, demonstrates the contribution of the tendency of the descents from the first point to the variance (Figure 1).[23] After the first point, the contribution of every factor to the variance decreases and the contributions of the variances to be added are observed to be quite similar. A one-factor structure was confirmed based on the eigenvalue and variance percentages as well as the data gathered from the graph and the results of EFA (Figure 1) (Table 3).

GFI index above 0.80, AGFI value above 0.85, CMIN/df below 5, and RMSEA value below 0.08 are required for good fit.[24,25] The fit indices in accordance with the results of the first-level multifactorial CFA analysis show that the index values are sufficient (Table 4).

After CFA, the t-statistics used to determine the significance of the factors revealed that all of them were significant statistically ($p<0.05$) (Table 5). The outcomes of the first level CFA shows that the factor load

values for the 7-item scale range from 0.56 to 0.77 (Figure 2). The CFA results in our investigation supported Turkish FCV-19S unidimensional structure. When compared to different versions of the scale (0.68 to 0.90 for the Italian version; 0.72 to 0.80 for the Bangla version; 0.48 to 0.72 for the Turkish version and 0.52 to 0.81 for another Turkish version; 0.62 to 0.84 for the Arabic version; 0.64 to 0.66 for the original Iran version), each study produced different results.[2,11,16,26-28]

In our study, the CR value for testing the reliability of the measurement model was 0.85. The CR of latent variables in the measurement model must be greater than 0.70,[13] which supports the test reliability in our study.

A significant positive correlation was observed with anxiety and depression as examined by the HAD Scale and HAS (Table 6), and this result is consistent with other results in the literature.[11,16,27,28] This finding demonstrates that the translated scale has contemporaneous validity. COVID-19 fear has revealed a relationship related to mental problems. In the later stages of infectious epidemics that cause pandemics such as COVID-19, negative psychological processes (e.g. anxiety, depression) also affect individuals psychologically.

The Cronbach alpha value for the entire scale and each individual item can be computed.[29] According to Bayram[30], the Cronbach's alpha value determined for all elements shows the scale's overall reliability, with 0.70 being widely acknowledged as the minimal number. According to our study, the Cronbach's alpha (0.83), similar to the original scale (α=0.82), were slightly lower than the Italian (α=0.87) Bangladesh (α=0.87), Arab (α=0.88), Israel (α=0.86) and Turkish (α=0.85, α=0.86 and α=0.89) versions and higher than the Eastern European (α=0.80) version.[2,5,11,12,16,26-28,31]

The similarity of two measures taken at different periods determines the coherence of a test or scale.[13] The test-retest procedure was applied to establish the scale's invariance over time in our study, The evaluation of the Pearson correlation coefficiency between two measurements revealed a high level of correlation ($r=0.734$; $p<0.05$). This demonstrates that the scale is stable over time. Furthermore, both readings were sufficiently reliable and above 0.70 (α^2=0.819, α1=0.844).

CONCLUSION

Finally, the current research with nursing students revealed that the Turkish version of the scale is a valid and accurate measurement tool. This measurement tool can be applied in studies to assess COVID-19 fear. The use of an objective assessment method to assess COVID-19 fear is vital for comprehending the association between COVID-19 fear and other mental problems, including anxiety and depression, as well as for preventing these problems. Although the scale's validity and reliability were previously tested with several groups in the Turkish community, it is asserted that the study be repeated with other health professionals and risk groups, in addition to nursing students.

MAIN POINTS

- Fear and concern were sparked by the rapid spread of COVID-19 infection in a brief period of time, which resulted in deaths. COVID-19 infection is particularly dangerous for nurses and nursing candidates who are responsible for patient care.
- It is critical to assess the COVID-19 concerns of nursing students, who will be future nurses. FCV-19S can be utilized in studies to assess COVID-19-related fear.
- The use of an objective assessment method to assess COVID-19 fear is vital for apprehending the relationship between COVID-19 fear and other mental problems, including anxiety and depression, as well as preventing these problems.

Ethics

Ethics Committee Approval: The approval of the was obtained from Kırklareli University Research Ethics Board (approval number: 54001588-199-E.7748, P0211R00).

Informed Consent: Students were informed about the study on the first page of the data collection form, and they were asked to click the "I approve" button on the screen with the online questionnaire if they approved to participate in the study. Participants who filled out the online form were considered to have agreed to attend the study. All students presented informed consent.

Peer-review: Internally peer-reviewed.

Authorship Contributions

Concept: B.B.Ö., Design: B.B.Ö., N.Ö., Data Collection or Processing: B.B.Ö., A.A.S., Analysis or Interpretation: B.B.Ö., N.Ö., A.A.S., F.T., Literature Search: B.B.Ö., N.Ö., A.A.S., F.T., Writing: B.B.Ö., N.Ö., A.A.S., F.T.

Conflict of Interest: The authors declared that they have no conflict of interest.

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

REFERENCES

1. Cucinotta D, Vanelli M. WHO Declares COVID-19 a Pandemic. Acta Biomed. 2020; 91(1): 157-60.
2. Haktanir A, Seki T, Dilmaç B. Adaptation and evaluation of Turkish version of the fear of COVID-19 Scale. Death Stud. 2022; 46(3): 719-27.
3. Mamun MA, Griffiths MD. First COVID-19 suicide case in Bangladesh due to fear of COVID-19 and xenophobia: Possible suicide prevention strategies. Asian J Psychiatr. 2020; 51: 102073.
4. Taylor S, Landry CA, Paluszek MM, Fergus TA, McKay D, Asmundson GJG. Development and initial validation of the COVID Stress Scales. J Anxiety Disord. 2020; 72: 102232.
5. Tzur Bitan D, Grossman-Giron A, Bloch Y, Mayer Y, Shiffman N, Mendlovic S. Fear of COVID-19 scale: Psychometric characteristics, reliability and validity in the Israeli population. Psychiatry Res. 2020; 289: 113100.
6. Mertens G, Gerritsen L, Duijndam S, Salemink E, Engelhard IM. Fear of the coronavirus (COVID-19): Predictors in an online study conducted in March 2020. J Anxiety Disord. 2020; 74: 102258.
7. Chen Q, Liang M, Li Y, Guo J, Fei D, Wang L, et al. Mental health care for medical staff in China during the COVID-19 outbreak. Lancet Psychiatry. 2020; 7(4): e15-6. Erratum in: Lancet Psychiatry 2020; 7(5): e27.
8. Cao W, Fang Z, Hou G, Han M, Xu X, Dong J, et al. The psychological impact of the COVID-19 epidemic on college students in China. Psychiatry Res. 2020; 287: 112934.

9. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020; 395: 497-506. Erratum in: Lancet. 2020.

10. Savitsky B, Findling Y, Ereli A, Hendel T. Anxiety and coping strategies among nursing students during the covid-19 pandemic. Nurse Educ Pract. 2020; 46: 102809.

11. Satici B, Gocet-Tekin E, Deniz ME, Satici SA. Adaptation of the Fear of COVID-19 Scale: Its Association with Psychological Distress and Life Satisfaction in Turkey. Int J Ment Health Addict. 2021; 19(6): 1980-8.

12. Korukcu O, Ozkaya M, Faruk Boran O, Boran M. The effect of the COVID-19 pandemic on community mental health: A psychometric and prevalence study in Turkey. Health Soc Care Community. 2021; 29(5): e204-13.

13. Alpar CR. Applied statistics and validity-reliability with examples from Sports, Health and Education Sciences. Ankara: Detay Publishing, 2016 (Turkish).

14. Carlos WG, Dela Cruz CS, Cao B, Pasnick S, Jamil S. Novel Wuhan (2019-nCoV) Coronavirus. Am J Respir Crit Care Med. 2020; 201(4): P7-8.

15. Huang L, Xu F, Liu H. Emotional responses and coping strategies of nurses and nursing college students during COVID-19 outbreak. MedRxiv. 2020.

16. Ahorsu DK, Lin CY, Imani V, Saffari M, Griffiths MD, Pakpour AH. The Fear of COVID-19 Scale: Development and Initial Validation. Int J Ment Health Addict. 2022; 20(3): 1537-45.

17. Zigmond AS, Snaith RP. The hospital anxiety and depression scale. Acta Psychiatr Scand. 1983; 67(6): 361-70.

18. Aydemir Ö, Güvenir T, Küey L, Kültür S. The validity and reliability study of the Turkish version of the hospital anxiety and depression scale. Turkish Journal of Psychiatry. 1997; 8(4): 280-7.

19. Aydemir Ö, Kirpinar İ, Sati T, Uykur B, Cengisiz C. Reliability and Validity of the Turkish Version of the Health Anxiety Inventory. Noro Psikiyatr Ars. 2013; 50(4): 325-31.

20. Salkovskis PM, Rimes KA, Warwick HM, Clark DM. The Health Anxiety Inventory: development and validation of scales for the measurement of health anxiety and hypochondriasis. Psychol Med. 2002; 32(5): 843-53.

21. Davis LL. Instrument review: Getting the most from a panel of experts. Appl Nurs Res. 1992; 5(4): 194-7.

22. Büyüköztürk Ş. Factor analysis: basic concepts and using to development scale. Educational Management in Theory and Practice. 2002; 32: 470-83.

23. Çokluk Ö, Şekercioğlu G, Büyüköztürk Ş. Multivariate Statistics for Social Sciences: SPSS and Lisrel Applications. Ankara: Pegem Academy Publishing, 2012 (Turkish).

24. Simon D, Kriston L, Loh A, Spies C, Scheibler F, Wills C, et al. Confirmatory factor analysis and recommendations for improvement of the Autonomy-Preference-Index (API). Health Expect. 2010; 13(3): 234-43.

25. Hooper D, Coughlan J, Mullen MR. Structural equation modelling: Guidelines for determining model fit. EJBRM. 2008; 6: 53-60.

26. Alyami M, Henning M, Krägeloh CU, Alyami H. Psychometric Evaluation of the Arabic Version of the Fear of COVID-19 Scale. Int J Ment Health Addict. 2021; 19(6): 2219-32.

27. Soraci P, Ferrari A, Abbiati FA, Del Fante E, De Pace R, Urso A, et al. Validation and Psychometric Evaluation of the Italian Version of the Fear of COVID-19 Scale. Int J Ment Health Addict. 2022; 20(4): 1913-22.

28. Sakib N, Bhuiyan AKMI, Hossain S, Al Mamun F, Hosen I, Abdullah AH, et al. Psychometric Validation of the Bangla Fear of COVID-19 Scale: Confirmatory Factor Analysis and Rasch Analysis. Int J Ment Health Addict. 2022; 20(5): 2623-34.

29. Kılıç S. Cronbach's alpha reliability coefficient. JMOOD. 2016; 6(1): 47-8.

30. Bayram N. Data Analysis with SPSS in Social Sciences, Bursa: Ezgi Bookstore, 2004.

31. Reznik A, Gritsenko V, Konstantinov V, Khamenka N, Isralowitz R. COVID-19 Fear in Eastern Europe: Validation of the Fear of COVID-19 Scale. Int J Ment Health Addict. 2021; 19(5): 1903-8.

RESEARCH ARTICLE

CYPRUS
JOURNAL OF MEDICAL SCIENCES

DOI: 10.4274/cjms.2021.2021-129
Cyprus J Med Sci 2023;8(3):197-204

The Prevalence of Dermal and Respiratory Symptoms among Greenhouse Agricultural Workers: A Surveillance Study

Derya Adıbelli, Adem Sümen

Department of Public Health Nursing, Akdeniz University Faculty of Kumluca Health Sciences Faculty, Antalya, Türkiye

Abstract

BACKGROUND/AIMS: This study was conducted to examine the prevalence of dermal and respiratory symptoms among workers in greenhouse agriculture.

MATERIAL AND METHODS: This cross-sectional study was conducted with 529 greenhouse workers in a district center between March and September 2020. The data collection form was developed by researchers to collect the research data. Descriptive statistics and logistic regression analysis were used in the analysis.

RESULTS: 41.8% of the participants stated that they used gloves and 25.8% of them used masks while working, but 7.0% of them reported that they did not use any personal protective equipment. It was found that working for 8 h or more per day was associated with respiratory distress, itching-rashing on skin, and nose-eye problems, while working for 25 days or more per month was associated with respiratory distress and cough complaints ($p<0.05$). Working in the tasks of seed sowing, pesticide application/spraying, and grafting was found to be associated with wheezing, coughing, itching-rashing on skin, and nose-eye problems ($p<0.05$). Those who did not wear protective equipment suffered from wheezing, and those who did not use a mask had bronchitis, cough, wheezing, itching-rashing on skin, and nose-eye problems ($p<0.05$).

CONCLUSION: Working conditions, a type of work done in greenhouse agriculture, and failure to use personal protective equipment increase the prevalence of dermal and respiratory symptoms.

Keywords: Greenhouse agriculture, skin, respiratory, symptoms, surveillance

INTRODUCTION

Greenhouse agriculture gains intensity in the Mediterranean Region in our country. While the control of chemicals used in greenhouse agriculture was mostly up to the producer's choice in previous years, the usage dosage, prevalence, and interval of chemicals have been implemented under the principles determined by the ministry and strict supervision control in recent years. Chemical drugs used on the crop to obtain efficient and good quality crops and to offer them to buyers can cause various health problems among greenhouse agricultural workers. Since the primary exposure is through the respiration and skin, these areas are the most affected areas of the body.[1] When the data on health problems of workers in greenhouse agriculture was analyzed, it was seen that pesticides triggered the inflammatory response and caused

***This study was presented as an oral report in the International Symposium on Multidisciplinary Approach toward the Disadvantaged Groups between the dates of 22 and 24 October 2020.**

To cite this article: Adıbelli D, Sümen A. The Prevalence of Dermal and Respiratory Symptoms among Greenhouse Agricultural Workers: A Surveillance Study. Cyprus J Med Sci 2023;8(3):197-204

ORCID IDs of the authors: D.A. 0000-0001-9320-7083; A.S. 0000-0002-8876-400X.

Address for Correspondence: Derya Adıbelli
E-mail: derya.adibelli@antalya.edu.tr
ORCID ID: orcid.org/0000-0001-9320-7083

Received: 06.05.2021
Accepted: 28.09.2021

reactions by changing the esterase level in the cell membrane of immunocytes.[2,3] In a study on the health problems of women working in agriculture, it was found that the dermal diseases, asthma and other respiratory problems were common among them and their children,[4] and that the chemicals used in agriculture suppressed the immune system and increased the susceptibility to allergic reactions[5,6] and that the risk of autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus could increase.[7,8] Similarly, it was shown in the studies that exposure to pesticides reduced cholinesterase levels[9,10] and caused neurological and respiratory symptoms and skin reactions, and that low cholinesterase levels increased the asthma-related pulmonary inflammation.[11]

The most obvious effects of greenhouse cultivation on workers are asthma, allergic alveolitis, and dermatitis. Although the most common way of pesticide exposure is the skin,[12-14] especially the hand and face areas are affected.[15] Pesticides can be absorbed directly through the skin due to their physicochemical properties. Clothing that does not completely cover the body or has a permeable structure also leads to exposure through the skin. Since pesticides also contain respirable components,[16] the second most frequent way of exposure is the respiratory tract. After pesticide application, which is generally done indoors by spraying, the lack of ventilation and high temperature and humidity as well as failure to use protective equipment increase the level of exposure.[17,18] Furthermore, it is recommended not to apply pesticides when the ambient temperature is above 30 °C.[17] In studies examining the respiratory functions of greenhouse workers, it was reported that chronic cough, respiratory distress, rhinitis, bronchial hyperreactivity, and asthma-related symptoms were seen at a high rate[16,19-22] and that even non-occupational exposure caused respiratory problems.[23]

Public health nurses and their field studies have a great role in determining the health problems in the community and the prevalence and level of health problems developing among the working people. In this context, one of the important tasks of public health nursing is surveillance. Greenhouse agriculture is the main source of income for the people of the district in the region where the study was conducted and its use is high. Due to the observation of dermal and respiratory symptoms among those working in this field, the study was planned to examine the dermal and respiratory problems of those working in greenhouse agriculture. The following research questions were sought:

1. What are the working conditions of the workers in the greenhouse (daily and monthly working hours, work they do, use of personal protective equipment, ...)?

2. Did workers experience skin and respiratory problems while working in the greenhouse?

3. Do working conditions impact skin and respiratory symptoms?

MATERIALS AND METHODS

Population and Sample

This cross-sectional study was conducted with individuals working in greenhouse agriculture in a district center between March and September 2020. The population of the research consists of 3210 people (Kumluca District Directorate of Agriculture, 2019 data) located in the Kumluca district center and registered as working in greenhouse agriculture. The sample of the research consists of people working in greenhouse agriculture over the age of 18 living in the district center and accepting to participate in the study. It was aimed to includeat least 344 workers in greenhouse agriculture according to the calculation of the number of sample whose population was known, and the study was completed with 529 people who could be reached. When the square of 0.30 is taken as 0.09 as the medium effect size for the zeroth order correlation coefficient (R^2),[24] it is seen that the sample size has 90% power at 95% confidence interval (post-hoc) (G*Power 3.1.9).

Data Collection and Tools

The data of this study, which is a field study, was collected by face-to-face interview in the greenhouses where individuals worked (data collection was suspended upon starting the pandemic process and the data collection continued when the normalization process started. In this process, in accordance with the coronavirus measures, absolute attention was paid to social distance, use of masks, and hygiene. The workers' breaks and lunch times were preferred as the data collection time. A data collection form developed by the researchers in line with the literature was used to collect research data.[9,25] Before the main data of the study were collected, a preliminary study was conducted with 17 people and the comprehensibility of the questions was examined.

Data Collection Form: It consists of three parts as sociodemographic characteristics, professional characteristics, and health status and 24 questions (Professional questions: "At what age did you start working in the greenhouse?", "How many hours do you work in the greenhouse per day?", "How many days do you work in the greenhouse monthly?", "What work do you do in the greenhouse?", "What protective equipment do you use while working in the greenhouse? ", "What do you do with the emptied boxes of pesticides?", "What do you think causes your health problems?" Health-related questions: "Have you experienced/are you experiencing any of the problems of respiratory distress, bronchitis, cough, wheezing, itching/rashing on hands/face, or itching, watering, pain on nose/eyes?", "Did your health problem begin after you started working in the greenhouse?", "Does working in the greenhouse increase the severity of your health problem?", "Do other people with whom you work in the same place have similar diseases/problems?", "Do your complaints decrease when you do not work in the greenhouse?"). In the literature review conducted within the scope of the research subject, a standardized measurement tool related to dermal symptoms was not found, and a respiratory questionnaire about respiratory problems was obtained (Saint George Respiratory Questionnaire). When the scope and content of the questionnaire were examined, it was seen that it was mostly aimed at patients with COPD, and therefore it could not be used as a data collection tool in this study.

The data collection form used in the study was examined by three academicians, other than researchers, who were experts in public health nursing, before the study in terms of content validity, and the results were examined using the Davis technique. In the Davis technique, expert opinions are analyzed as follows: a) appropriate, b) needs minor revision, c) needs major revision, d) not appropriate The Content Validity Index (CVI) is obtained by dividing the sum of A and B in all expert forms by the total number of experts. If the CVI is greater than 80%, the question is sufficient in terms of content validity.[26] The average CVI score of the form was found to be 98.1%.

Ethical Approval

Informed consent was obtained from the participants. The study was conducted in accordance with the Declaration of Helsinki principles (revised in Brazil in 2013). The approval of the Akdeniz University Faculty of Medicine Clinical Research Ethics Committee was obtained to conduct the study (approval number: KAEK-282).

Statistical Analysis

The data were evaluated in the Statistical Package for Social Science 25.0 (IBM Corp.; Armonk, NY, USA) program, and descriptive statistics and logistic regression analysis were used in the analysis of the data. The cases where the type 1 error level is below 5% was considered as statistical significance.

RESULTS

A total of 529 people working in greenhouse agriculture participated in the study. 51.0% of the participants were women, 44.2% were primary school graduates, and the average age is 36.03±14.03. More than half of the participants (54.3%) are working in their family/own business and their monthly income is averagely 569.67±397.54 dolars. 25.5% of the participants said they smoked and 19.1% said they drank alcohol.

The average age for the participants to start working in the greenhouse was found as 16.86±7.62, the number of working days per month as 21.48±7.75 and the number of working hours per day as 7.75±1.99. It was observed that they mostly worked in April (11.6%), May (11.9%), and June (11.2%) in the greenhouse and were mostly engaged in harvesting/gathering crops (22.9%), pesticide application (19.4%), and weeding (19.3%). 41.8% of the participants stated that they used gloves and 25.8% of them used masks while working, but 7.0% of them reported that they did not use any personal protective equipment. It was seen that the reasons for the failure to use protective materials included the unavailability of such materials (39.3%), and considering them uncomfortable (23.2%) and unnecessary (23.2%). It was determined that the emptied pesticide boxes were mostly thrown into garbage bins on the streets (34.0%) and sent for recycling (31.6%). 6.6% of the participants reported that they wore the clothes used while working in the greenhouse also in their daily life and 53.5% of them consumed food and beverages in the greenhouse (Table 1).

Information on the health problems experienced by the participants in the study is given in Figure 1, and the problems of itching, rashing, swelling, etc. one hands/face (open areas of the body) were found to be experienced the most by a 48.8% rate. It is followed by nose or eye problems (itching, watering, burning, pain, etc.) with a 45.7% rate, cough with a 39.1% rate, and wheezing/shortness of breath with a 22.1% rate (Figure 1). The participants stated that dermal problems were mostly caused by contact with products (38.9%) and respiratory problems were due to dust/dirt (31.9%), while both dermal and respiratory problems were reported to result from pesticides (21.4%), chemical fertilizers (19.3%) and hot working environment (17.0%) (Figure 2).

In the logistic regression analysis, working for 8 h or more per day was found to be associated with respiratory distress [odd ratio: (OR): 2.04, p=0.02], itching-rashing on skin (OR: 15.27, p=0.01) and nose-eye problems (OR: 5.93, p=0.003), while working for 25 days or more per

Table 1. Certain professional characteristics of participants (n=529)

Characteristics	Min.-Max.	M ± SD
Age to start working in greenhouse	7-55	16.86±7.62
The number of working hours in greenhouse per day	2-12	7.75±1.99
The number of working days in greenhouse per month	3-30	21.48±7.75
	n	%
Working months in greenhouse (n=2343)*		
Winter (December-January-February)	584	25.0
Spring (March-April-May)	740	31.6
Summer (June -July -August)	534	22.8
Autumn (September-October-November)	485	20.8
Type of work in greenhouse		
Family business/own business	287	54.3
Land tenure/annual renting	77	14.6
Seasonal worker	44	8.3
Daily wage	88	16.6
Partner	33	6.2
Works in greenhouse (n=1907)*		
Seed sowing	272	14.3
Weeding	369	19.3
Pesticide application/spraying	370	19.4
Grafting	154	8.1
Harvesting/gathering crop	437	22.9
Product packaging	237	12.4
Other (uprooting, irrigation)	68	3.6
Protective equipment used during work (n=1033)*		
I do not use personal protective equipment	72	7.0
Apron	100	9.7
Boots	87	8.4
Gloves	432	41.8
Mask	266	25.8
Safety goggles	56	5.4
All	20	1.9
Reason for not using protective equipment (n=328)*		
The unavailability of equipment	129	39.3
Considering them uncomfortable	76	23.2
I do not think they are protective	21	6.4
Considering them unnecessary	76	23.2
Considering them expensive	26	7.9
Emptied boxes of pesticides		
I leave them in the greenhouse where I work	30	5.7
I throw them into the garbage bins on the streets	180	34.0
I bury them in the ground	16	3.0
I send them for recycling	167	31.6
I burn them in fire	91	17.2
I wash them and use for other purposes	34	6.4
I sell them for scrap	11	2.1
Wearing work clothes in daily life		
Yes	35	6.6
No	494	93.4
Food consumption in greenhouse		
Yes	283	53.5
No	246	46.5

*Multiple options were checked. Min.: Minimum, Max.: Maximum, SD: Standard deviation.

month was found to be associated with respiratory distress (OR: 0.27, p=0.04) and cough complaints (OR: 6.80, p=0.02) (Table 2).

In the multivariate analysis examining the relationship between the type of work done in greenhouse agriculture and health problems, the seed sowing work was found to be associated with respiratory distress (OR: 1.69, p=0.01), wheezing (OR: 1.71, p=0.01), itching-rashing on skin (OR: 2.93, p=0.001) and nose-eye problems (OR: 2.15, p=0.001). Pesticide application/spraying was found to be associated with bronchitis (OR: 8.04, p=0.04) and nose-eye problems (OR: 1.66, p=0.02), while grafting was found to be associated with cough (OR: 0.62, p=0.02), itching-rashing on skin (OR: 0.34, p=0.001) and nose-eye problems (OR: 0.29, p=0.001). Considering the relationship between the use of personal protective equipment and health problems, it was discovered that wheezing (OR: 0.32, p=0.005) was experienced by those not wearing protective equipment; and bronchitis (OR: 0.48, p=0.03), cough (OR: 0.48, p=0.001), wheezing (OR: 0.50, p=0.005), itching-rashing on skin (OR: 0.42, p=0.001) and nose-eye problems (OR: 0.51, p=0.001) were experienced by those not wearing mask (Table 3).

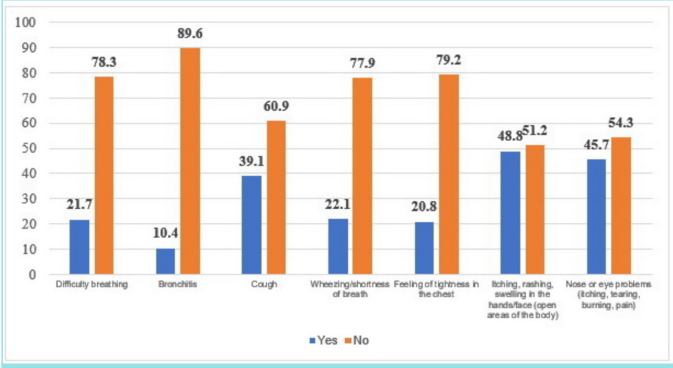


Figure 1. Health problems experienced by the participants.

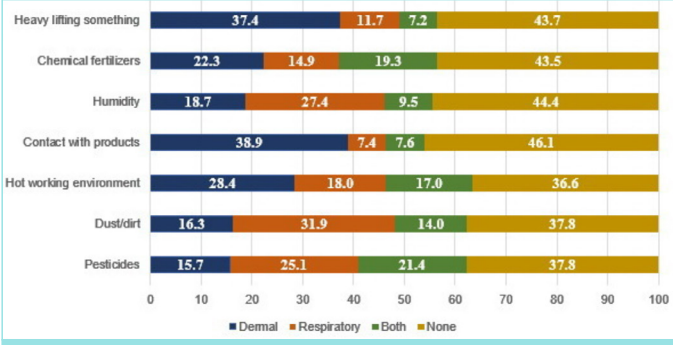


Figure 2. Factors that cause health problems for the participants dermal and respiratory tract.

Forty-four point eight percent of the individuals participating in the study stated that their health problems occurred after they started working in the greenhouse, and 42.3% of them reported an increase in the severity of their problems while working in the greenhouse, and 39.1% of them said similar health problems were seen among other people working in the same place, and 45.9% had a decrease in their complaints on the days when they did not work in the greenhouse.

DISCUSSION

The results of this study report the dermal and respiratory symptoms experienced by those working in greenhouse agriculture and the variables associated with them. It is seen that the average age at which the participants started working in greenhouse agriculture is less than 18 years. Workers work in greenhouse agriculture approximately 3/4 of the month and 1/3 of the day. Similarly, in a study, the age of starting to work in greenhouse agriculture was found to be 15.5.[1] In this study, some participants reported a much earlier age to start greenhouse agriculture as family business. Greenhouse agriculture is one of the most dangerous lines of business for child labour.[27] In cases where greenhouse agriculture is done as a family business, the child may be exposed to this environment at a very early age, including infancy, and chemical exposure may predispose to respiratory and dermal symptoms.

It was seen that the participants mostly worked in the greenhouse in spring and early summer, and they mostly did the work of gathering crops, applying pesticides, and weeding. About half of the participants stated that they used gloves and one quarter of them used masks while working, but some of them reported that they did not use any personal protective equipment. It was seen that the failure to use protective materials resulted from the unavailability of such materials and considered them uncomfortable and unnecessary. The application interval of pesticides varies seasonally. While pesticides are applied at the lowest rate in May-June, the highest usage is in October-November.[9] In a study conducted with agricultural workers, it was stated that female and male workers working in the greenhouse used gloves the most, and when they did not, they were not provided with them, and they could not use personal protective equipment because of their high cost.[25] Huyen et al.[28] found that all workers used masks and could not use other equipment (gloves, boots, goggles…) due to their insufficiency. The fact that protective equipment is considered uncomfortable and unnecessary by the workers besides the reasons of high cost and unavailability of them indicates a lack of awareness among the workers. Creating this awareness and raising the awareness of workers about the use of equipment should be one of the priority initiatives in terms of agricultural health.

Table 2. Logistic regression analysis for the relationship between working time and health problems in greenhouse agriculture (n=529)

Variables	Odds' ratios, (95% GA)				p*
	Respiratory distress	Cough	Itching-rashing	Nose-eye problems	
The number of working hours in greenhouse per day	2.04 (0.40-14.60)[a]		15.27 (1.71-26.48)[b]	5.93 (1.83-19.20)[c]	0.023[a] 0.015[b] 0.003[c]
The number of working days in greenhouse per month	0.27 (0.04-1.71)[x]	6.80 (1.23-34.48)[y]			0.044[x] 0.028[y]

*p<0.05, [a]8 hours, [b,c]10 hours, [x,y]25-30 days, for the monthly working day variable, the reference category has been changed to "last".

Table 3. Logistic regression analysis for the relationship between work type and equipment usage and health problems in greenhouse agriculture (n=529)

Variables	Odds' ratios (95% GA)						p*
	Respiratory distress	Bronchitis	Cough	Wheezing	Itching-rashing	Nose-eye problems	
Seed sowing	1.69 (1.11-2.58)[a]			1.71 (1.09-2.68)[b]	2.93 (1.97-4.37)[c]	2.15 (1.45-3.19)[d]	0.015[a] 0.019[b] 0.000[c] 0.000[d]
Pesticide application/ spraying		8.04 (2.36-27.40)[a]				1.66 (1.07-2.57)[b]	0.044[a] 0.022[b]
Grafting			0.62 (0.41-0.95)[a]		0.34 (0.21-0.54)[b]	0.29 (0.18-0.46)[c]	0.028[a] 0.000[b] 0.000[c]
Personal protective equipment (PPE)** not used		0.31 (0.09-1.02)[a]		0.32 (0.14-0.71)[b]			0.054[a] 0.005[b]
Mask		0.48 (0.24-0.93)[a]	0.48 (0.32-0.72)[b]	0.50 (0.31-0.80)[c]	0.42 (0.28-0.63)[d]	0.51 (0.34-0.76)[e]	0.030[a] 0.000[b] 0.005[c] 0.000[d] 0.001[e]

*p<0.05, **Personal Protective Equipment, [a,b,c,d,e]No, for variables with an OR value less than 1, the reference category is changed to "last".

It was determined in the study that the emptied pesticide boxes were mostly thrown into garbage bins on the streets and sent for recycling. Similarly, in a study, it was revealed that the majority of the workers threw the emptied pesticide boxes in the nearby garbage bins, and 20% of them dumped them anywhere.[28] In the same study, when workers were asked about the reasons for their behavior, they stated that the municipal garbage container was too far from the agricultural areas and it was difficult and time consuming to transport the emptied boxes there.[28] Nowadays, when waste management and plastic waste recycling have become extremely important, it is seen that these plastic wastes containing chemical residues are unconsciously left to nature and there is a serious lack of awareness among agricultural workers.

A low percentage of the participants stated that they wore the clothes used while working in the greenhouse in their daily life, and more than half of them consumed food and beverages in the greenhouse. Avoiding the use of the same clothes is very important in terms of preventing the contamination of other family members and preventing secondary exposure. Mouth is one of the ways of taking pesticides into the body, and it makes us think that the participants of the research are either unaware of or insensitive about the harmful effects of consuming food and beverage in the greenhouse to human health.

In the research, itching, rashing, swelling, etc. on hands/face (open areas of the body) were found to be experienced the most by the participants. It is followed by nose or eye problems (itching, watering, burning, pain, etc.) with a 45.7% rate, cough with a 39.1% rate, and wheezing/shortness of breath with a 22.1% rate. In a study conducted with farmers, it was reported that symptoms such as allergies, nasal congestion, and wheezing were observed frequently after the use of pesticides.[29] In one study, it was reported that pesticides used in agriculture can be absorbed by eyes in amounts that can cause serious or even fatal disease.[30] Granular pesticides can pose a special danger to the eyes depending on the size and weight of the particles.[31] If pesticides are applied with electrical equipment, the granules can bounce off vegetation or other surfaces at high speed, causing serious eye damage.[32] Eye protection is also needed when measuring or mixing concentrated or highly toxic pesticides. Protective face shields or goggles should be worn when spraying pesticides or to prevent eye contact with powders.

In the study, the workers think that dermal problems are mostly caused by contact with products and respiratory problems are due to dust/dirt, while both dermal and respiratory problems are reported to result from pesticides, chemical fertilizers, and a hot working environment. Pesticide exposure was reported to be commonly associated with eye irritation, skin infection/rashing, and dry throat.[33] Fungicides, a type of pesticide, have been reported to cause high rates of irritation and dermal sensitivity on the skin and mucous membranes.[34] In a study, a significant difference was found in terms of dermal symptoms between the hand by which pesticide was applied and other parts of the body.[35] In some of the current literature information, it was determined that agricultural workers had asthma,[36] chronic obstructive pulmonary disease[37,38] and pulmonary hypersensitivity.[39] In studies performed with workers working in flower cultivation in greenhouses, respiratory symptoms were also reported to be common.[40-42] Inhalation of pesticide aerosols or vapors can directly damage the airways or interact with irritant receptors in the airway mucosa, resulting in the release of chemical mediators that trigger neurogenic inflammation.[20] Huyen et al.[28] reported that dermal symptoms were common among farmers due to their constant contact with pesticides. Entering into the greenhouse with daily clothes and using them while working, although at a low rate, and failure to clean hands and face sufficiently after working in the greenhouse may make workers sensitive in terms of dermal and eye diseases.

In the study, daily and monthly working time was found to be associated with dermal and respiratory symptoms. Similarly, a study conducted in Spain found a positive correlation between working time in the greenhouse and rhinitis.[43] Quansah et al.[19] discovered in their research that the prevalence of chronic cough and wheezing was higher among

agricultural workers who applied pesticides more than 30 days a year. The length of the working period (hours and days) seems to trigger dermal and respiratory symptoms by increasing the time of exposure to chemical agents in the greenhouse.

In the study, it was determined that the tasks of seed sowing, pesticide application/spraying, and grafting in the greenhouse were associated with dermal and respiratory symptoms. A research performed in China reported that pesticide exposure was mostly seen among workers engaged in sowing, plant growing, and pesticide application.[44] Another study found that respiratory and dermal symptoms were mostly seen in female workers engaged in cutting and weeding tasks. In the same study, it was discovered that the prevalence of respiratory symptoms was high among male workers engaged in pesticide spraying.[25] Research conducted with female agricultural workers in Africa revealed that pesticide spraying had a positive relationship with ocular-nasal symptoms.[45] The biological mechanism by which some pesticides cause respiratory symptoms and asthma is not fully understood. Exposure to high concentrations of pesticides is thought to cause excessive mucus secretion and contraction of the smooth muscle of the respiratory tract, resulting in shortness of breath, wheezing, and cough.[46]

The study revealed that those not using any protective equipment suffered from wheezing and those not wearing masks experienced bronchitis, coughing, wheezing, itching-rashing on skin, and nose-eye problems. Personal protective equipment is an important approach to reducing respiratory exposure in agricultural activities.[47] One study reported that a large proportion of pesticide poisonings were caused by the failure to use personal protective equipment.[48] While many workers are unaware of the risks associated with pesticide use, the lack of training and equipment to safely use pesticides increases health risk.[49] It has been reported that workers must use hand, head, eye, foot, ear and respiratory protectors that are defined as appropriate personal protective equipment while working with pesticides to prevent their exposure to the harmful effects of pesticides, and that skin exposure to pesticides can be prevented by 98% with the proper use of these protective materials.[50] The use of protective equipment among agricultural workers may vary by years and region. In a study, it was found that 93% of workers used protective equipment during pesticide applications and this use was preferred mostly by young men,[51] while Esechie and Ibiyato[12] discovered that the use of personal protective equipment by greenhouse workers was insufficient. The research performed in our country revealed that the rate of using personal protective equipment during pesticide applications varied between 13-41%.[40,52,53] The awareness of the use of personal protective equipment and the risks of pesticides varies by age and gender.[54,55] Reviewing the literature, the high rate of occupational diseases and health problems among agricultural workers shows that personal protection is inadequate or faulty, and those applying pesticides have insufficient knowledge about storage, application, and disposal of these products.[56-58]

Approximately half of the participants in the study stated that their health problems occurred after they started working in the greenhouse, that their problems increased while working in the greenhouse, and that similar health problems were also seen among other people working in the same place, and that their complaints decreased on the days not when they did not work in the greenhouse. These findings suggest that the working environment triggers health problems among workers. The data by the Turkish Statistical Institute show that 18.2% of total employment is in the agricultural sector.[59] The results of this study are important to guide workers rendering services in agricultural activities, to elaborate plans for raising social awareness, and to form a basis for the steps to be taken in this regard.

Study Limitations

The beginning of the pandemic process created a limitation in terms of data collection. Because people with respiratory problems are thought to be not working in the greenhouse due to the risk of contracting coronavirus during the pandemic and normalization process, these people may not have been reached during the data collection process. Also, the findings obtained from the study are limited to the sample of the research.

CONCLUSION

In conclusion, factors such as working conditions, type of work done in greenhouse agriculture, and failure to use personal protective equipment increase the prevalence of dermal and respiratory symptoms among workers. Greenhouse agriculture should be paid attention because it is a common line of business in our country and the main source of income for a large part of the society, and hence improvements should be made in working environments.

- Public health professionals should provide training on the use of personal protective equipment and the impact of pesticides on human health, environmental health, and the future to the workers; and training on the health effects of working conditions to the employers and agricultural companies; and training on the use of personal protective equipment to the pesticide vendors.

- Working times can be standardized at the directorate or ministerial level rather than individual decisions.

- Measures to reduce exposure to chemical and biological hazards should be considered. Scientific research covering exposure measurements and objective health examinations should be conducted among greenhouse agricultural workers.

- Encouraging and guiding agricultural workers to use biological agents to eradicate pests and plant diseases will help minimize workersexposure to pesticides, reduce negative impacts on the environment and save production costs.

- The use of machines in the application of pesticides can reduce the direct contact of workers with these pesticides. Machines can also reduce spraying time, labor and production costs.

MAIN POINTS

- Working for 8 h or more per day and working for 25 days or more per month were associated with skin and respiratory problems.
- The type of work done in greenhouse agriculture increases the prevalence of dermal and respiratory symptoms among workers.
- Failure to use personal protective equipment increases the prevalence of dermal and respiratory symptoms among workers.

ETHICS

Ethics Committee Approval: The approval of the Akdeniz University Faculty of Medicine Clinical Research Ethics Committee was obtained to conduct the study (approval number: KAEK-282).

Informed Consent: Informed consent was obtained from the participants.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

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

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. Nuraydın A, Bilek Ö, Kenziman AK, Korkusuz MA, Atagün Aİ, Çakar NÖ, et al. The Mersin Greenhouse Workers Study. Surveillance of Work-related Skin, Respiratory, and Musculoskeletal Diseases. Ann Glob Health. 2018; 84(3): 504-11.
2. Dhouib I, Jallouli M, Annabi A, Marzouki S, Gharbi N, Elfazaa S, et al. From immunotoxicity to carcinogenicity: the effects of carbamate pesticides on the immune system. Environ Sci Pollut Res Int. 2016; 23(10): 9448-58.
3. Banks CN, Lein PJ. A review of experimental evidence linking neurotoxic organophosphorus compounds and inflammation. Neurotoxicology. 2012; 33(3): 575-84.
4. Bowers Y, London L, Holtman Z, Messina S, Arendse B, Lindoor E, et al. Obstacles to the rights of access to health care for farm worker women in the Western Cape. Critical Health Perspectives. 2009;(1). Available from: http://www.salearningnetwork.uct.ac.za/sites/default/files/image_tool/images/386/publications/papers/chp_2009_no1_obstacles_to_health_of_farm_worker_women_final_v.pdf
5. Chalubinski M, Kowalski ML. Endocrine disrupters--potential modulators of the immune system and allergic response. Allergy. 2006; 61(11): 1326-35.
6. Jeebhay MF, Baatjies R, Chang YS, Kim YK, Kim YY, Major V, et al. Risk factors for allergy due to the two-spotted spider mite (Tetranychus urticae) among table grape farm workers. Int Arch Allergy Immunol. 2007; 144(2): 143-9.
7. Parks CG, Walitt BT, Pettinger M, Chen JC, de Roos AJ, Hunt J, et al. Insecticide use and risk of rheumatoid arthritis and systemic lupus erythematosus in the Women's Health Initiative Observational Study. Arthritis Care Res (Hoboken). 2011; 63(2): 184-94.
8. Simoniello MF, Contini L, Benavente E, Mastandrea C, Roverano S, Paira S. Different end-points to assess effects in systemic lupus erythematosus patients exposed to pesticide mixtures. Toxicology. 2017; 376: 23-9.
9. García-García CR, Parrón T, Requena M, Alarcón R, Tsatsakis AM, Hernández AF. Occupational pesticide exposure and adverse health effects at the clinical, hematological and biochemical level. Life Sci. 2016; 145: 274-83.
10. Suemizu H, Sota S, Kuronuma M, Shimizu M, Yamazaki H. Pharmacokinetics and effects on serum cholinesterase activities of organophosphorus pesticides acephate and chlorpyrifos in chimeric mice transplanted with human hepatocytes. Regul Toxicol Pharmacol. 2014; 70(2): 468-73.
11. Dalvie MA, Jeebhay MF, London L, Rother HA. Health effects due to pestiside exposure among rural women in Western Cape, Final Report to women on Farms Project. 2010. Available from: http://www.wfp.org.za/publications/general-reports/doc_view/77-pesticides-final-report.html
12. Esechie JO, Ibitayo OO. Pesticide use and related health problems among greenhouse workers in Batinah Coastal Region of Oman. J Forensic Leg Med. 2011; 18(5): 198-203.
13. Kim KH, Kabir E, Jahan SA. Exposure to pesticides and the associated human health effects. Sci Total Environ. 2017; 575: 525-35.
14. Anderson SE, Meade BJ. Potential health effects associated with dermal exposure to occupational chemicals. Environ Health Insights. 2014; 8(Suppl 1): 51-62.
15. Jalilian H, Neghab M, Tatar M, Taheri S. Respiratory and Dermal Symptoms and Raised Serum Concentrations of Biomarkers of Oxidative Stress among Pesticide Retailers. Int J Occup Environ Med. 2018; 9(4): 194-204.
16. Amaral AF. Pesticides and asthma: challenges for epidemiology. Front Public Health. 2014; 2: 6.
17. How EPA protects workers from pesticide risk. Available from: https://www.epa.gov/pesticide-worker-safety/how-epa-protects-workers-pesticide-risk
18. Macfarlane E, Carey R, Keegel T, El-Zaemay S, Fritschi L. Dermal exposure associated with occupational end use of pesticides and the role of protective measures. Saf Health Work. 2013; 4(3): 136-41.
19. Quansah R, Bend JR, Abdul-Rahaman A, Armah FA, Luginaah I, Essumang DK, et al. Associations between pesticide use and respiratory symptoms: A cross-sectional study in Southern Ghana. Environ Res. 2016; 150: 245-54.
20. Hernández AF, Parrón T, Alarcón R. Pesticides and asthma. Curr Opin Allergy Clin Immunol. 2011; 11(2): 90-6.
21. Henneberger PK, Liang X, London SJ, Umbach DM, Sandler DP, Hoppin JA. Exacerbation of symptoms in agricultural pesticide applicators with asthma. Int Arch Occup Environ Health. 2014; 87(4): 423-32.
22. Hoppin JA, Umbach DM, London SJ, Henneberger PK, Kullman GJ, Alavanja MC, et al. Pesticides and atopic and nonatopic asthma among farm women in the Agricultural Health Study. Am J Respir Crit Care Med. 2008; 177(1): 11-8.
23. Ye M, Beach J, Martin JW, Senthilselvan A. Pesticide exposures and respiratory health in general populations. J Environ Sci (China). 2017; 51: 361-70.
24. Tabachnick BG, Fidell LS. Using Multivariate Statistics. 6th ed. New York: Pearson, 2013.
25. Hanssen VM, Nigatu AW, Zeleke ZK, Moen BE, Bråtveit M. High Prevalence of Respiratory and Dermal Symptoms Among Ethiopian Flower Farm Workers. Arch Environ Occup Health. 2015; 70(4): 204-13.
26. Davis LL. Instrument review: Getting the most from a panel of experts. Appl Nurs Res. 1992; 5(4): 194-7.
27. Öngel S. Çocuk İşçiliği Gerçeği Raporu. 2015. Available from: http://disk.org.tr/2015/04/disk-ar-turkiyede-cocuk-isciligi-gercegi-raporu-2015/
28. Huyen VN, Song VN, Thuy NT, Dung LTP, Hoan LK. Effects of pesticides on farmers' health in Tu Ky district, Hai Duong province, Vietnam. Sustainable Futures. 2020; 2: 100026.
29. Kongtip P, Techasaensiri B, Nankongnab N, Adams J, Phamonphon A, Surach A, et al. The Impact of Prenatal Organophosphate Pesticide Exposures on Thai Infant Neurodevelopment. Int J Environ Res Public Health. 2017; 14(6): 570.
30. Gilden RC, Huffling K, Sattler B. Pesticides and health risks. J Obstet Gynecol Neonatal Nurs. 2010; 39(1): 103-10.
31. Jaga K, Dharmani C. Ocular toxicity from pesticide exposure: A recent review. Environ Health Prev Med. 2006; 11(3): 102-7.

32. Fareed M, Kesavachandran CN, Pathak MK, Bihari V, Kuddus M, Srivastava AK. Visual disturbances with cholinesterase depletion due to exposure of agricultural pesticides among farm workers. Toxicol Environ Chem 2012; 94(8): 1601-9.

33. Dhananjayan V, Ravichandran B. Occupational health risk of farmers exposed to pesticides in agricultural activities. Curr Opin Environ Sci Health. 2018; 4: 31-7.

34. Bolognesi C, Merlo FD. Pesticides: Human health effects. Elsevier: 2019.p.438-53.

35. Bozdoğan NY, Bozdoğan AM, Daglioglu N, Erdem T. Determination of dermal exposure of operator in greenhouse spraying. Agricultural Mechanization in Asia. Africa and Latin America. 2017; 48(1): 33-8.

36. Wells AD, Poole JA, Romberger DJ. Influence of farming exposure on the development of asthma and asthma-like symptoms. Int Immunopharmacol. 2014; 23(1): 356-63.

37. Fontana L, Lee SJ, Capitanelli I, Re A, Maniscalco M, Mauriello MC, Iavicoli I. Chronic Obstructive Pulmonary Disease in Farmers: A Systematic Review. J Occup Environ Med. 2017; 59(8): 775-88.

38. Guillien A, Soumagne T, Dalphin JC, Degano B. COPD, airflow limitation and chronic bronchitis in farmers: a systematic review and meta-analysis. Occup Environ Med. 2019; 76(1): 58-68.

39. Quirce S, Vandenplas O, Campo P, Cruz MJ, de Blay F, Koschel D, et al. Occupational hypersensitivity pneumonitis: an EAACI position paper. Allergy. 2016; 71(6): 765-79.

40. Ergonen AT, Salacin S, Ozdemir MH. Pesticide use among greenhouse workers in Turkey. J Clin Forensic Med. 2005; 12(4): 205-8.

41. Zuskin E, Schachter EN, Mustajbegovic J. Respiratory function in greenhouse workers. Int Arch Occup Environ Health. 1993; 64(7): 521-6.

42. Del Prado-Lu JL. Pesticide exposure, risk factors and health problems among cutflower farmers: a cross sectional study. J Occup Med Toxicol. 2007; 2: 9.

43. Riu E, Monsó E, Marin A, Magarolas R, Radon K, Morera J, et al. Occupational risk factors for rhinitis in greenhouse flower and ornamental plant growers. Am J Rhinol. 2008; 22(4): 361-4.

44. Ding SM, Zhang X. Control route and pollution factor of applications of pesticide. Chin Agric Mech. 2010; 37: 32-4.

45. Ndlovu V, Dalvie MA, Jeebhay MF. Asthma associated with pesticide exposure among women in rural Western Cape of South Africa. Am J Ind Med. 2014; 57(12): 1331-43.

46. Mamane A, Baldi I, Tessier JF, Raherison C, Bouvier G. Occupational exposure to pesticides and respiratory health. Eur Respir Rev. 2015; 24(136): 306-19.

47. Casey ML, Mazurek JM. Respirator Use Among US Farm Operators with Asthma: Results From the 2011 Farm and Ranch Safety Survey. J Agromedicine. 2017; 22(2): 78-88.

48. An X, Ji X, Jiang J, Wang Y, Wu C, Zhao X. Potential dermal exposure and risk assessment for applicators of Chlorothalonil and Chlorpyrifos in cucumber greenhouses in China. Hum Ecol Risk Assess. 2015; 21(4): 972-85.

49. Groot MJ, Van't Hooft KE. The Hidden Effects of Dairy Farming on Public and Environmental Health in the Netherlands, India, Ethiopia, and Uganda, Considering the Use of Antibiotics and Other Agro-chemicals. Front Public Health. 2016; 4: 12.

50. Eğri N, Çakar İ, Ceylan S, Kürkçü EA, Çavdar B, Arslan Tatar ÇP, et al. Occupational health and safety guide in greenhouse agriculture. T.R. Ministry of Labor and Social Security, Directorate of Occupational Health and Safety. Ankara. 2013.

51. Schenker MB, Orenstein MR, Samuels SJ. Use of protective equipment among California farmers. Am J Ind Med. 2002; 42(5): 455-64.

52. Yavuz H, Simsek Z, Akbaba M. Health-risk behaviors in agriculture and related factors, southeastern Anatolian region of Turkey. J Agromedicine. 2014; 19(4): 364-72.

53. Şahin G, Uskun E, Ay R, Öztürk M. Knowledge, attitudes and behaviors of those working in apple cultivation on pesticides. TAF Prev Med Bull. 2010; 9(6): 633-44.

54. Jallow MFA, Awadh DG, Albaho MS, Devi VY, Thomas BM. Pesticide risk behaviors and factors influencing pesticide use among farmers in Kuwait. Sci Total Environ. 2017; 574: 490-8.

55. Wang W, Jin J, He R, Gong H. Gender differences in pesticide use knowledge, risk awareness and practices in Chinese farmers. Sci Total Environ. 2017; 590-1: 22-8.

56. Elibariki R, Maguta MM. Status of pesticides pollution in Tanzania - A review. Chemosphere. 2017; 178: 154-64.

57. Sharifzadeh MS, Damalas CA, Abdollahzadeh G. Perceived usefulness of personal protective equipment in pesticide use predicts farmers' willingness to use it. Sci Total Environ. 2017; 609: 517-23. Retraction in: Sci Total Environ. 2020; 717: 137472.

58. Sankoh AI, Whittle R, Semple KT, Jones KC, Sweetman AJ. An assessment of the impacts of pesticide use on the environment and health of rice farmers in Sierra Leone. Environ Int. 2016; 94: 458-66.

59. Turkish Statistics Institute-TSI (2019). Labor statistics, March 2019. Available from: https://data.tuik.gov.tr/Bulten/Index?p=Isgucu-Istatistikleri-2019-33784

RESEARCH ARTICLE

CYPRUS
JOURNAL OF MEDICAL SCIENCES

DOI: 10.4274/cjms.2021.2021-41
Cyprus J Med Sci 2023;8(3):205-210

YouTube Videos Provide Poor Information on High Tibial Osteotomy

Kerem Yıldırım[1,2], **Mehmet Yalçınozan**[3]

[1]Department of Orthopaedics and Traumatology, İstanbul Gelisim University Faculty of Health Sciences, İstanbul, Türkiye
[2]Department of Orthopaedics and Traumatology, Private Beyzadeoğlu Clinic, İstanbul, Türkiye
[3]Department of Orthopaedics and Traumatology, Near East University Faculty of Medicine, Mersin, Türkiye

Abstract

BACKGROUND/AIMS: To examine the informative features and qualities of YouTube videos on high tibial osteotomy (HTO) in terms of the informative reliability of the videos.

MATERIALS AND METHODS: The included videos were evaluated individually using two orthopedic surgeons using modified DISCERN score and JAMA Benchmark score. Videos were categorized according to publishing year, video type, video length, number of views, and view rates. Any correlation between these categories and JAMA and modified DISCERN scores were analyzed.

RESULTS: Twenty-six videos were available for evaluation. Mean-modified DISCERN and JAMA scores were 2.6 and 2.0 for observer 1 and 2.5 and 1.9 for observer 2, respectively. Median view rates of the videos published between 2008-2011, 2012-2015, and 2015-2020 were 2559.00, 3314.0, and 7458.00, respectively ($p=0.003$). Other variables showed no difference compared to the publishing year groups ($p>0.05$). JAMA scores by both observers were positively correlated with video length. A weak positive correlation between mean JAMA score and view rate and a positive correlation between modified DISCERN scores by both observers and video length were found. Mean-modified DISCERN scores and video length values had a strong correlation ($p=0.001$; $r=0.609$). A significant correlation was found between JAMA and modified DISCERN scores. Mean-modified DISCERN scores showed a moderate increase correlated with the mean JAMA score increase ($p=0.017$; $r=0.463$).

CONCLUSION: Although the view rate has increased over time, the informative quality and reliability of YouTube videos concerning HTO are low and did not improve over the last decade.

Keywords: High tibial osteotomy, YouTube, JAMA Benchmark score, modified DISCERN score, osteoarthritis

INTRODUCTION

Medial compartment osteoarthritis (MCOA) of the knee is a common and challenging pathology in young and active patients. High tibial osteotomy (HTO) was developed to restore the mechanical axis of the knee, decrease the load on the medial joint compartment, and delay the progression of MCOA.[1-4] Due to favorable outcomes[5-7] in appropriately indicated young and active patients and technical developments, HTO has recently become more popular as a corrective and joint-preserving surgery. HTO being a very technically detailed operation,[8] any patient offered HTO should be informed in detail before surgery.

YouTube is a global social network and an open source of information with over two billion monthly logged-in users in over 100 countries around the world, across 80 languages, and is a rapidly growing visual database with over 500 h of content uploaded every minute.[9]

Although started as a recreational and entertainment platform; YouTube, due to patient interest, has become a platform for medical

To cite this article: Yıldırım K, Yalçınozan M. YouTube Videos Provide Poor Information on High Tibial Osteotomy. Cyprus J Med Sci 2023;8(3):205-210

ORCID IDs of the authors: K.Y. 0000-0003-1624-6438; M.Y. 0000-0002-2772-1137.

Address for Correspondence: Kerem Yıldırım
E-mail: drkeremyildirim@gmail.com
ORCID ID: orcid.org/0000-0003-1624-6438

Received: 28.02.2021
Accepted: 29.08.2021

information for academicians and colleagues as well as patients. Many studies have revealed that medical information on orthopaedic as well as other medical fields found on YouTube is of low quality and liability, misleading and poor.[10-16]

The aim of this study was to examine the informative features and qualities of the videos with a high number of views that were shared on YouTube about HTO surgery as a treatment option in young patients with medial knee osteoarthritis in terms of the informative efficiency of the videos on disease and treatment options.

While YouTube is a very popular video platform with no supervision by healthcare professionals, this study hypothesized that the videos on YouTube were providing poor medical information on HTO.

MATERIALS AND METHODS

A search with the keywords "high tibial osteotomy", "HTO", "proximal tibial osteotomy", "tibia osteotomy" and "knee osteotomy" was performed on YouTube on October 8, 2020. Non-English videos, repeated videos, inhumane videos, videos without any audio or text narration, and videos that were viewed less than 10,000 times were excluded. As the primary results, the included videos were evaluated individually by two orthopaedic surgeons using two different scoring systems: modified DISCERN score and JAMA Benchmark score. For secondary results, the videos were categorized according to publishing year, video type (animation, live surgery, patient diary, etc.), video length, number of views, and view rates. Any correlation between these categories and JAMA and modified DISCERN scores were analyzed.

DISCERN is a scoring system developed at Oxford and used to evaluate the quality of health care information, originally made up of 16 questions.[17] Establishing a scoring system for clarity, reliability, bias/balance, providing additional information, and uncertainty criteria, Singh et al.[15] modified DISCERN for the evaluation of YouTube. The reliability of information was scored from 0 to 5 (reliability score) based on 5 questions for the reliability and completeness of information. A higher modified DISCERN score means higher reliability.

The JAMA benchmarks were published as a suggestion for basic quality standards for internet information on health care.[18] It evaluates four features that must be clearly visible on a website: authorship (writers and contributors), attribution of references (references for all content and copyright information), disclosure (potential conflict of interest of the website), and currency (the dates on which the content was uploaded and updated). Scores are between 0 and 4, in which a higher score indicates higher video reliability.

The study was conducted in accordance with the Declaration of Helsinki Principles.

Statistical Analysis

Data were analyzed with IBM® SPSS® Statistics 23 (IBM®, Armonk, NY, United States). The conformity to normal distribution was examined using the Shapiro-Wilk test. The Kruskal-Wallis test was used to compare quantitative data that were not normally distributed to groups of three or more. Spearman's rho correlation coefficient was used to examine the relationship between non-normally distributed quantitative variables. The in-class correlation coefficient was used to examine the agreement between the first and second experts. Analysis results are mean ± standard deviation for quantitative data presented as deviation and median (minimum-maximum). The significance level was taken as $p<0.05$.

RESULTS

When the exclusion criteria were applied, 26 videos were available for evaluation. 19.2% of the videos were uploaded in 2011 and 42.3% of the videos were live surgery recordings. Descriptive statistics for categorical variables are given in Table 1. Mean-modified DISCERN and JAMA Benchmark scores were 2.6 and 2.0 for observer 1 and 2.5 and 1.9 for observer 2, respectively. The mean length of the videos was 538.2 sec., whereas the shortest was 16.0 sec. and the longest was 3131.0 sec. The maximum view rate of the videos was 18979.0 views per year (v/yr), minimum was 928.0 v/yr, and the mean view rate was 6198.2 v/yr. (Table 2). Video types and median value of video lengths had significant difference ($p=0.013$). The median values of animated videos, live surgery recordings, and other types were 187.00 sec., 606.00 sec., and 301.50 s, respectively. Video type and other variablesmedian values showed no difference ($p>0.050$) (Table 3).

Video publishing year groups and view rate variables showed a statistically significant difference ($p=0.003$). The median view rates of the videos published between 2008-2011, 2012-2015 and 2015-2020 was 2559.00, 3314.0, and 7458.00, respectively. Other variables showed no significant difference compared to the publishing year groups ($p>0.050$) (Table 4).

Total JAMA Benchmark scores by observer 1 ($p=0.015$; $r=0.473$) and observer 2 ($p=0.041$; $r=0.403$) were significantly positively correlated with video length values. A weak positive correlation between mean

Table 1. Descriptive statistics of categorical variables of videos

	Frequency (n)	Percentage (%)
Year		
2008	4	15.4
2011	5	19.2
2012	3	11.5
2013	1	3.8
2014	2	7.7
2015	1	3.8
2016	4	15.4
2017	4	15.4
2018	1	3.8
2019	1	3.8
Type		
Live surgery	11	42.3
Animation	5	19.2
Clinical outcome	3	11.5
Specialist narrative	2	7.7
Digital planning tutorial (for-profit)	1	3.8
Explanation on bone model	1	3.8
Patient diary	1	3.8
Seminar	1	3.8
Webinar (mixed)	1	3.8

JAMA benchmark and view rate values was found (p=0.047; r=0.393). A positive correlation between modified DISCERN scores by observer 1 (p<0.001; r=0.639) and observer 2 (p=0.003; r=0.563) and video length values was found. Mean-modified DISCERN scores and video length values had a statistically significant strong correlation (p=0.001; r=0.609) (Table 5).

The statistical analysis of JAMA (ICC=0,860) and DISCERN (ICC=0.867) scores showed a strong correlation between both observers (Table 6). A significant correlation was also found between the mean JAMA Benchmark and mean-modified DISCERN scores. Mean-modified DISCERN scores showed a moderate increase correlated with the mean JAMA Benchmark score increase (r=0.463; p=0.017).

DISCUSSION

The most important finding of this study is that the informative quality and reliability of YouTube videos concerning HTO are low and that they did not improve over the last decade, which proves our hypothesis true. In 2017, a study analyzing the quality of YouTube videos on anterior cruciate ligament injury and reconstruction[10] showed that the videos on YouTube were of low quality with a mean modified DISCERN score between 2.2 and 2.3 and a mean JAMA score between 2.3 and 2.5. Another study in 2016 analyzed the informative quality of YouTube videos on the diagnosis and treatment of hip arthritis[13] and showed that 86% of the videos provided poor information capacity, while only 3% provided excellent quality. The study concluded that YouTube is a poor source of accurate information about the diagnosis and treatment of hip arthritis. Several recent studies have researched the informative quality of YouTube videos on several common orthopedic problems and procedures, such as rehabilitation and return to sports after anterior cruciate ligament reconstruction, developmental dysplasia of the hip, total hip/knee arthroplasty, posterior cruciate ligament injuries, rotator cuff injuries, and treatment of Bankart lesions. All referred studies concluded that the informative/educational quality and reliability of YouTube videos were poor.[19-24] Among a large number of studies, we did not find any study that analyzed YouTube content concerning HTO. The results of our study were no exception to other studies mentioned above. Similar to the results of other studies analyzing videos on other

Table 2. Descriptive statistics of quantitative variables

	Mean	Standard deviation	Median	Minimum	Maximum
JAMA Benchmark-total (observer 1)	2.0	0.4	2.0	1.0	3.0
JAMA Benchmark-total (observer 2)	1.9	0.5	2.0	1.0	3.0
JAMA Benchmark-mean	2.0	0.4	2.0	1.0	3.0
Modified DISCERN (observer 1)	2.6	1.6	3.0	0.0	5.0
Modified DISCERN (observer 2)	2.5	1.4	3.0	0.0	5.0
Modified DISCERN mean	2.6	1.5	3.0	0.0	5.0
Views (n)	30224.2	21498.0	23681.0	11136.0	93933.0
Length (seconds)	538.2	646.9	343.0	16.0	3131.0
View Rate (view per year)	6198.2	5406.7	4361.0	928.0	18979.0

Table 3. The comparison of quantitative variables by video type

	Animation	Live surgery	Others	p*
JAMA Benchmark-total (observer 1)	1.80±0.84	2.09±0.30	2.10±0.32	0.441
	2.00 (1.00-3.00)	2.00 (2.00-3.00)	2.00 (2.00-3.00)	
JAMA Benchmark-total (observer 2)	1.60±0.55	2.00±0.45	2.00±0.47	0.244
	2.00 (1.00-2.00)	2.00 (1.00-3.00)	2.00 (1.00-3.00)	
JAMA Benchmark-mean	1.70±0.67	2.05±0.35	2.05±0.37	0.581
	2.00 (1.00-2.50)	2.00 (1.50-3.00)	2.00 (1.50-3.00)	
Modified DISCERN (observer 1)	2.00±1.41	3.45±0.82	2.00±1.89	0.064
	3.00 (0.00-3.00)	4.00 (2.00-4.00)	1.50 (0.00-5.00)	
Modified DISCERN (observer 2)	2.00±1.41	3.18±0.87	2.00±1.76	0.136
	3.00 (0.00-3.00)	3.00 (2.00-4.00)	2.00 (0.00-5.00)	
Modified DISCERN-mean	2.00±1.41	3.32±0.78	2.00±1.76	0.068
	3.00 (0.00-3.00)	3.50 (2.00-4.00)	2.00 (0.00-5.00)	
Views (n)	27780.80±15273.45	34356.00±27321.63	26900.80±17726.88	0.893
	26835.00 (12547.00-52952.00)	24324.00 (11260.00-93933.00)	22758.00 (11136.00-72566.00)	
Length (seconds)	156.60±90.22	671.64±413.08	582.30±929.65	**0.013**
	187.00 (16.00-255.00)[a]	606.00 (73.00-1662.00)[b]	301.50 (20.00-3131.00)[ab]	
View rate (view per year)	7139.60±6089.71	7611.55±6757.48	4172.70±2547.19	0.559
	4917.00 (2241.00-17650.00)	4540.00 (1462.00-18979.00)	3160.00 (928.00-8062.00)	

*Kruskal-Wallis test. [a,b]: There is no difference between the same letters, mean ± standard deviation, median (minimum-maximum).

Table 4. Comparison between video publishing years and quantitative variables

	2008-2011	2012-2015	2015 and later	p*
JAMA Benchmark-total (observer 1)	1.89±0.33	2.00±0.00	2.20±0.63	0.291
	2.00 (1.00-2.00)	2.00 (2.00-2.00)	2.00 (1.00-3.00)	
JAMA Benchmark-total (observer 2)	1.67±0.50	2.00±0.00	2.10±0.57	0.133
	2.00 (1.00-2.00)	2.00 (2.00-2.00)	2.00 (1.00-3.00)	
JAMA Benchmark means	1.78±0.36	2.00±0.00	2.15±0.58	0.106
	2.00 (1.00-2.00)	2.00 (2.00-2.00)	2.00 (1.00-3.00)	
Modified DISCERN (observer 1)	2.00±1.80	2.86±1.68	3.00±1.15	0.453
	2.00 (0.00-4.00)	4.00 (0.00-4.00)	3.00 (1.00-5.00)	
Modified DISCERN (observer 2)	1.89±1.54	2.57±1.62	3.00±1.15	0.306
	2.00 (0.00-4.00)	3.00 (0.00-4.00)	3.00 (1.00-5.00)	
Modified DISCERN-mean	1.94±1.59	2.71±1.60	3.00±1.15	0.342
	2.00 (0.00-4.00)	3.00 (0.00-4.00)	3.00 (1.00-5.00)	
Views (n)	28323.00±18528.14	33712.86±28539.39	29493.10±20541.85	0.999
	24324.00 (11136.00-72566.00)	26517.00 (11260.00-93933.00)	21074.00 (12547.00-75919.00)	
Length (seconds)	318.22±302.62	617.57±538.18	680.70±901.07	0.330
	277.00 (16.00-811.00)	396.00 (49.00-1662.00)	331.00 (130.00-3131.00)	
View rate (view/year)	2855.11±2099.26	5542.43±6054.25	9665.90±5289.12	**0.003**
	2559.00 (928.00-8062.00)[a]	3314.00 (1875.00-18786.00)[ab]	7458.00 (4182.00-18979.00)[b]	

*Kruskal-Wallis test. [a,b]: There is no difference between the same letters, mean ± standard deviation, median (minimum-maximum).

Table 5. Relationship between scores, views, length and view rate values

		Views	Length (s)	View rate
JAMA Benchmark-total (observer 1)	r	0.265	0.356	0.385
	p	0.190	0.074	0.052
JAMA Benchmark-total (observer 2)	r	0.050	**0.473**	0.321
	p	0.808	**0.015**	0.110
JAMA Benchmark-mean	r	0.147	**0.403**	**0.393**
	p	0.474	**0.041**	**0.047**
Modified DISCERN (observer 1)	r	-0.009	**0.639**	0.123
	p	0.966	**0.001**	0.551
Modified DISCERN (observer 2)	r	0.024	**0.563**	0.242
	p	0.909	**0.003**	0.233
Modified DISCERN-mean	r	0.024	**0.609**	0.193
	p	0.908	**0.001**	0.344

r: Spearman's rho correlation coefficient.

Table 6. The consistency of JAMA and DISCERN scores between observers

	ICC (95% CI)	p
JAMA	0.860 (0.688-0.937)	<0.001
DISCERN	0.867 (0.973-16.805)	<0.001

CI: Confidence interval.

orthopedic issues, our results showed that YouTube videos concerning HTO were poor with a mean JAMA score of 2 and a mean modified DISCERN score of 2.6.

Cassidy et al.[10] showed that neither the video quality or source, nor the number of views had no correlation with the recorded scores. Other studies[22-24] stated that video content uploaded by medical professionals/academicians had higher informative quality and reliability. A study that analyzed video content quality on YouTube concerning Bankart lesion and its treatment showed that there was no difference in DISCERN or JAMA scores according to video type.[19] Similarly, we found no significant difference after the comparison of the scores of three different groups of video types. The type of the video did not have any effect on the scores, which means that animations, live surgery videos, and other types (webinar, seminar, patient diary etc.) had similar low informative quality and reliability. Moreover, animations and live surgery videos had similar view rates. In contrast to live surgery videos, animations were significantly shorter in time. A recent study analyzing YouTube videos on arteriovenous malformations showed that videos featuring animations had higher popularity compared to other types of videos.[25] The practical translation of this may be that short animations may be enough to inform patients instead of recording live surgery videos and not jeopardizing sterility and concentration during surgery.

Another result of this study was that video length and view rate showed positive correlations with both JAMA and modified DISCERN scores. A study analyzing the quality of testicular cancer videos on YouTube revealed a positive correlation between video length and both JAMA and DISCERN scores.[26] Similarly, another study on the informative quality of YouTube videos on hallux valgus found a positive correlation between video length and DISCERN score.[27] This may be explained either by the fact that answers to questions that need answering for higher quality and reliability require more time; or that any information that comes up incidentally as the video gets longer addresses the required answer to a question.

Videos from three different time periods (2008-11, 2012-15 and 2015-19) had similar poor JAMA and modified DISCERN scores. This may be interpreted as that orthopaedic society failed to improve itself in terms of self-expression and education on digital media in the last decade. View rates, however, increased in time from 2559 views per

year between 2008 and 2011 to 9666 between 2015 and 2019. This may be explained by society's increasing habit of using digital streaming platforms and/or increasing use of the Internet by patients to research health-related issues.[28]

Moreover, the number of YouTube videos uploaded between 2015 and 2019 comprises 38% of all included videos, with this period having the highest number of videos. Similarly, the view rate increased from 3314 per year between 2011 and 2015 to 7458 between 2015 and 2019. A search on PubMed with the keywords "HTO AND proximal tibial osteotomy" between 2008 and 2019 resulted in 281 studies, where 182 (68%) of them were published between 2015 and 2019. The increase in the number of videos, view rate, and number of studies published in PubMed may be regarded as a confirmation of the "re-interest" in HTO recently.

In addition, the consistency in the comparison of JAMA and DISCERN scores indicates the reliability, precision, and value of these scales in their evaluative qualities. Similarly, a recent study that evaluated the quality of YouTube videos on spondylolisthesis showed that DISCERN, JAMA, and Global Quality Score scores correlated among themselves.[29]

Study Limitations

There are several limitations to our study. First of all, this study evaluates videos only on a single platform, namely YouTube. Videos on other popular digital platforms might have provided different results. On the other hand, because of the lack of validated tools specifically designed to evaluate online video content, we used the JAMA Benchmark scoring system that has been developed for written content, not for video content and the modified DISCERN scoring system that was developed for YouTube video content.[15] Another limitation of the scoring systems used in this study is that both systems are the observer dependent. Moreover, by selecting videos with a view number over 10.000, we may have overlooked videos that may be probably of higher quality but with a smaller number of views.

CONCLUSION

Although the view rate has increased over time, the informative quality and reliability of YouTube videos concerning HTO are low and did not improve over the last decade.

MAIN POINTS

- Although the view rate has increased over time, the informative quality and reliability of YouTube videos concerning HTO are low and did not improve over the last decade.
- Over time, the change in the social media usage habits of the population is also seen in the field of health. It seems that the increase in viewer demand for informative videos on this subject has also increased the number of shared videos over time.
- JAMA or modified DISCERN sores can be used for the evaluation or standardization of informative videos.

ETHICS

Ethics Committee Approval: Ethics committee form was not required.

Informed Consent: Informed consent was not required.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: K.Y., M.Y., Design: K.Y., M.Y., Materials: K.Y., Data Collection and/or Processing: K.Y., Analysis and/or Interpretation: K.Y., M.Y., Literature Search: K.Y., Writing: K.Y., M.Y., Critical Review: M.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. Jackson JP, Waugh W. Tibial osteotomy for osteoarthritis of the knee. J Bone Joint Surg Br. 1961;43-B:746-51.
2. Jung WH, Takeuchi R, Chun CW, Lee JS, Ha JH, Kim JH, et al. Second-look arthroscopic assessment of cartilage regeneration after medial opening-wedge high tibial osteotomy. Arthroscopy. 2014; 30(1): 72-9.
3. Koshino T, Wada S, Ara Y, Saito T. Regeneration of degenerated articular cartilage after high tibial valgus osteotomy for medial compartmental osteoarthritis of the knee. Knee. 2003; 10(3): 229-36.
4. Thambiah MD, Tan MKL, Hui JHP. Role of High Tibial Osteotomy in Cartilage Regeneration - Is Correction of Malalignment Mandatory for Success? Indian J Orthop. 2017; 51(5): 588-99.
5. Bonasia DE, Dettoni F, Sito G, Blonna D, Marmotti A, Bruzzone M, et al. Medial opening wedge high tibial osteotomy for medial compartment overload/arthritis in the varus knee: prognostic factors. Am J Sports Med. 2014; 42(3): 690-8.
6. Day M, Wolf BR. Medial Opening-Wedge High Tibial Osteotomy for Medial Compartment Arthrosis/Overload. Clin Sports Med. 2019; 38(3): 331-49.
7. Noyes FR, Mayfield W, Barber-Westin SD, Albright JC, Heckmann TP. Opening wedge high tibial osteotomy: an operative technique and rehabilitation program to decrease complications and promote early union and function. Am J Sports Med. 2006; 34(8): 1262-73.
8. Pehlivanoglu T, Yildirim K, Beyzadeoglu T. High Tibial Osteotomy. In: Nikolopoulos DD, Safos GK, Michos J, editors. Tibia Pathology and Fractures, IntechOpen; 2020.p.109-31.
9. YouTube. (2020). https://blog.youtube/press/
10. Cassidy JT, Fitzgerald E, Cassidy ES, Cleary M, Byrne DP, Devitt BM, et al. YouTube provides poor information regarding anterior cruciate ligament injury and reconstruction. Knee Surg Sports Traumatol Arthrosc. 2018; 26(3): 840-5.
11. Gokcen HB, Gumussuyu G. A Quality Analysis of Disc Herniation Videos on YouTube. World Neurosurg. 2019; 124: e799-e804.
12. Kaicker J, Dang W, Mondal T. Assessing the quality and reliability of health information on ERCP using the DISCERN instrument. Health Care Curr Rev. 2013; 1(1): 1-4.
13. Koller U, Waldstein W, Schatz KD, Windhager R. YouTube provides irrelevant information for the diagnosis and treatment of hip arthritis. Int Orthop. 2016; 40(10): 1995-2002.
14. O'Neill SC, Baker JF, Fitzgerald C, Fleming C, Rowan F, Byrne D, et al. Cauda equina syndrome: assessing the readability and quality of patient information on the Internet. Spine (Phila Pa 1976). 2014; 39(10): E645-9.
15. Singh AG, Singh S, Singh PP. YouTube for information on rheumatoid arthritis--a wakeup call? J Rheumatol. 2012; 39(5): 899-903.

16. Smith PE, McGuire J, Falci M, Poudel DR, Kaufman R, Patterson MA, et al. Analysis of YouTube as a Source of Information for Diabetic Foot Care. J Am Podiatr Med Assoc. 2019; 109(2): 122-6.

17. Charnock D, Shepperd S, Needham G, Gann R. DISCERN: an instrument for judging the quality of written consumer health information on treatment choices. J Epidemiol Community Health. 1999 ; 53(2): 105-11.

18. Silberg WM, Lundberg GD, Musacchio RA. Assessing, controlling, and assuring the quality of medical information on the Internet: Caveant lector et viewor--Let the reader and viewer beware. JAMA. 1997; 277(15): 1244-5.

19. Akpolat AO, Kurdal DP. Is quality of YouTube content on Bankart lesion and its surgical treatment adequate? J Orthop Surg Res. 2020; 15(1): 78.

20. Kunze KN, Cohn MR, Wakefield C, Hamati F, LaPrade RF, Forsythe B, et al. YouTube as a Source of Information About the Posterior Cruciate Ligament: A Content-Quality and Reliability Analysis. Arthrosc Sports Med Rehabil. 2019; 1(2): e109-14.

21. Kuru T, Erken HY. Evaluation of the Quality and Reliability of YouTube Videos on Rotator Cuff Tears. Cureus. 2020; 12(2): e6852.

22. Ng MK, Emara AK, Molloy RM, Krebs VE, Mont M, Piuzzi NS. YouTube as a Source of Patient Information for Total Knee/Hip Arthroplasty: Quantitative Analysis of Video Reliability, Quality, and Content. J Am Acad Orthop Surg. 2021; 29(20): e1034-44.

23. Oztermeli A, Karahan N. Evaluation of YouTube Video Content About Developmental Dysplasia of the Hip. Cureus. 2020; 12(8): e9557.

24. Springer B, Bechler U, Koller U, Windhager R, Waldstein W. Online Videos Provide Poor Information Quality, Reliability, and Accuracy Regarding Rehabilitation and Return to Sport After Anterior Cruciate Ligament Reconstruction. Arthroscopy. 2020; 36(12): 3037-47.

25. Krakowiak M, Szmuda T, Fercho J, Ali S, Maliszewska Z, Słoniewski P. YouTube as a source of information for arteriovenous malformations: A content-quality and optimization analysis. Clin Neurol Neurosurg. 2021; 207: 106723.

26. Duran MB, Kizilkan Y. Quality analysis of testicular cancer videos on YouTube. Andrologia. 2021; 53(8): e14118.

27. Sari E, Umur LF. Quality Analysis of Hallux Valgus Videos on YouTube. J Am Podiatr Med Assoc. 2021; 111(5).

28. Cassidy JT, Baker JF. Orthopaedic Patient Information on the World Wide Web: An Essential Review. J Bone Joint Surg Am. 2016; 98(4): 325-38.

29. Yaradilmis YU, Evren AT, Okkaoglu, MC, Ozturk O, Haberal B, Ozdemir M. Evaluation of quality and reliability of YouTube videos on spondylolisthesis. Interdisciplinary Neurosurgery. 2020; 22: 100827.

RESEARCH ARTICLE

CYPRUS
JOURNAL OF MEDICAL SCIENCES

DOI: 10.4274/cjms.2023.2023-18
Cyprus J Med Sci 2023;8(3):211-215

The Effect of Resveratrol Administration on Element Metabolism in Bone Tissue of Acute Swimming Exercised Rats

Dilek Arslangil[1], Saltuk Buğra Baltacı[1], Ömer Ünal[2]

[1]Department of Physiology, Selçuk University Faculty of Medicine, Konya, Türkiye
[2]Department of Physiology, Kırıkkale University Faculty of Medicine, Kırıkkale, Türkiye

Abstract

BACKGROUND/AIMS: In this study, we investigated how acute exercise affects bone element levels in resveratrol supplemented rats.

MATERIAL AND METHODS: The rats used in the study were divided into 4 groups with equal numbers (7 animals in each group). Control (group 1), swimming control (group 2), resveratrol (group 3), resveratrol + swimming (group 4). At the end of the four-week application, the animals were sacrificed under general anesthesia and bone tissue samples were removed. Zinc, magnesium, copper, iron, lead, cobalt, molybdenum, chromium, manganese, phosphorus, calcium and selenium levels were determined in bone tissue samples taken.

RESULTS: The bone zinc, iron, calcium, phosphorus, magnesium and boron levels of group 3 who received resveratrol supplementation were higher than all other groups ($p<0.05$). The lowest magnesium, calcium, and phosphorus values were obtained in the swimming groups (groups 2 and 4) ($p<0.05$). The levels of other measured elements did not differ between the groups.

CONCLUSION: According to the findings of this study: 1) Both resveratrol supplementation and acute swimming exercise cause changes in bone element metabolism. 2) Resveratrol supplementation changes bone element levels independent of exercise. 3) Resveratrol supplementation has a protective and/or regulating effect on bone element metabolism.

Keywords: Resveratrol supplementation, acute exercise, bone tissue, elements, rat

INTRODUCTION

Along with nutritional behaviors, sports and physical activity are considered as an important component in maintaining a healthy lifestyle.[1] Many studies have shown that moderate exercise may be beneficial for primary and secondary diseases, especially heart diseases,[2] disorders in carbohydrate metabolism, especially diabetes,[3] metabolic syndrome[4] and diseases such as Alzheimer's[5,6] caused by hippocampal degeneration.

Hormesis is a concept that has been proposed to explain the ability to maintain life at low concentrations of toxic agents and the adaptive cases developed against these agents.[7] The adaptation mechanism exhibited by metabolism against physical exercise can also be evaluated within the context of this concept. Sports and exercise are recommended to maintain a healthy lifestyle. However, it has also been shown that sports and physical exercise can cause oxidative stress and thus tissue damage.[7] Therefore, intensive efforts are made to develop dietary strategies for tissue damage caused by sports and exercise.[7] In recent year, ability of resveratrol in modulation of physical performance and prevention of oxidative damage has been studied.[7] Resveratrol administration in elderly mice has been shown to prevent lipid peroxidation.[8] Similar findings have been demonstrated by Dolinsky et

To cite this article: Arslangil D, Baltacı SB, Ünal Ö. The Effect of Resveratrol Administration on Element Metabolism in Bone Tissue of Acute Swimming Exercised Rats. Cyprus J Med Sci 2023;8(3):211-215

ORCID IDs of the authors: D.A. 0000-0003-4413-2833; S.B.B. 0000-0002-9752-7508; Ö.Ü. 0000-0001-6155-6780.

Address for Correspondence: Ömer Ünal
E-mail: omerunal@kku.edu.tr
ORCID ID: orcid.org/0000-0001-6155-6780

Received: 28.02.2023
Accepted: 11.01.2023

al.[9] In a study on 14 athletes, resveratrol administration was reported to prevented lipid peroxidation caused by exercise.[10] It is mentioned that resveratrol supplement may delay aging by improving health and quality of life in the aging process, in addition to its performance enhancing effect in athletes and physically active individuals.[11,12] Consistently, it was demonstrated in elderly mice model that resveratrol increases strength in exercise[8] and may prolong lifetime.[13,14] In the mentioned events it is obvious that muscular function is likely to be largely activated by resveratrol therapy.[15] The above reports of researchers on the subject show that resveratrol administration is beneficial in rodents with suppressed physical performance. But it is also noteworthy that resveratrol supplementation increased exercise capacity and physical performance not only in experimental animals with chronic health problems but also in healthy young adult mice and rats.[16,17] Similarly, it has been demonstrated that resveratrol increased muscle strength and functions in experimental animals specially raised for exercise types requiring endurance.[18] More importantly, in addition to the positive effects of resveratrol on exercise,[19] its important effects on cardiac tissue have also been shown in two different rodent models. In conclusion, administration of resveratrol with regular exercise[17] has sustainable positive effects not only on cardiovascular functions but also on the muscle activity.

Contrary to the mentioned effects of resveratrol, studies on its effects on element metabolism in exercise are scarce in the literature. Therefore, the aim of this study how acute exercise affects bone element levels in resveratrol-supplemented rats.

MATERIAL AND METHODS

Experimental Design

Adult male rats (Wistar) used in this study were obtained from Necmettin Erbakan University Kombassan Experimental Medicine Research and Application Center. This study was conducted in accordance with the Declaration of Helsinki. The study protocol was approved by the Experimental Animals Ethics Board of Necmettin Erbakan University's Kombassan Experimental Medicine Research and Application Center (approval number: 2013-183).

The rats used in the study were divided into 4 groups with equal numbers (7 animals in each group). Control (group 1), swimming control (group 2), resveratrol (group 3), resveratrol + swimming (group 4).

Rats in group 1 and 2 were fed standard rat chow. The rats in groups 3 and 4 received resveratrol "Sigma R5010" (10 mg/kg/day) supplementation in drinking water for 4 weeks in addition to the standard diet. The rats in groups 2 and 4 were given 30 min of acute swimming exercise at the end of the 4 weeks of practice.

The rats in the study groups were fed standard rat chow not less than ten grams per hundred grams of their body weight. The food of the experimental animals was obtained from the center where the study was conducted as normal rat food (as pellets). All animals were maintained at the same room temperature (21±1 °C) and on the same light/dark cycle (12 h light and 12 h dark cycle) throughout the study.

Swimming Exercise

Swimming exercises in rats were performed in an 80 cm long and 50 cm wide swimming pool made of heat-resistant polyethylene. To minimize stress factors in animals, acute swimming exercises were performed as 30-minute swimming exercises in a single session. It is accepted that swimming exercises between 30 and 60 min cause moderate stress in rats.[20]

Informed Consent

Following the four-week applications, 30-minute acute swimming exercises were applied to the animals in a single session. All animals were sacrificed 24 h after the acute swimming exercise and bone tissue samples were taken. General anesthesia was administered to all animals (with intramuscular administration of a combination of Ketalar, Parke-Davis and xylazine "Rompun, Bayer") to avoid animal suffering.

Elemental Analysis in Bone Tissue

At the end of the four-week practice, the animals were sacrificed under general anesthesia 24 h after 30 min of acute swimming exercise, and bone tissue samples were taken. Analysis of Zn, Pb, Co, Mo, Mg, Mn, P, Cu, Fe, Ca, Se and B elements in bone tissue samples was performed by the anomical emission method (inductively coupled plasma atomic-emission spectrometer). Results were calculated as µg/gram tissue. All elemental analysis in bone tissue were performed following the National Institute of Standards and Technology 1547 procedure.[21]

Statistical Analysis

Statistical evaluation of the data obtained was carried out using SPSS v. 21.0 package software, and the arithmetic mean and standard deviation of all parameters were calculated. Data were determined to show a normal distribution with Shapiro-Wilk test. One-Way variance analysis was used to determine differences between the groups, and the least significant difference test was used to find the group causing difference. $P<0.05$ values were considered statistically significant.

RESULTS

The highest bone zinc level was obtained in group 3, independent of swimming exercise, who received resveratrol supplementation ($p<0.05$). Acute swimming exercise led to the suppression of bone zinc levels in swimming groups (group 2 and group 4) with and without resveratrol supplementation ($p<0.05$). The lowest iron values in bone tissue were obtained in the swimming groups (groups 2 and 4) ($p<0.05$). There was no difference between the groups in terms of copper and selenium values (Table 1).

Table 1. Zn, Fe, Cu and Se levels in bone tissue of experimental animals (µg/gram tissue)

Groups	Zn	Fe	Cu	Se
G1 (control)	38.08±6.05[b]	18.10±8.30[a]	0.42±0.10	1.66±0.48
G2 (swimming control)	23.37±2.52[c]	8.78±3.76[b]	0.64±0.40	2.17±1.84
G3 (resveratrol)	53.22±12.52[a]	17.72±7.99[a]	0.44±0.19	2.23±0.50
G4 (resveratrol + swimming)	17.82±2.88[c]	8.86±2.65[b]	0.42±0.33	1.77±0.37

*Means with different superscripted letters in the same column are statistically significant a<b<c ($p<0.05$).

Resveratrol supplementation (group 3) increased bone calcium, phosphorus, and magnesium levels independent of swimming exercise ($p<0.05$). When compared with the control group, acute exercise led to a significant suppression of calcium, phosphorus, and magnesium levels in the bone in the swimming groups (groups 2 and 4) with and without resveratrol supplementation ($p<0.05$). There was no difference between the groups in terms of lead values in bone tissue (Table 2).

Resveratrol supplementation increased bone boron levels in group 3 ($p<0.05$). There was no difference between the groups in terms of Co, Cd, and Mo values in bone tissue (Table 3).

DISCUSSION

The highest levels of zinc, magnesium, calcium and phosphorus were obtained from the resveratrol only group, and the lowest levels of zinc, magnesium, calcium and phosphorus were obtained from the swimming groups and resveratrol + swimming group. Regular and long-term moderate exercise increase mineral content, and in conclusion this type of exercise is beneficial for bone mineralization.[22,23] On the other hand, many researchers have shown that exhausting exercises or the exercises performed until exhaustion negatively affect element metabolism in bone tissues.[24-26] In their study, Nielsen[26] pointed out that loss of bone minerals, especially zinc, occurred in rats that were subjected to tiring and exhausting exercise, and that especially loss of zinc in the bone tissue may cause osteopenia. Again, a single swimming exercise performed until exhaustion was reported to increase the loss of both magnesium and iron in the bone tissue.[24,25] In the present study, our finding of decreased magnesium, calcium, zinc and iron levels (in swimming control and resveratrol + swimming groups) is consistent with the results of above mentioned studies. Interestingly, in this study we found that levels of zinc, magnesium and calcium that were decreased in the swimming groups (swimming control and resveratrol + swimming groups) were increased in resveratrol alone group, and there was no change in bone iron levels. This finding is important in terms of indicating that resveratrol administration with a dose of 10 mg/kg for 4 weeks may lead to an increase in the mineral content of bones. In the present study, boron levels in bone tissue were higher in the resveratrol alone group compared to all other groups. Boron administration was reported to decrease calcium excretion, increase estrogen levels, and prevent bone loss in women in the menopausal period.[27-29] In a study, 3 mg/day boron was given to postmenopausal women, and it was found that urinary spillage of Mg, Ca, and P was decreased with boron supplementation, with these decreases being more prominent in the Mg diet.[29] Researchers state that magnesium and boron are essential for optimal calcium metabolism and elderly men and postmenopausal women need these minerals in order to be protected against losses in bone mass.[30,31] In our study, high boron levels in the bone tissue obtained in the resveratrol alone group are an important finding given the effects of boron element on bone metabolism. It was proposed that resveratrol has a bone tissue protecting effect, which occurs by stimulation of osteoblastic activity, and that resveratrol administration is critical to the prevention of age-related bone losses.[32] Similar finding was also reported by Wu et al.[32] Zhao et al.[33] Demonstrated that resveratrol administration has a protective effect on bone loss in osteoporosis. Increased levels of magnesium, calcium, zinc, and boron values that we obtained in resveratrol administration alone seem as a very crucial finding, emerge as an important result which will provide original and interesting information especially in the relationship between bone metabolism and resveratrol.

CONCLUSION

When the results of this study are examined as a whole; 1) Resveratrol administration changed element levels in the bone tissue independently of exercise. 2) It should be underlined that resveratrol administration has a protective and/or regulatory effect on bone metabolism independent of exercise.

MAIN POINTS

- Resveratrol administration changes element levels in the bone tissue independently of exercise.
- It should be underlined that resveratrol administration has a protective and/or regulatory effect on bone metabolism independent of exercise.
- In future studies, it can be suggested that the relationship between resveratrol administration and bone metabolism should be examined not only in terms of element metabolism but also in terms of histological changes.

Table 2. Ca, P, Mg and Pb levels in bone tissue of experimental animals (µg/gram tissue)

Groups	Ca	P	Mg	Pb
G1 (control)	53.05±12.9[b]	30.50±5.16[b]	11.50±2.41[b]	0.10±0.03
G2 (swimming control)	33.91±7.93[c]	14.76±1.30[c]	7.84±1.97[c]	0.34±0.12
G3 (resveratrol)	83.91±24.14[a]	42.92±15.30[a]	15.22±5.35[a]	0.37±0.20
G4 (resveratrol + swimming)	31.53±17.82[c]	15.18±2.15[c]	5.70±1.87[c]	0.16±0.07

*Means with different superscripted letters in the same column are statistically significant a<b<c ($p<0.05$).

Table 3. Co, Cd, Mo and B levels in bone tissue of experimental animals (µg/gram tissue)

Groups	Co	Cd	Mo	B
G1 (control)	0.048±0.03	0.011±0.01	0.06±0.03	0.15±0.01[b]
G2 (swimming control)	0.021±0.01	0.020±0.03	0.08±0.04	0.19±0.03[b]
G3 (resveratrol)	0.053±0.02	0.010±0.01	0.10±0.02	0.83±0.18[a]
G4 (resveratrol + swimming)	0.020±0.01	0.011±0.02	0.05±0.02	0.29±0.05[b]

*Means with different superscripted letters in the same column are statistically significant a<b ($p<0.05$).

Acknowledgments: Statistics of this article Selçuk University Faculty of Sport Sciences Lecturer Prof. Dr. It was made by Suleyman Patlar.

ETHICS

Ethics Committee Approval: The study protocol was approved by the Experimental Animals Ethics Board of Necmettin Erbakan University's Kombassan Experimental Medicine Research and Application Center (approval number: 2013-183).

Informed Consent: Patient approval has not been obtained as it is performed on animals.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

DISCLOSURES

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

Financial Disclosure: This study was supported by the Scientific Research Projects Coordinatorship of Selçuk University (SUBAPK; project no: 1420132013).

REFERENCES

1. Tiller NB, Sullivan JP, Ekkekakis P. Baseless Claims and Pseudoscience in Health and Wellness: A Call to Action for the Sports, Exercise, and Nutrition-Science Community. Sports Med. 2023; 53(1): 1-5.
2. Baltaci SB, Mogulkoc R, Baltaci AK. Resveratrol and exercise. Biomed Rep. 2016; 5(5): 525-30.
3. Robertson I, Wai Hau T, Sami F, Sajid Ali M, Badgujar V, Murtuja S, et al. The science of resveratrol, formulation, pharmacokinetic barriers and its chemotherapeutic potential. Int J Pharm. 2022; 618: 121605.
4. Bertram MY, Lim SS, Barendregt JJ, Vos T. Assessing the cost-effectiveness of drug and lifestyle intervention following opportunistic screening for pre-diabetes in primary care. Diabetologia. 2010; 53(5): 875-81.
5. Lemos GO, Torrinhas RS, Waitzberg DL. Nutrients, Physical Activity, and Mitochondrial Dysfunction in the Setting of Metabolic Syndrome. Nutrients. 2023; 15(5): 1217.
6. Dal-Ros S, Zoll J, Lang AL, Auger C, Keller N, Bronner C, et al. Chronic intake of red wine polyphenols by young rats prevents aging-induced endothelial dysfunction and decline in physical performance: role of NADPH oxidase. Biochem Biophys Res Commun. 2011; 404(2): 743-9.
7. O'Connor E, Mündel T, Barnes MJ. Nutritional Compounds to Improve Post-Exercise Recovery. Nutrients. 2022; 14(23): 5069.
8. DeWeerdt S. Food: The omnivore's labyrinth. Nature. 2011; 471(7339): S22-4.
9. Dolinsky VW, Jones KE, Sidhu RS, Haykowsky M, Czubryt MP, Gordon T, et al. Improvements in skeletal muscle strength and cardiac function induced by resveratrol during exercise training contribute to enhanced exercise performance in rats. J Physiol. 2012; 590(11): 2783-99.
10. Dolinsky VW, Rogan KJ, Sung MM, Zordoky BN, Haykowsky MJ, Young ME, et al. Both aerobic exercise and resveratrol supplementation attenuate doxorubicin-induced cardiac injury in mice. Am J Physiol Endocrinol Metab. 2013; 305(2): E243-53.
11. Durbin SM, Jackson JR, Ryan MJ, Gigliotti JC, Alway SE, Tou JC. Resveratrol supplementation preserves long bone mass, microstructure, and strength in hindlimb-suspended old male rats. J Bone Miner Metab. 2014; 32(1): 38-47.
12. Hakki SS, Dundar N, Kayis SA, Hakki EE, Hamurcu M, Kerimoglu U, et al. Boron enhances strength and alters mineral composition of bone in rabbits fed a high energy diet. J Trace Elem Med Biol. 2013; 27(2): 148-53.
13. Hart N, Sarga L, Csende Z, Koltai E, Koch LG, Britton SL, et al. Resveratrol enhances exercise training responses in rats selectively bred for high running performance. Food Chem Toxicol. 2013; 61: 53-9.
14. Hurley BF, Hanson ED, Sheaff AK. Strength training as a countermeasure to aging muscle and chronic disease. Sports Med. 2011; 41(4): 289-306.
15. Lagouge M, Argmann C, Gerhart-Hines Z, Meziane H, Lerin C, Daussin F, et al. Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1alpha. Cell. 2006; 127(6): 1109-22.
16. Malaguti M, Angeloni C, Hrelia S. Polyphenols in exercise performance and prevention of exercise-induced muscle damage. Oxid Med Cell Longev. 2013; 2013: 825928.
17. Marchal J, Pifferi F, Aujard F. Resveratrol in mammals: effects on aging biomarkers, age-related diseases, and life span. Ann N Y Acad Sci. 2013; 1290: 67-73.
18. Sung MM, Byrne NJ, Robertson IM, Kim TT, Samokhvalov V, Levasseur J, et al. Resveratrol improves exercise performance and skeletal muscle oxidative capacity in heart failure. Am J Physiol Heart Circ Physiol. 2017; 312(4): H842-53.
19. McDonald R, Hegenauer J, Saltman P. Age-related differences in the bone mineralization pattern of rats following exercise. J Gerontol. 1986; 41(4): 445-52.
20. Mercken EM, Carboneau BA, Krzysik-Walker SM, de Cabo R. Of mice and men: the benefits of caloric restriction, exercise, and mimetics. Ageing Res Rev. 2012; 11(3): 390-8.
21. Murase T, Haramizu S, Ota N, Hase T. Suppression of the aging-associated decline in physical performance by a combination of resveratrol intake and habitual exercise in senescence-accelerated mice. Biogerontology. 2009; 10(4): 423-34.
22. Naghii MR, Samman S. The role of boron in nutrition and metabolism. Prog Food Nutr Sci. 1993; 17(4): 331-49.
23. Navas FJ, Córdova A. Effect of magnesium supplementation and training on magnesium tissue distribution in rats. Biol Trace Elem Res. 1996; 53(1-3): 137-45. Erratum in: Biol Trace Elem Res. 1996; 55(1-2): 213.
24. Navas FJ, Córdova A. Iron distribution in different tissues in rats following exercise. Biol Trace Elem Res. 2000; 73(3): 259-68.
25. Nielsen FH, Hunt CD, Mullen LM, Hunt JR. Effect of dietary boron on mineral, estrogen, and testosterone metabolism in postmenopausal women. FASEB J. 1987; 1(5): 394-7.
26. Nielsen FH. Micronutrients in parenteral nutrition: boron, silicon, and fluoride. Gastroenterology. 2009; 137(5 Suppl): S55-60.
27. NIST Standard reference materials catalog 1995- 96. NIST Special Publication 260, Standards Reference Material Programme, U.S. Department of Commerce, Gaithersburg, MD 20899-0001, USA.
28. Oscai LB, Molé PA. Laboratory techniques involving small animals in exercise physiology research. Exerc Sport Sci Rev. 1975; 3: 351-70.
29. Rayalam S, Della-Fera MA, Baile CA. Synergism between resveratrol and other phytochemicals: implications for obesity and osteoporosis. Mol Nutr Food Res. 2011; 55(8): 1177-85.
30. Schrauwen P, Timmers S. Can resveratrol help to maintain metabolic health? Proc Nutr Soc. 2014; 73(2): 271-7.

31. Seco C, Revilla M, Hernández ER, Gervás J, González-Riola J, Villa LF, et al. Effects of zinc supplementation on vertebral and femoral bone mass in rats on strenuous treadmill training exercise. J Bone Miner Res. 1998; 13(3): 508-12.

32. Wu RE, Huang WC, Liao CC, Chang YK, Kan NW, Huang CC. Resveratrol protects against physical fatigue and improves exercise performance in mice. Molecules. 2013; 18(4): 4689-702.

33. Zhao L, Wang Y, Wang Z, Xu Z, Zhang Q, Yin M. Effects of dietary resveratrol on excess-iron-induced bone loss via antioxidative character. J Nutr Biochem. 2015; 26(11): 1174-82.

RESEARCH ARTICLE

CYPRUS
JOURNAL OF MEDICAL SCIENCES

DOI: 10.4274/cjms.2023.2022-33
Cyprus J Med Sci 2023;8(3):216-219

Evaluation of Hepatitis B Surface Antigen, Anti-HCV, and Anti-HIV Seroprevalence in Patients Operated in the Orthopedics and Traumatology Department

Serkan Güler, Hakan Yolaçan, Erdem Aras Sezgin

Department of Orthopaedics and Traumatology, Aksaray Training and Research Hospital, Aksaray, Türkiye

Abstract

BACKGROUND/AIMS: Occupational exposure to blood-borne viruses is a major concern for surgeons, nurses, and operating room personnel. We aimed to determine the hepatitis B surface antigen (HBsAg), anti-hepatitis C virus (anti-HCV), and human immunodeficiency virus (anti-HIV) seroprevalence of patients who were operated in the orthopedic service and to determine the prevalence of patients with positive serology, especially those in an orthopedics unit.

MATERIAL AND METHODS: In our study, patients who were operated in the orthopedic unit between January 1, 2021 and January 1, 2022 were retrospectively analyzed. HBsAg, anti-HCV, and anti-HIV serologies of the participants as well as age, gender, and case subgroups were recorded. The surgeries performed on the participants were analyzed in 9 subgroups: foot-ankle, general orthopedics, trauma, sports surgery-arthroscopy, pediatric orthopedics, hand-wrist, arthroplasty, spine, and oncological surgery.

RESULTS: We included 2006 patients in the study. According to the case grouping, 26 were foot-ankle, 202 general orthopedics, 642 trauma, 366 sports surgery-arthroscopy, 2 pediatric orthopedics, 145 hand-wrist, 582 arthroplasty, 12 spine, and 29 oncology. According to the ELISA results, 77 (3.8%) patients were seropositive, 64 (3.2%) were positive for HBsAg, and 13 (0.6%) were positive for anti-HCV. Anti-HIV positivity and co-infection were not detected in the participants. HBsAg (p=0.025) and anti-HCV (p=0.031) seropositivity were significantly higher in the group that underwent surgery due to trauma compared to other case subgroups.

CONCLUSION: HBsAg and anti-HCV seropositivity were higher in patients who underwent surgery for trauma than in those who underwent surgery for other reasons.

Keywords: Orthopedic surgery, seropositivity, seroprevalence

INTRODUCTION

Occupational exposure to blood-borne viruses is a major concern for surgeons, nurses, and operating room personnel. These include hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV). Contaminated sharp instruments increase the risk of transmission of these viruses. The World Health Organization stipulates that more than two million injuries occur in 35 million healthcare workers each year. Moreover, 66,000 HBV, 16,000 HCV, and 1,000 HIV infections occurred due to these injuries.[1] Screening for HBV, HCV, and HIV is vital for healthcare professionals working in surgical centers and emergency departments.[2] The use of advanced protective measures to limit occupational exposure and the disposal of seropositive wastes generated during surgery is of utmost importance.[3]

The aim of our study was to determine the incidence of HBsAg, anti-HCV, and anti-HIV positivity in patients who were operated on in the

To cite this article: Güler S, Yolaçan H, Sezgin EA. Evaluation of Hepatitis B Surface Antigen, Anti-HCV, and Anti-HIV Seroprevalence in Patients Operated in the Orthopedics and Traumatology Department. Cyprus J Med Sci 2023;8(3):216-219

ORCID IDs of the authors: S.G. 0000-0002-3424-4244; H.Y. 0000-0002-2449-9745; E.A.S. 0000-0001-5869-6571.

Address for Correspondence: Serkan Güler
E-mail: drserkanguler@hotmail.com
ORCID ID: orcid.org/0000-0002-3424-4244

Received: 30.07.2022
Accepted: 28.02.2023

orthopedic service and to determine which sub-branch of orthopedics is prevalent in patients who were found positive. After these determinations, it is aimed to create a new perspective on the concerns of surgeons and measures such as the dissemination of protective equipment to address these concerns.

The hypothesis of our study is that the incidence of HBsAg, anti-HCV, and anti-HIV positivity in patients who underwent orthopedic elective surgery is lower than in patients who underwent surgery due to trauma.

MATERIALS AND METHODS

Participants who were operated in the orthopedics and traumatology service between January 1, 2021 and January 1, 2022 were retrospectively analyzed. All patients operated during the study period were included in the study. Patients whose HBsAg, anti-HCV, and anti-HIV tests were not requested in the pre-operative period. We analyzed the surgeries in nine subgroups, including foot-ankle, general orthopedics, trauma, sports surgery-arthroscopy, pediatric orthopedics, hand-wrist, arthroplasty, spine, and oncological surgery. Amputation, implant removal, ingrown toenails, soft tissue infection surgeries, and foreign body removal surgeries were evaluated in the general orthopedics group. HBsAg, anti-HCV, and anti-HIV serologies as well as age, gender, and case subgroups of 2006 patients who met the criteria were recorded.

In our hospital, blood collection for serology is routine in the pre-operative period, and blood samples were studied in the microbiology laboratory with the macro-ELISA system (Architect-Abbott, USA).

This study was approved by the Ethics Committee of Aksaray University Training and Research Hospital (approval number: 2022/08-06, date: 21.04.2022). There is no information about the patient in the submitted manuscript.

Statistical Analysis

SPSS version 26 package program was used for statistical analysis of the data. Kolmogorov-Smirnov test was used to evaluate the homogeneity of the data. Chi-square test was used to compare categorical variables and statistical results were evaluated according to 0.05 significance level.

RESULTS

We evaluated 2006 patients in the study. Among these, 1,110 were female and 896 were male. The mean age of the patients was calculated as 50.03 (1-95) years. According to the case grouping, 26 were foot-ankle, 202 general orthopedics, 642 trauma, 366 sports surgery-arthroscopy, 2 pediatric orthopedics, 145 hand-wrist, 582 arthroplasty, 12 Two of them were spine and 29 of them were oncology group. According to the ELISA results, 77 (3.8%) of patients were seropositive, 64 of them (3.2%) were positive for HBsAg and 13 (0.6%) were positive for anti-HCV. Anti-HIV positivity and co-infection was not detected in any of the patients (Table 1).

HBsAg was positive in 38 female patients and 26 male patients (Table 2). No significant correlation was found between gender and HBsAg seropositivity (p=0.509). Anti-HCV positivity was detected in 7 females and 6 males (Table 2). No significant correlation was found between gender and anti-HCV seropositivity (p=0.914). Similarly, no significant correlation was found between age with HBsAg and anti-HCV seropositivity (p=0.658).

HBsAg seropositivity was detected in 4 general orthopedic cases, 35 trauma cases, 6 surgery-arthroscopy cases, 4 hand-wrist cases, and 15 arthroplasty cases (Table 3). The rate of detecting HBsAg seropositivity in the group that underwent surgery for trauma was significantly higher than that in the other groups that underwent surgery (p=0.025).

Anti-HCV seropositivity was detected in 11 trauma cases, 1 surgery-arthroscopy case, and 1 arthroplasty case (Table 3). Anti-HCV seropositivity rate was significantly higher in the group that underwent surgery due to trauma than in the other groups that underwent surgery (p=0.031).

DISCUSSION

The number of HBsAg, anti-HCV, and anti-HIV seropositive individuals detected during admission to the hospital was too high to be underestimated.[4] Blood-borne viruses are a serious concern for surgeons and healthcare professionals. In this study, we evaluated the magnitude and distribution of HBsAg, anti-HCV, and anti-HIV seroprevalence according to case subgroups in patients operated upon in the orthopedics and traumatology units.

Table 1. Number and percentage of patients by serological marker type

Serological marker type	The number and percentage of patients (n=2006)
HBsAg	64 (3.2)
Anti-HCV	13 (0.6)
Anti-HIV	0 (0.0)
HBsAg and anti-HCV	0 (0.0)
HBsAg or anti-HCV	77 (3.8)

HBsAg: Hepatitis B surface antigen, HCV: Hepatitis C virus, HIV: Human immunodeficiency virus.

Table 2. HBsAg and anti-HCV seroprevalence by gender

	HBsAg		Anti-HCV	
	Negative	Positive	Negative	Positive
Female	1,072	38	1,103	7
Male	870	26	890	6
Total	1,942	64	1,993	13

HBsAg: Hepatitis B surface antigen, HCV: Hepatitis C virus.

Table 3. HBsAg and anti-HCV seroprevalence by case subgroups

	HBsAg		Anti-HCV	
	Negative	Positive	Negative	Positive
Foot-ankle	26	0	26	0
General orthopedics	198	4	202	0
Trauma	607	35	631	11
Sports surgery-arthroscopy	360	6	365	1
Pediatric orthopedics	2	0	2	0
Hand-wrist	141	4	145	0
Arthroplasty	567	15	581	1
Spine	12	0	12	0
Oncology	29	0	29	0
Total	1942	64	1993	13

HBsAg: Hepatitis B surface antigen, HCV: Hepatitis C virus.

According to survey data, injuries occur in approximately 7% of surgeries,[5,6] and 87% of surgeons have a history of percutaneous injury throughout their careers.[7]

Routine screening for viral markers is recommended in developed countries.[3] Having knowledge about seropositivity before surgery allows for timely implementation of precautions such as protective goggles and waterproof gowns. In addition, healthcare workers can start prophylaxis immediately after any exposure. The role of universal measures in prevention is at the highest level, and prevention is always better than cure.[8,9] Surgical assistants have the highest risk in this regard that can be reduced with orientation training.[10]

In an epidemiological seroprevalence study conducted in Türkiye, HBsAg seropositivity was 2-7%.[11] In another study, seropositivity was 2.21% for HBsAg, 0.56% for anti-HCV, and 0.0008% for anti-HIV.[12] In a meta-analysis study, 34 European cities were evaluated and the prevalence of HBsAg was 0.1-5.6% and anti-HCV prevalence was 0.4-5.2% in this study.[13] In the study conducted by Pneumaticos et al.[14], 1,628 patients who were operated on in the orthopedics and traumatology service were evaluated and seropositivity was found in 66 (4.0%) of them. HCV positivity was found in 34 (2.0%) of them, HBV positivity in 30 (1.8%), and HIV positivity in 2 (0.1%) of them. In addition, this study did not evaluate which case groups had higher seropositivity.[14] In our study, we found 64 (3.2%) seropositivity for HBsAg and 13 (0.6%) for anti-HCV, and no patient was anti-HIV positive. In our study, while HBsAg seropositivity was higher, anti-HCV seropositivity was lower. In addition, anti-HIV seropositivity was not detected in our study. In addition to these, in our study, in addition to the seropositivity rates in all operated patients, the relationship between case groups and seropositivity was investigated.

According to the results, no significant relationship was found between gender and age with seropositivity ($p>0.05$). The seropositivity detected in patients who underwent surgery due to trauma was significantly higher than that detected in elective surgeries ($p<0.05$).

Study Limitations

The most important limitation of our study was its retrospective design and the relatively lower number of cases in some case subgroups. In addition, the lack of evaluation of demographic data such as socioeconomic status, education, ethnic identity, occupation, previous blood transfusion, and history of drugs is the shortcoming of our study. The most important advantage of our study is that the number of cases is higher than other studies in the literature, and it is the first study conducted in the field of orthopedics and traumatology by dividing case subgroups. Thus, we recommend that multicenter and meta-analysis studies be performed with more cases in the future.

CONCLUSION

In this study, HBsAg and anti-HCV seropositivity were higher in patients who underwent surgery for trauma than in those who underwent surgery for other reasons. It is thought that the reason for this is that the mobilization of trauma patients is more limited, surgical indications are clearer, and alternative treatment methods are more limited, making trauma patients more irrefutable for physicians. However, the physician is more flexible in deciding of surgery in case groups that underwent elective surgery, that is, in patients who underwent elective surgery. For this reason, if HBsAg, anti-HCV, or anti-HIV seropositivity is detected in elective surgeries left to the discretion of the physician, the physician may abandon the decision to perform surgery. This explains why seropositivity is lower in these case groups. To prevent this situation, we think that it is crucial to provide and use protective materials such as glasses, liquid-proof surgical clothing, and barrier gloves in addition to orientation training.

MAIN POINTS

- HBsAg and anti-HCV seropositivity were higher in patients who underwent surgery for trauma than in those who underwent surgery for other reasons.
- In addition to orientation training, it is very important to provide and use protective materials such as glasses, liquid-proof surgical clothing, and barrier.
- Taking protective measures and providing protective equipment can help reduce the concerns of surgeons and operating room personnel about contamination.

ETHICS

Ethics Committee Approval: This study was approved by the Ethics Committee of Aksaray University Training and Research Hospital (approval number: 2022/08-06, date: 21.04.2022).

Informed Consent: There is no information about the patient in the submitted manuscript.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: S.G., H.Y., E.A.S., Design: S.G., H.Y., E.A.S., Data Collection and/ or Processing: S.G., H.Y., E.A.S., Analysis and/ or Interpretation: S.G., H.Y., E.A.S., Literature Search: S.G., H.Y., E.A.S., Writing: S.G., H.Y., E.A.S.

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. Prüss-Üstün A, Rapiti E, Hutin Y. Sharps Injuries: Global Burden of Disease from Sharps Injuries to Health-care Workers, WHO Environmental Burden of Disease Series. Geneva: World Health Organization; 2003.
2. Albrecht E, Frascarolo P, Meystre-Agustoni G, Farron A, Gilliard N, Darling K, et al. An analysis of patients' understanding of 'routine' preoperative blood tests and HIV screening. Is no news really good news? HIV Med. 2012; 13(7): 439-43.
3. Ahmed R, Bhattacharya S. Universal screening versus universal precautions in the context of preoperative screening for HIV, HBV, HCV in India. Indian J Med Microbiol. 2013; 31(3): 219-25.
4. U.S. Public Health Service. Updated U.S. Public Health Service Guidelines for the Management of Occupational Exposures to HBV, HCV, and HIV and Recommendations for Postexposure Prophylaxis. MMWR Recomm Rep. 2001; 50(RR-11): 1-52.
5. Tokars JI, Chamberland ME, Schable CA, Culver DH, Jones M, McKibben PS, et al. A survey of occupational blood contact and HIV infection among

orthopedic surgeons. The American Academy of Orthopaedic Surgeons Serosurvey Study Committee. JAMA. 1992; 268(4): 489-94.

6. Tokars JI, Bell DM, Culver DH, Marcus R, Mendelson MH, Sloan EP, et al. Percutaneous injuries during surgical procedures. JAMA. 1992; 267(21): 2899-904.
7. Lowenfels AB, Wormser GP, Jain R. Frequency of puncture injuries in surgeons and estimated risk of HIV infection. Arch Surg. 1989; 124(11): 1284-6.
8. Gounden YP, Moodley J. Exposure to human immunodeficiency virus among healthcare workers in South Africa. Int J Gynaecol Obstet. 2000; 69(3): 265-70.
9. Chogle NL, Chogle MN, Divatia JV, Dasgupta D. Awareness of post-exposure prophylaxis guidelines against occupational exposure to HIV in a Mumbai hospital. Natl Med J India. 2002; 15(2): 69-72.
10. Lee JJ, Kok SH, Cheng SJ, Lin LD, Lin CP. Needlestick and sharps injuries among dental healthcare workers at a university hospital. J Formos Med Assoc. 2014; 113(4): 227-33.
11. Tozun N, Ozdogan O, Cakaloglu Y, Idilman R, Karasu Z, Akarca U, et al. Seroprevalence of hepatitis B and C virus infections and risk factors in Turkey: a fieldwork TURHEP study. Clin Microbiol Infect. 2015; 21(11): 1020-6.
12. Avcıküçük H, Süzük S, Kavak M. HbsAg, Anti-Hepatitis C Virus and Anti-HIV Seroprevalence Among Patients Admitted to Our Hospital between 2005 and 2013. Viral Hepat J. 2014; 20(3): 125-30.
13. Hahné SJ, Veldhuijzen IK, Wiessing L, Lim TA, Salminen M, Laar Mv. Infection with hepatitis B and C virus in Europe: a systematic review of prevalence and cost-effectiveness of screening. BMC Infect Dis. 2013; 13: 181.
14. Pneumaticos SG, Savvidou C, Tsiakalos A, Sipsas NV. Seroprevalence of HIV, HBV and HCV in orthopaedic patients at a tertiary hospital in Greece. European Journal of Orthopaedic Surgery & Traumatology. 2012; 22(1): 57-60.

RESEARCH ARTICLE

CYPRUS
JOURNAL OF MEDICAL SCIENCES

DOI: 10.4274/cjms.2023.2022-30
Cyprus J Med Sci 2023;8(3):220-228

Knowledge, Attitudes and Behaviors Regarding Occupational Risks and Standard Precautions Among Healthcare Workers in North Cyprus: A Descriptive Cross-sectional Study

Ahmet Körceğez[1], Özen Asut[2]

[1]Department of Internal Medicine, Famagusta General Hospital, Famagusta, North Cyprus
[2]Department of Public Health, Near East University Faculty of Medicine, Nicosia, North Cyprus

Abstract

BACKGROUND/AIMS: The occupational risks of healthcare workers (HCW) are well established. The aim of this study was to assess the knowledge, attitudes and behaviors of healthcare professionals regarding occupational risks and standard precautions.

MATERIAL AND METHODS: This cross-sectional study was conducted in three hospitals in North Cyprus, aiming to access HCW with a questionnaire developed by the researchers, with 3 knowledge, 9 perception and attitude, 22 behavior, and 10 health hazard items. The data were analyzed using SPSS18.0. Descriptive statistics with univariate and bivariate analyses were performed with significance level set as $p<0.05$.

RESULTS: The response rate was 80.7%, and 83% of the participants considered the institutional precautions to be unsatisfactory, while 57.5% described their work as very risky, 50.7% reported experiencing at least one sharps injury, and 37.1% reported exposure to chemicals during their professional activities. The nursing profession and night shift work were predictors of higher stress and risk perceptions and occupational injury. The vaccination rates of the recommended vaccines were low. The technician profession and working in the state institution were predictors of lower rates of hepatitis vaccination and the female gender for lower rates of tetanus vaccination. Only 18.4% of the participants reported regular mask use and 50.6% effective gloves use while contacting patients. Nurses followed by physicians exhibited better performance regarding personal protective equipment use. Compliance with standard precautions was unsatisfactory in general: responses manifested levels of correct knowledge as 40.5%, correct attitudes as 53.9%, and correct behaviors as 52.1%.

CONCLUSION: A definite need to develop educational and administrative interventions to improve compliance with standard precautions was established. The public health authorities were informed about the outputs.

Keywords: Healthcare workers, occupational risk and injury, standard precautions, personal protective equipment use, North Cyprus

INTRODUCTION

Healthcare workers (HCW) are all individuals engaged in work actions with the intent of improving the health of the people. Healthcare facilities around the world employ about 60 million workers. HCWs include medical doctors, nurses, laboratory technicians, pharmacists and providers of health management and supportive services such as drivers, cleaners and cooks.[1] HCWs are exposed to a broad variety of risks including biological, chemical, physical, ergonomic and psychosocial hazards; fire and explosion risks and electrical hazards.[2,3] Violence

To cite this article: Körceğez A, Asut Ö. Knowledge, Attitudes and Behaviors Regarding Occupational Risks and Standard Precautions Among Healthcare Workers in North Cyprus: A Descriptive Cross-sectional Study. Cyprus J Med Sci 2023;8(3):220-228

ORCID IDs of the authors: A.K. 0000-0002-3254-3347; Ö.A. 0000-0002-9604-4037.

Address for Correspondence: Ahmet Körceğez
E-mail: ahmetfikri2000@yahoo.com
ORCID ID: orcid.org/0000-0002-3254-3347

Received: 29.07.2022
Accepted: 24.01.2023

against health workers, burnout, and musculoskeletal disorders are also widespread. To their own health and safety, the occupational health problems of HCWs are closely related to patients and the community health.[1]

Infectious diseases in general pose a major threat to the health of HCWs globally. In a systematic review covering 10 years, increased risk from infectious diseases concentrated among specific professions including HCWs.[4] For example, measles infection was found to occur 13-14 times more frequently among health workers compared to the normal population. Consequently, immunization is one of the specific preventive measures for the health of HCWs. Currently, a routine vaccination program is recommended for all workers in healthcare facilities, while some vaccines are only suggested under certain circumstances. Influenza, chickenpox, measles-mumps-rubella (MMR), tetanus, and hepatitis B vaccines are recommended for all health professionals.[5,6] MMR vaccination is recommended for all HCWs in some European countries, USA, Canada, and Australia and is compulsory for all HCWs in Finland.[6] On the other hand, there is a consensus that typhoid, hepatitis A, meningococcus, and BCG vaccines should be provided for HCWs where the risk is high for these conditions.[6,7] Effective immunization programs have resulted in a decline in infectious diseases among HCWs recently.

Low adherence to standard precautions is one of the main reasons for the impact of infectious diseases on the health of HCWs. The primary reasons for low adherence include insufficient education, inadequate provision of protective equipment, and hazardous working conditions.[1,4,8] Standard precautions include both individual and community level measures. Pre- and in-service training of health professionals on workplace safety, warning signs, and other measures for increasing awareness are among basic preventive approaches. Another major preventive measure is the use of appropriate personal protective equipment (PPE). PPE is designed to protect workers from various work-related health hazards that may affect their health and safety.[4] The main forms of PPEs specifically for use by HCWs are masks, gloves, aprons, lead aprons, face shields, and goggles. However, knowledge and awareness of the risks and precautions do not necessarily result in compliance with preventive measures. In one study, although 97% of the participants were fully aware of the benefits of using PPEs, only 25% were actually in adherence with the standard precautions.[9]

The European Union program of 2010 (32/EU program) has introduced precautions for the prevention of cutting and piercing wounds among HCWs. The preventive measures recommended are education, escalating awareness, healthy and careful use and disposal of cutting and piercing equipment and continuous and effective use of PPEs.[10]

The most prominent risks for healthcare professionals in Türkiye have been identified as infections, exposure to radiation, anesthetics and chemicals, injuries, violence, exhausting and strenuous working conditions, high workload, and neuromusculoskeletal traumas.[11] Similar conditions are observed to be prevalent in North Cyprus as well although there is a lack of evidence-based research on the issue.[12]

The results of research on the assessment of the knowledge and behaviors of HCWs toward universal precautions have highlighted low levels of knowledge about control measures and poor adherence with the precautions.[13-16] Some studies have demonstrated the positive impact of policies like infection control programs, periodic training and infection reporting systems.[14]

The only study on the health and safety of HCWs in North Cyprus was published in 2021. The study illustrated the conditions in a university hospital in Kyrenia.[17] In this study, 57.5% of the HCWs had a satisfactory level of correct knowledge, 37.3% had satisfactory positive attitudes, and 30.9% had satisfactory practices toward standard precautions. Occupation was a predictor, and doctors were less likely to have satisfactory knowledge and practice compared with nurses. The findings revealed substandard adherence to standard precautions among HCWs.[17]

The objective of the current study was to assess the working conditions and health hazards of HCWs in addition to their knowledge levels, attitudes, and practices with respect to occupational risks and standard precautions.

MATERIALS AND METHODS

Study Design and Setting

This cross-sectional study was conducted among HCWs in Famagusta city of North Cyprus. The study setting included a state hospital and two private hospitals. A purposive sampling method was used, and the aim was to access all HCWs of the hospitals with a questionnaire. The time frame of the data collection period was 01.11.2016 to 31.05.2017.

The participants were divided into 4 groups for analysis purposes: Group 1-physicians, group 2-nurses, group 3-technicians and other professions, group 4-cleaning staff. Group 3 included laboratory, radiology and anesthesia technicians, physiotherapists, dietitians, security guards, and ambulance drivers.

The approval of the Ethics Committee of the Near East University and the permissions of the three hospital administrations were obtained. (Near East University Ethics Committee Report (approval number: 2016/40-330, date: 20.10.2016). Informed consent of the participants was obtained before the interview.

Data Collection Method and the Study Tool

The study tool was a questionnaire developed and administered face to face by the researchers. The questionnaire was designed after a thorough literature search of previous studies.[18,19]

The questionnaire consisted of 55 questions in five sections, including demographic and professional features, perceptions of occupational risks and diseases, history of occupational accidents and diseases, compliance with standard precautions, and PPE use. The first section including medical history consisted of 13 questions on the socio-demographics of the participants.

The perceptions of occupational risks and disease were covered by 5 questions, while the history of occupational diseases and accidents was investigated by 4 questions. Standard precautions section including knowledge, attitude and practice items consisted of 28 questions, of which 9 were on PPE use. Radiology technicians were questioned about the use of lead aprons, while doctors and nurses were questioned about their behaviors regarding invasive intervention practices with 5 additional questions.

The study tool included 3 knowledge: 5 perception: 4 attitude: 22 behavior items and 10 health hazard event items.

Statistical Analysis

The data were entered, cleaned and analyzed using IBM SPSS (Statistical Package for the Social Sciences) version 18.0 (SPSS Inc, Chicago, IL, USA). The total scores of the participants' knowledge, attitude, and behavior responses were divided into 2 categories as satisfactory and unsatisfactory. The ratios of the total correct responses were calculated for each item group. A satisfactory level of knowledge, attitude, and behavior was set as the answer choice representing the best practice on the issue related to the question.

Univariate analyses were conducted for descriptive statistics including means, frequency, and percentages to describe the characteristics of the study sample. Bivariate analyses were performed to calculate the relationships between the categorical variables regarding satisfactory levels of knowledge, attitude, and behavior using Pearson's chi-square test and Fisher's exact test. The significance level was set as $p<0.05$.

Terms and definitions were accepted in compliance with the literature.[18,20]

RESULTS

Of the 409 HCWs employed at the three hospitals, 330 completed the questionnaire, with a response rate of 80.7%.

Socio-economic and Professional Characteristics and Working Conditions of the Participants

The socio-economic and professional characteristics of the participants are presented in Table 1. Of the participants, 67.9% were female, 54.2% were under 40 years of age, and the distribution according to profession revealed 43.6% nurses, 22.2% physicians, 12.1% cleaning staff and 11.6% technicians. The mean age was 38.0±8.8, median: 38, minimum and maximum: 20-71. The data about other professions are presented in Table 1. Of the total participants, 30.5% were daily smokers, 7.3% were occasional smokers and 5.5% were former smokers. Notably, smoking rates were 36.8% among nurses and 26.0% among physicians.

Of all participants, 62.1% were employees of the public hospital, while 37.9% were working at the two private hospitals. The mean weekly working duration was 42 h per week. HCWs work more than 40 h per week comprised 36.0% of the total for the public hospital compared to 82.5% for the two private hospitals. The employees of the private hospitals worked significantly more hours weekly than those of the public hospital ($\chi^2=65.6$, $p<0.001$).

The analysis of the working conditions revealed that 53.8% worked night shifts and 45% had eight or more night shifts per month. Nurses comprised the professional group with the highest rate of night shifts of 69.3% because of the general employment system for nurses in North Cyprus.

Occupational Injuries and Diseases

The participants' history of occupational accidents and injuries is presented in Table 2.

About half of the participants (50.7%) had experienced accidents or were injured while working. Furthermore, 3.4% had experienced chemical

Table 1. The socio-economic and professional characteristics of healthcare workers participating in the study (Famagusta, 2017)

Socio-economic/professional features	n	%
Age (n=330)		
<40 years of age	179	54.2
≥40 years of age	151	45.8
Sex (n=330)		
Male	106	32.1
Female	224	67.9
Profession (n=330)		
Physician	73	22.2
Nurse	144	43.6
Laboratory technician	20	6.1
Radiology/surgery technician	18	5.5
Physiotherapist	5	1.5
Dietitian	2	0.6
Ambulance driver	9	2.7
Transporter	13	3.9
Caregiver	6	1.8
Cleaning staff	40	12.1
Workplace (n=330)		
State hospital	205	62.1
Private hospital 1	54	16.4
Private hospital 2	71	21.5

Table 2. History of occupational accidents and injuries experienced by the participants (Famagusta, 2017)

Injury history	n	%
Piercing injury during work (n=322)		
Never	154	47.8
Once	54	16.8
Occasionally	104	32.3
Frequently	5	1.6
Don't remember	5	1.6
Chemical splash (n=326)		
Never	205	62.9
Occasionally	111	34.0
Frequently	10	3.1
Chemical burns (n=319)		
Yes	11	3.4
Type of chemical burn (n=10)		
Chemical splash to eyes	4	40.0
Chemical exposure to oral mucosa	2	20.0
Chemical splash to face	3	30.0
The exposure of respiratory system to waste anesthetic gases	1	10.0
The status of PPE use during chemical burn accident (n=10)		
No PPE	9	90.0
Don't remember	1	10.0
Allergies during work (n=323)		
Latex glove allergy	72	22.3
Drug allergy	21	6.5
Other	10	3.1

PPE: Personal protective equipment.

burns, 90% of whom were without PPEs during the accidents. Of the total, 32.3% indicated occasionally experiencing sharp injuries.

Among the physicians and nurses, 65.6% stated that they had experienced injuries at least once while performing injections. The nurses reported the highest rate of experiencing chemical exposure with 47.5% and piercing injury with 66.2%. Frequencies of penetrating injury history among the occupation members were 66.2% for the nurses, 58.3% for the physicians, and 17.8% for the cleaning staff (significantly lower for the cleaning staff with $p<0.001$). A history of chemical exposure and sharp injury was highest among nurses.

Working Conditions

Significant differences were demonstrated between the night- and day-shift workers for some occupational conditions including skin health problems related to latex glove use (66.9% versus 47.9% respectively, $p=0.01$). The frequencies of sharps - related injuries and chemical exposure were also higher for those who had night-shift work compared to day-shift work (60.2% versus 39.3%, $p=0.01$ for sharps injuries and 43% versus 29% for chemical exposures).

Perceptions of the Participants Related to Their Working Conditions

Approximately 90% of the participants described their work as stressful (89.7%). Among the cleaning staff, a significantly lower proportion ($\chi^2=23.43$, $p<0.001$) described their work as stressful than the other groups. Regarding professional risks, 57.5% of the total participants perceived their working conditions as very risky and 36.0% as moderately risky. The perceptions of the nurses about their own professional risks were the highest with 69.9%.

On the other hand, the proportion of participants who described their work as high risk was significantly higher for night-shift workers (70.3%) compared to day-shift workers (41.5%) ($p<0.001$). Similarly, state hospital workers' risk perception of their job was significantly higher than private hospital workers (65.0% versus 45.1%, $\chi^2=15.87$, $p<0.001$).

Of the participants, only 17% perceived infection prevention measures at their workplace to be satisfactory. State hospital HCWs rated these measures as unsatisfactory with a rate of 98% and private hospital workers with 57.9%, where the difference was significant ($\chi^2=86.42$, $p<0.001$). Likewise, night-shift workers reported a significantly higher rate of unsatisfactory responses compared to day-shift workers in this respect (87.6% versus 69.2% respectively, $p<0.001$).

Knowledge on Infection Prevention Measures

The question about the duration of handwashing was responded correctly by 77.9% of the nurses, 70.0% of the cleaning staff, 63.9 % of doctors, and 60.6% of group 4. Only 24% of the participants responded satisfactorily to the question about hepatitis B's transmission and hepatitis C.

The results of the survey regarding safe handling and disposal of syringes indicated that only 16.7% of the doctors, 19.2% of the nurses, and 18% in total of the two groups provided the correct answers. Moreover, 83.3% of the physicians, 80.8% of the nurses, and 82% in total stated that re-capping of the needle sticks was the correct behavior, the difference between the two groups being non-significant ($p=0.782$).

Behaviors Regarding Infection Prevention Measures

Of the participants, 50.5% reported consistent handwashing before examining or caring for a patient, while 6.0% stated they never washed their hands before touching a patient. On the other hand, 81.8% indicated regular handwashing after examining or caring for a patient, while 0.6% stated they never washed their hands after contacting a patient.

Vaccination Status

Vaccination rates in general were low among the participants; 28% of the participants had received no vaccinations at all during their professional career. The behaviors of the participants regarding vaccinations are presented in Table 3.

Of the total participants, 83.9% were vaccinated for hepatitis B. The participants vaccinated for hepatitis B, tetanus, and influenza comprised 6.4% of the respondents. The frequency of hepatitis B-vaccinated professionals was 78.5% among the participants from the state hospital compared to 92.7% for the private hospitals, the difference being significant.

The behaviors of the participants regarding getting tested and vaccinated for some infections according to professions are presented in Table 4.

Hepatitis B vaccination status was found to be 98.6% among physicians, 79.8% among nurses, and 91.4% among the cleaning staff. Group 3 professionals, including technicians and other professionals, had a significantly lower rate with 71.2% (Table 4).

Only 17.4% of the participants reported being vaccinated at least once for influenza during their professional career. There was a significant difference between state hospital workers (21.5%) and private sector workers (10.8%) regarding influenza vaccination ($\chi^2=5.81$, $p=0.016$), but vaccination rates were unsatisfactory for both groups.

Table 3. The vaccination status of the participants (Famagusta, 2017)

Vaccination status	n	%
Tetanus vaccination in the last 5 years (n=326)	171	52.5
Hepatitis B lifelong vaccination (n=329)	276	83.9
Vaccinations during professional career (n=311)		
None	87	28.0
Hepatitis B	181	58.2
Tetanus	101	32.5
Influenza	50	16.1
Hepatitis A	2	0.6
Other (MMR, pneumococcus, measles, meningococcus)	8	2.5
Multiple vaccinations during professional career (n=311)		
Hepatitis B only	87	28.0
Hepatitis B + tetanus	59	19.0
Hepatitis B + influenza	13	4.2
Hepatitis B + tetanus + influenza	20	6.4
Tetanus only	18	5.8
Influenza only	13	4.2
Tetanus + influenza	4	1.3
Hepatitis A + hepatitis B	2	0.6

MMR: Measles-mumps-rubella.

Similarly, state hospital HCWs were vaccinated for tetanus during their professional career significantly more than private hospital workers (40.8% versus 25.0%) as well (χ^2=8.6, p=0.004). Of the participants, 52.5% had been vaccinated for tetanus in the last 5 years. Regarding genders, 46.1% of the female participants and 65.7% of the male participants reported being vaccinated for tetanus during the last five years, the difference being significant (χ^2=13.38, p<0.001). On the other hand, tetanus vaccination was highest among physicians with 54.2% and lowest among the cleaning staff with 18.2% (χ^2=17.7, p=0.007).

- Attitudes and behaviors regarding PPE use.

- The behaviors of the participants regarding the use of PPE in general are displayed in Table 5. The frequency of those wearing gloves regularly when contacting a patient was 48.6% and 95.8% of these reported they renewed the gloves for every patient. Conversely 2.8% of the total reported they never wore gloves in professional practice. There were no significant differences between HCWs 40 years of age and above and those 40 years of age below 40 years regarding mask and glove use attitudes and behaviors and experiencing sharps injuries. However, other precautions such as vaccinations for tetanus and hepatitis B were implemented significantly higher among professionals 40 years and over.

Of the total, only 18.4% reported regular and 23.5% frequent use of masks during close contact with the patients. Of the mask users, 52.9% thought that masks should be renewed for every patient, while 39.3% believed that changing a mask daily would be sufficient. Notably 20% of the participants never wore masks while in close contact with a patient.

Among radiology technicians, 72.7% responded that they rarely wore lead aprons while being exposed to X-rays, while the rest reported frequent use during exposure.

- The use of goggles among participants was low as 75.6% had never used goggles and 29.3% responded that goggles use is not needed at all.

The distribution of the attitudes and behaviors of the participants regarding PPE use regarding professions is demonstrated in Table 6.

Of the nurses, 100% expressed the opinion that gloves should be used during work consistently. The cleaning staff comprised the group with the lowest frequency regarding the necessity of glove use with 85.4%.

On the other hand, the behaviors of the participants differed from their attitudes: Only 55.6% of the nurses and 49.8% of the doctors indicated that they used gloves consistently when working with patients. Of those wearing gloves, 88.7% of the doctors and 71.0% of the nurses changed gloves for every patient.

The rates of regular mask use were 16% among nurses and 20% among physicians. The regular mask use rate was highest among the cleaning staff with 33%. Mask use was significantly higher among the cleaning staff compared to other professions (p<0.001).

General evaluation of the knowledge, attitude and behavior responses about compliance with standard precautions manifested that the level of correct knowledge was 40.5% in total, for attitudes 53.9% and behaviors (or practices) 52.1%. Vaccination status of the participants demonstrated that 28% had not been vaccinated at all by any vaccine after they started their professional career.

DISCUSSION

In this cross-sectional study, the knowledge, attitudes and behaviors of HCW on occupational health and standard precautions were investigated, along with their working conditions and occupational hazards. The results indicated that private hospital employees worked significantly more hours than state hospital workers. Nurses had the highest rates of health hazards. As a consequence, job risk perception among nurses was higher than that among other professional groups. While more than half of the total participants perceived their job as very risky, the rate of this perception was highest among nurses with 70%. The higher smoking rate among nurses of 36.8% may be attributed to all these unfavorable factors. The predictors of higher stress and risk perceptions were night shift work, the nursing profession, and working in the state hospital.

Most of the participants had been previously tested for hepatitis B and C by screening tests and the rates were highest for physicians, followed by nurses. Similarly, the frequency of hepatitis C screening was 69% in a study in Türkiye.[21]

Table 4. The distribution of the participants according to professions regarding their behaviors on being tested and vaccinated for some infections (Famagusta, 2017)

"S" status	Profession physician		Nurse		Group 3*		Cleaners		(%), χ^2	p
	n	%	n	%	n	%	n	%		
Tested for HIV in previous year (n=329)	29	39.7	46	32.2	27	40.3	16	34.8		0.083
Tested for hepatitis B&C (n=320)	69	97.2	126	88.1	44	66.7	37	86.0	21.88	<0.001
Hepatitis B infection status (n=318)										
Vaccinated	72	98.6	115	79.8	47	7 1.2	42	91.4		
Not infected, not vaccinated	1	1.4	23	16.0	13	19.7	2	4.3		
Infected and immunized	0	0	0	0	1	1.5	0	0		
Hepatitis B carrier	0	0	1	0.7	0	0	0	0		
No information	0	0	5	3.5	5	7.6	2	4.3		
Tetanus vaccine in the last 5 years (n=324)	42	57.5	66	47.5	38	57.6	25	54.3		0.462
PPD skin test (n=323)	39	54.2	64	45.4	23	34.8	8	18.2	17.7	0.007

*Group 3: Laboratory, radiology, anesthesia technician; physiotherapist, dietician, security guard, ambulance driver. HIV: Human immunodeficiency virus, PPD: Purified Protein Derivative.

Table 5. The behaviors of the participants regarding their use of personal protective equipment (Famagusta, 2017)		
Behavior		
Frequency of mask use (n=310)	**n**	**%**
Always	57	18.4
Frequently	73	23.5
Rarely	155	50.0
Never	25	8.1
Frequency of glove use (n=311)		
Always	151	48.6
Frequently	104	33.4
Rarely	46	14.8
Never	10	3.2
Frequency of apron use (n=301)		
Always	64	21.3
Frequently	33	11.0
Rarely	98	32.5
Never	106	35.2
Frequency of use of goggles (n=283)		
Always	13	4.6
Frequently	9	3.2
Rarely	47	16.6
Never	214	75.6
Frequency of lead apron use (n=11)*		
Always	0	0
Frequently	3	27.3
Rarely	8	72.7
Never	0	0
*Only radiology technicians.		

Table 6. The attitudes and behaviors of the participants regarding the use of PPEs according to professions (Famagusta, 2017)										
Attitude and behavior	**Profession**									
	Physician		**Nurse**		**Group 3***		**Cleaner**		**χ^2**	**p**
	n	**%**	**n**	**%**	**n**	**%**	**n**	**%**		
Attitude regarding glove use (n=313)										
Should always be worn during working hours	69	97.2	135	100	61	92.4	35	85.4		
No need to wear gloves all the time	2	2.8	0	0.0	5	7.6	6	14.6		
Attitude regarding mask use (n=308)									7.88	0.049
Should always be used during working hours	30	42.9	81	61.8	31	47.7	21	50.0		
No need to use all the time	40	57.1	50	38.2	34	52.3	21	50.0		
Behavior regarding glove use (n=306)									5.33	0.149
Uses all working hours	29	49.8	75	55.6	28	42.4	19	48.7		
Does not use regularly	42	59.2	60	44.4	33	57.6	20	51.3		
Mask use behavior (n=310)									8.05	0.045
Uses all working hours	14	20.0	22	16.3	8	12.1	13	33.3		
Glove use when touching a patient (n=316)									13.53	0.004
Regularly	25	35.2	78	54.9	26	39.4	24	64.9		
Mask use when contacting a patient (n=320)									26.79	<0.001
Regularly	16	21.9	22	15.3	10	15.4	20	52.6		
Changing gloves per patient (n=193)**										0.379
Always	55	88.7	93	71.0						
Occasionally	7	11.3	38	29.0						
Handwashing duration (n=314)									8.15	0.043
Correct	46	63.9	106	77.9	40	60.6	28	70.0		
Incorrect	26	36.1	30	22.1	26	39.4	12	30.0		
*Group 3: Laboratory, radiology, anesthesia technicians; physiotherapist, dietitian, security guard, ambulance driver. **Only physicians and nurses. PPE: personal protective equipment.										

On the other hand, vaccination rates in general were low in the current study, as 28% of the respondents had received no vaccines at all after starting the profession. Hepatitis B vaccination had the highest lifetime rate among other vaccines, although 12% were still not vaccinated for hepatitis B at all. Hospital type and profession were predictors of hepatitis B vaccination, with significantly lower rates for state hospital workers and the group including laboratory technicians.

Two studies among HCWs investigating hepatitis B vaccination rates reported these rates as 83.1% and 50.4% respectively.[7,21] In the review of Haviari et al.[6], the rates of hepatitis B vaccination were reported in the range of 63-95%. The review also reported pertussis immunization rates of 14-73% and MMR rates of 87-97%. However, pertussis and MMR vaccination rates in our study were very low, contrary to international findings.

Although influenza vaccination has been recommended for all health professionals in many countries, only 16.1% of the participants in our study reported receiving the vaccine at least once after starting their profession. Hospital type was a predictor for influenza vaccination with significantly higher rates for state hospital workers. Likewise HCWs who were vaccinated for influenza in the previous year comprised only 4.3% in a study in Türkiye.[7]

In a meta-analysis published in 2011, the vaccination rates for seasonal influenza among health professionals were 7.5-63.0%.[5] Influenza vaccination rates among HCWs were determined to be 60% in a study conducted in South Korea[22] and 51.4% among primary HCWs in Jerusalem.[23] In the review by Haviari et al.[6], influenza vaccination ranged from 15 to 90%. To conclude, influenza vaccination rates among HCWs exhibit a broad variation depending on the national and local circumstances.[6] The vaccination status established in this study is far from satisfactory, except for a relatively higher rate for hepatitis B. Our study points to the urgent need for interventions to escalate the vaccination rates in North Cyprus.

Of the total, slightly more than half of the participants responded that they had experienced sharps injuries at least once during their professional life, with the highest rate being among nurses with 66.2%. The nursing profession, night shift work, and working in a private hospital were predictors of more injuries and accidents. Consistent with our findings, other studies have shown that nurses were exposed to penetrating and cutting and other work-related injuries more frequently than other HCWs.[24,25] The frequencies observed among nurses ranged from 61% to 89%, as demonstrated by a number of studies.[21,24,26,27]

The increase in the frequency of injuries during night shift work has also been reported.[28] Likewise, injuries mostly occur during the later periods of prolonged working hours and among HCWs with less professional experience.[26,29]

Regarding injury type, 15.7% of all injuries were found to be due to invasive interventions in one study.[30] A study from Serbia reported that 60.6% of HCWs had experienced at least one needle stick injury during their professional practice. Nurses had a higher risk of needle stick injuries than doctors. Among the factors contributing to needle stick injuries, recapping needles and decontamination/cleaning instruments after surgery were more frequent among nurses, whereas use of a needle before an intervention was more common among doctors.[27]

In our study, the frequency of chemical spill accidents at least once was 37.1%, rising up to 43% during night shifts, while the frequency of chemical burns was 3.4%. The comparison of the professions showed that 48.5% of the nurses, 35% of the physicians, and 23% of the other groups had experienced chemical spills. In a study conducted in Cameroon, 36.7% of HCWs mentioned they had been exposed to blood or body fluids in the last three months, with frequencies of 43% among nurses, 16.4% among physicians, and 5.5% among laboratory workers. On the other hand, other studies reported more frequent injuries among physicians.[31,32] Distribution according to injury type in another study exhibited frequencies of exposures as blood or body fluid spills 60.3%, needle stick injury 28.7% and cuts 10.9%.[33]

Skin health problems were experienced by 58.3% of the participants and 22% reported latex glove allergies in the current study. The reasons for refraining from glove use were examined in a study where 65.3% of the participants indicated allergic conditions as the cause of their reluctance to wear gloves. The level of latex allergy was stated as 11.8%[7] and 57%[34] in two different studies.

PPE use behaviors were investigated in depth in the current study. The attitudes of the nurses were better than those of the other professions regarding PPE use. The rates of satisfactory attitudes of the nurses concerning regular mask and glove use were high; all of the nurses shared the opinion that gloves should be used consistently during work hours. However, their behaviors were not in compliance with their attitudes. Similarly although 97% of the participants were fully aware of the benefits of using PPE, only 25% were actually practicing according to the safety rules in one study.[9]

The regular mask use rate was significantly higher among cleaning staff (33.3%) compared with physicians (22%) and nurses (16%). The low level of mask use among physicians and nurses remains a matter of concern. Glove use when contacting a patient was significantly lower among physicians with 35%, compared to 55% for nurses and 64% for cleaners. Regular glove use was highest among nurses. An important point to be considered in this respect is that the survey was conducted before the COVID-19 pandemic.

The correct response rates to the knowledge questions were not satisfactory since the expectation is that nurses and doctors will provide mostly correct answers. These included questions on the sufficient duration of handwashing, the route of the transmission of hepatitis B and C, and compliance with safe injection and disposal criteria. The results showed no significant differences between doctors and nurses in this regard. In a previous study conducted in Northern Cyprus, 57.5% of the HCWs had a satisfactory level of correct knowledge, 37.3% had satisfactory positive attitudes, and 30.9% had satisfactory practice toward standard precautions. Occupation was a predictor, and doctors were less likely to have satisfactory knowledge and practice compared with nurses.[17]

Study Limitations

One of the limitations of the study is that the survey was based on a convenience sample and hence, was not representative of all the health professionals in the country. Thus our study findings have limited generalizability. Also, the sample population was small and an interviewer-administered questionnaire may have relatively lower reliability compared to more objective measurements. The study was done before the start of the COVID-19 pandemic and hence the situation

might have changed regarding the use of some PPEs, specifically mask and glove use.

CONCLUSION

According to the results of this study, most participants had substandard levels of knowledge, attitudes, and behaviors toward standard precautions. The study results point to the need for educational programs on occupational health and safety precautions.

HCW have high risks of infection and other occupational diseases and accidents.

1. A country-wide comprehensive program for HCWs is a prerequisite for any progress on this issue.

2. In this context, pre- and periodic examinations and training programs unique to each occupational group on standard precautions should be mandatory for all HCWs and controlled by the health authority.

3. Future research should aim to investigate a more comprehensive sample of HCWs for a better view of the situation.

MAIN POINTS

- The predictors of higher stress and risk perceptions were night shift work, the nursing profession, and working in the state hospital.
- The nursing profession, night shift work, and working in a private hospital were predictors of higher rates of injuries and accidents.
- Profession and hospital type was predictors of hepatitis B vaccination, with significantly lower rates for state hospital workers and the group including laboratory technicians.
- Hospital type was a predictor of influenza vaccination with significantly higher rates for state hospital workers and the male gender for higher rates for tetanus vaccination.

ETHICS

Ethics Committee Approval: The approval of the Ethics Committee of the Near East University and the permissions of the three hospital administrations were obtained. (Near East University Ethics Committee Report (approval number: 2016/40-330, date: 20.10.2016).

Informed Consent: Informed consent of the participants was obtained before the interview.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: A.K., Ö.A., Design: A.K., Ö.A., Data Collection and/or Processing: A.K., Ö.A., Analysis and/or Interpretation: A.K., Ö.A., Literature Search: A.K., Ö.A., Writing: A.K., Ö.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. World Health Organization. Health worker occupational health. Published 2016. Available at: http://www.who.int/occupational_health/topics/hcworkers/en/ Last accessed 14 Jul 2019.
2. World Health Organization. WHO calls for healthy, safe and decent working conditions for all health workers amidst COVID-19 pandemic. 28 April 2020. Available at: who.int/news/item/28-02-2020-who-calls-for-healthy-safe-and-decent-working-conditions-for-all-healthworkersamidstCOVID-19-pandemic
3. Asmr Y, Beza L, Engida H, Bekelcho T, Tsegaye N, Aschale Y. Assessment of Knowledge and Practices of Standard Precaution against Blood Borne Pathogens among Doctors and Nurses at Adult Emergency Room in Addis Ababa, Ethiopia. Emerg Med Int. 2019; 2019: 2926415.
4. Haagsma JA, Tariq L, Heederik DJ, Havelaar AH. Infectious disease risks associated with occupational exposure: a systematic review of the literature. Occup Environ Med. 2012; 69(2): 140-6.
5. Aguilar-Díaz Fdel C, Jiménez-Corona ME, Ponce-de-León-Rosales S. Influenza vaccine and healthcare workers. Arch Med Res. 2011; 42(8): 652-7.
6. Haviari S, Bénet T, Saadatian-Elahi M, André P, Loulergue P, Vanhems P. Vaccination of healthcare workers: A review. Hum Vaccin Immunother. 2015; 11(11): 2522-37.
7. Sari T, Temocin F, Kose H. Attitudes of healthcare workers towards influenza vaccine. Klimik Dergisi. 2017; 30(2): 59-63.
8. Porto JS, Marziale MH. Reasons and consequences of low adherence to standard precautions by the nursing team. Rev Gaucha Enferm. 2016; 37(2): e57395. English, Portuguese. Erratum in: Rev Gaucha Enferm. 2016; 37(2): e57395x.
9. Eljedi A. Prevalence and response to occupational hazards among nursing students in Gaza Strip, Palestine: The role of personal protective equipment and safety regulations. Public Health Research. 2015; 5(1): 32-8.
10. De Carli G, Abiteboul D, Puro V. The importance of implementing safe sharps practices in the laboratory setting in Europe. Biochem Med (Zagreb). 2014; 24(1): 45-56.
11. Fisek G. Saglik personeli sagligi. (Health professionals' health.) Saglikta Bulusma Noktasi Calisan Guvenligi, Makaleler. 2011. [Accessed 15 June 2019]. Available at: https://gurhan.fisek.net/saglik-personelinin-sagligi (Turkish).
12. Isik IN, Atasoylu E. Occupational safety and health in North Cyprus: Evaluation of risk assessment. Safety Science. 2017; 94: 17-25.
13. Hosoglu S, Akalin S, Sunbul M, Otkun M, Ozturk R; Occupational Infections Study Group. Healthcare workers' compliance with universal precautions in Turkey. Med Hypotheses. 2011; 77(6): 1079-82.
14. Giard M, Laprugne-Garcia E, Caillat-Vallet E, Russell I, Verjat-Trannoy D, Ertzscheid MA, et al. Compliance with standard precautions: Results of a French national audit. Am J Infect Control. 2016; 44(1): 8-13.
15. Stein AD, Makarawo TP, Ahmad MF. A survey of doctors' and nurses' knowledge, attitudes and compliance with infection control guidelines in Birmingham teaching hospitals. J Hosp Infect. 2003; 54(1): 68-73.
16. Kuwabara T, Chayama K, Tanaka S, Oka S, Hiyama T, Yoshihara M. Compliance with standard precautions among gastrointestinal endoscopists and endoscopy nurses in Japan. Am J Infect Control. 2012; 40(1): 80.
17. Abuduxike G, Acar Vaizoglu S, Asut O, Cali S. An Assessment of the Knowledge, Attitude, and Practice Toward Standard Precautions Among Health Workers From a Hospital in Northern Cyprus. Saf Health Work. 2021; 12(1): 66-73.
18. Cutter J, Jordan S. Inter-professional differences in compliance with standard precautions in operating theatres: a multi-site, mixed methods study. Int J Nurs Stud. 2012; 49(8): 953-68.

19. Health Management Toolkit Reference 2.0. Health questionnaire. (Accessed: October 2016). Available at: https://www.ceca.co.uk/wp-content/uploads/legacy-media/91380/section_2_-_health_questionnaire.pdf

20. Centers for Disease Control and Prevention. Standard precautions. (Accessed: 18 Nov 2021). www.cdc.gov. June 18, 2018.

21. Kuruuzum Z, Elmali Z, Gunay S, Gunduz S, Yapan Z. Occupational injuries of health professionals caused by blood and body fluids. Mikrobiyoloji Bulteni. 2017; 42 :61-9.

22. Yoon HJ, Lim J, Choi B, Kim J, Kim J, Kim C, et al. Vaccination rates and related factors among health care workers in South Korea, 2009. Am J Infect Control. 2013; 41(8): 753-4.

23. Abramson ZH, Levi O. Influenza vaccination among primary healthcare workers. Vaccine. 2008; 26(20): 2482-9.

24. Tarantola A, Abiteboul D, Rachline A. Infection risks following accidental exposure to blood or body fluids in health care workers: a review of pathogens transmitted in published cases. Am J Infect Control. 2006; 34(6): 367-75.

25. Martin S, Larson E. Chemotherapy-handling practices of outpatient and office-based oncology nurses. Oncol Nurs Forum. 2003; 30(4): 575-81.

26. Martins A, Coelho AC, Vieira M, Matos M, Pinto ML. Age and years in practice as factors associated with needlestick and sharps injuries among health care workers in a Portuguese hospital. Accid Anal Prev. 2012; 47: 11-5.

27. Marković-Denić L, Branković M, Maksimović N, Jovanović B, Petrović I, Simić M, et al. Occupational exposures to blood and body fluids among health care workers at university hospitals. Srp Arh Celok Lek. 2013; 141(11-12): 789-93.

28. Samancioglu S, Unlu D, Akyol DA. Assessment of piercing injuries among intensive care nurses. Journal of Nursolog. 2013; 16(1): 43-9.

29. Moro PL, Moore A, Balcacer P, Montero A, Diaz D, Gómez V, et al. Epidemiology of needlesticks and other sharps injuries and injection safety practices in the Dominican Republic. Am J Infect Control. 2007; 35(8): 552-9.

30. McCormick RD, Maki DG. Epidemiology of needle-stick injuries in hospital personnel. Am J Med. 1981; 70(4): 928-32.

31. Wicker S, Jung J, Allwinn R, Gottschalk R, Rabenau HF. Prevalence and prevention of needlestick injuries among health care workers in a German university hospital. Int Arch Occup Environ Health. 2008; 81(3): 347-54.

32. Foster TM, Lee MG, McGaw CD, Frankson MA. Prevalence of needlestick injuries and other high risk exposures among healthcare workers in Jamaica. West Indian Med J. 2010; 59(2): 153-8.

33. Nouetchognou JS, Ateudjieu J, Jemea B, Mbanya D. Accidental exposures to blood and body fluids among health care workers in a Referral Hospital of Cameroon. BMC Res Notes. 2016; 9: 94.

34. Buss ZS, Fröde TS. Latex allergen sensitization and risk factors due to glove use by health care workers at public health units in Florianopolis, Brazil. J Investig Allergol Clin Immunol. 2007; 17(1): 27-33.

RESEARCH ARTICLE

CYPRUS
JOURNAL OF MEDICAL SCIENCES

DOI: 10.4274/cjms.2021.3347
Cyprus J Med Sci 2023;8(3):229-233

How does the Exponential Increase in Rocuronium Dose Effect the Train of Four Parameters in Rats Reversed with Sugammadex? An Animal Model

Halil Cihan Köse[1], Selin Güven Köse[1], Serdar Onur Aydın[2], Tamer Kuzucuoğlu[3], Ömer Taşargöl[4], Serkan Tulgar[5]

[1]Clinic of Anesthesiology and Pain Medicine, University of Health Sciences Türkiye, Dışkapı Yıldırım Beyazıt Training and Research Hospital, Ankara, Türkiye
[2]Clinic of Neurosurgery, University of Health Sciences Türkiye, İstanbul University, Ümraniye Training and Research Hospital, İstanbul, Türkiye
[3]Clinic of Anesthesiology, University of Health Sciences Türkiye, Kartal Dr. Lütfi Kırdar City Hospital, İstanbul, Türkiye
[4]Clinic of Anesthesiology and Reanimation, Dr. Burhan Nalbantoğlu State Hospital, Nicosia, North Cyprus
[5]Department of Anesthesiology, Maltepe University Faculty of Medicine, İstanbul, Türkiye

Abstract

BACKGROUND/AIMS: Sugammadex is a gamma cyclodextrin structured agent used for reversing the effect of steroidal neuromuscular blocking (NMB) agents. The first aim of this study was to evaluate the dose of rocuronium required to re-establish NMB when administered 2 min after its reversal with sugammadex in rats. Also, to monitor the onset times and durations of NMB achieved by variable doses of rocuronium after reversal with sugammadex.

MATERIALS AND METHODS: Thirty-five Sprague-Dawley rats were randomly divided into groups including control and four experimental groups. The control group was designed to determine the onset time and duration of NMB induced by 1.2 mg/kg rocuronium. In the control group, no sugammadex was applied. In the experimental groups, rocuronium (1.2 mg/kg) was reversed with sugammadex (4 mg/kg). Subsequently, experimental groups were administered various doses of rocuronium. Groups were named according to the rocuronium dose administered (group 2.4, group 3.6, group 4.8 and group 6.0). Rats in all groups were monitored with train of four.

RESULTS: In group 2.4, rocuronium did not ensure NMB. In group 3.6, NMB occurredin only 3 rats. All rats in groups 4.8 and 6.0 achieved complete NMB. There was no statistically significant difference in the onset time of NMB in 4.8 and 6.0 groups ($p<0.05$). The mean duration of NMB in the experimental groups was significantly shorter than that in the control group ($p<0.01$).

CONCLUSION: Sufficient muscle relaxation and intubation conditions could be achieved with 3.6 mg/kg, 4.8 mg/kg, and 6.0 mg/kg doses of rocuronium as short as 2 min after sugammadex.

Keywords: Sugammadex, rocuronium, neuromuscular block

To cite this article: Köse HC, Güven Köse S, Aydın SO, Kuzucuoğlu T, Taşargöl Ö, Tulgar S. How does the Exponential Increase in Rocuronium Dose Effect the Train of Four Parameters in Rats Reversed with Sugammadex? An Animal Model. Cyprus J Med Sci 2023;8(3):229-233

ORCID IDs of the authors: H.C.K. 0000-0003-1550-348X; S.G.K. 0000-0003-4293-7814; S.O.A. 0000-0002-6698-4685; T.K. 0000-0003-4416-4181; Ö.T. 0000-0003-1408-5503; S.T. 0000-0003-1996-7505.

Address for Correspondence: Halil Cihan Köse
E-mail: halilcihankose@hotmail.com
ORCID ID: orcid.org/0000-0003-1550-348X

Received: 18.12.2020
Accepted: 24.01.2021

INTRODUCTION

Sugammadex is a gamma cyclodextrin structured agent used for reversing the effect of steroidal neuromuscular blocking (NMB) agents such as rocuronium and vecuronium via encapsulation.[1] In clinical practice, sugammadex is widely used to reverse rocuronium-induced NMB in postoperative care and emergency medicine.

Reoperation within the early postoperative period and re-intubation due to respiratory complications or allergic reactions are undesired situations that require NMB.[2] Theoretically, agents other than steroid-structured NMB should be used to re-establish NMB after reversal with sugammadex.[3] However, rocuronium is an alternative choice because of its rapid onset time and minimal hemodynamic changes even at high doses.[4] There are several case reports and studies on rocuronium being reused after reversal with sugammadex.[5-8] In these studies, there is no data on the onset time or duration of NMB with rocuronium when it is usedto re-establish NMB after administration of sugammadex. The aim of this experimental animal study was to evaluate the dose of rocuronium required to re-establish NMB when administered 2 min after its reversal with sugammadex. Secondary end points of this study were to monitor the onset times and durations of NMB achieved by variable doses of rocuronium after reversal with sugammadex.

MATERIALS AND METHODS

Study Subjects and Study Design

This experimental, randomized, controlled, and blinded animal study was approved by the Yeditepe University Local Animal Studies Ethical Board (approval number: 478, date: 19.08.2015). All invasive procedures, anesthesia, animal care, etc. were conducted in accordance with international guidelines on experimental animal studies.[9]

Thirty-five female Sprague-Dawley rats weighing 180-300 gr were equally randomized into four experimental and one control groups. Rats were maintained at a temperature (22-24 ºC) and humidity-controlled environment with free access to food and water. Sugammadex (Bridion; 200 mg/2 mL, Schering-Plough, Türkiye) and rocuronium (esmeron; 50 mg/5 mL Merck Sharp Dohme Ilac, Türkiye) were the agents used in this study.

Anesthesia and procedural preparation: Following intraperitoneal ketamine administration (60 mg/kg), intravenous access was achieved using a 20-22 G cannula in the tail lateral vein. Rats were secured on the dissection tray supinely, and 1 L/m O_2 was administered. Following a 0.5 cm incision on the midline of the neck, surgical dissectors were used to locate the trachea and place a 18 G cannula as a tracheostomy (Figure 1a, b). Thereafter, 1 mL of serum physiology was administered to maintain hemodynamic stability due to possible blood loss.

The effect of NMB was monitored using train of four (TOF) (TOF-WATCH S, Oragon; Dublin, Ireland). The TOF stimulating part was placed using platinum needles neighboring the sciatic nerve and its receiving part was placed in a pocket formed with surgical scissors between the gastrocnemius muscle and skin (Figure 1c). Stimulation with 2 Hz 0.2 ms was administered for 1.5 seconds every 15 seconds. Supramaximal current was determined to be T1/T4: 1.0 for the mentioned muscle groups.

Intravenous rocuronium (1.2 mg/kg) was administered to all rats with TOF measurements taken every 15 seconds. Rats were placed on 850 NEMI Scientific mechanical ventilators (respiratuar rate: 80-100, tidal volume 10 mL/kg) when their respiratory effort was lost. The control group was designed to determine the onset time and duration of NMB induced by 1.2 mg/kg rocuronium. In the control group, no sugammadex was applied.

In all experimental groups, rocuronium (1,2 mg/kg) was applied, then time until TOF <0.2 was recorded as *t1*. Sugammadex at a dose of

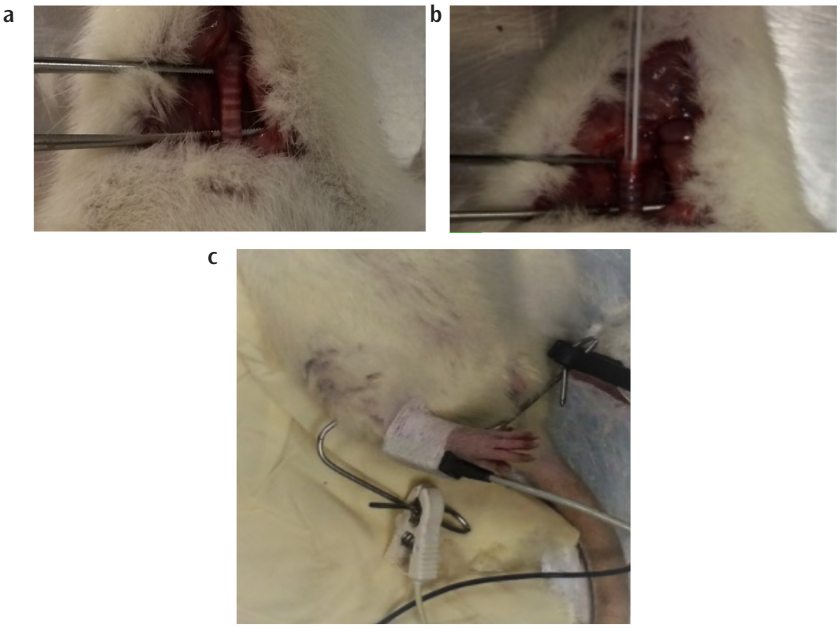


Figure 1. Steps of our study

4 mg/kg was applied when TOF <0.2. After that, the time between the administration of sugammadex and TOF >0.9 was observed and recorded as *t2*. Two minutes after TOF >0.9, groups were administered various doses of rocuronium. Groups were named according to the rocuronium dose performed (group 2.4, group 3.6, group 4.8 and group 6.0). After second doses of rocuronium were applied in experimental groups, the time until TOF <0.2 was defined as *t3*. The duration of action of rocuronium applied to the experimental groups for the second time and the duration of action of rocuronium applied to the control group was defined as *t4*. All steps of our study have been demonstrated in Figure 1.

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics 16 (IBM SPSS, Türkiye). Kolmogorov-Smirnov test was used to determine the normal distribution. One-Way ANOVA with post-hoc Tukey HSD was used to compare the groups. Statistical significance was set as $p<0.05$.

RESULTS

The average age of rats was 137.70±4.06 (133-145 days) and average weight was 187.24±15.43 Gr (171-208). There was no difference between the groups with regard to age, weight, basal body temperature, or basal respiratory weight ($p>0.05$).

There was no statistically significant difference between the groups with regards to *t1* and *t2*. NMB was achieved in no rats in group 2.4 and only 3 rats in group 3.6, whereas all rats in groups 4.8 and 6.0 achieved complete NMB. When groups 3.6, 4.8, and 6.0 were compared according to *dt3*, no statistically significant difference was found. While a statistically significant difference was found between the experimental and control groups for *t4*, there was no difference between the experimental groups (Table 1).

The relationship between time and mean TOF values after the second dose of rocuronium the following sugammadex administration is shown in Figure 2. Comparison of *t4* between groups is shown with box plot in Figure 3 and Table 2.

DISCUSSION

This experimental study demonstrated that increasing dosages of rocuronium can be used for the re-establishment of NMB after the reversal of rocuronium-induced NMB with sugammadex. However, re-used rocuronium has a shorter duration of action when it is performed after sugammadex administration.

Rocuronium is the most commonly used steroid-structured NMB agent. The use of steroidal neuromuscular agents for general anesthesia after the reversal of NMB with sugammadex is controversial. If NMB is required after routine dosage of rocuronium (0.6 mg/kg) has been reversed by 4 mg/kg of sugammadex, 1.2 mg/kg of rocuronium or 0.6 mg/kg of rocuronium can be applied at the 5th minute or 4th hour respectively.[3]

The timing and time to the effect of recurrent doses of rocuronium are open to debate. In a case report, 0.6 mg/kg of rocuronium was

Table 1. Comparison of *t1, t2, t3, t4* times according to groups and number of curarized rats per group

	Group 2.4	Group 3.6	Group 4.8	Group 6	Control group	p
t1 (sec.)	77.14±21.38	74.3±22.3	71.4±25.4	77.1±21.4	68.6±18.6	0.935
t2 (sec.)	68.57±19.51	71.4±25.4	88.6±19.5	80.0±28.3	-	0.395
t3 (sec.)	-	145.0±26.9	120.0±23.1	100.0±24.6	-	0.080
Curarized rats (n)	0/7	3/7	7/7	7/7	-	-
t4 (sec.)	-	310.0±57.7	311.4±33.8	360.0±86.4	514.3±53.8	0.001

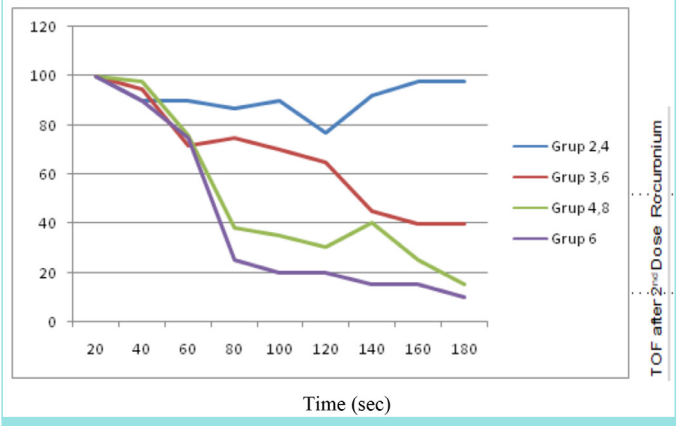


Figure 2. Relationship between time and TOF values after the second dose of rocuronium following sugammadex administration.

TOF: Train of four.

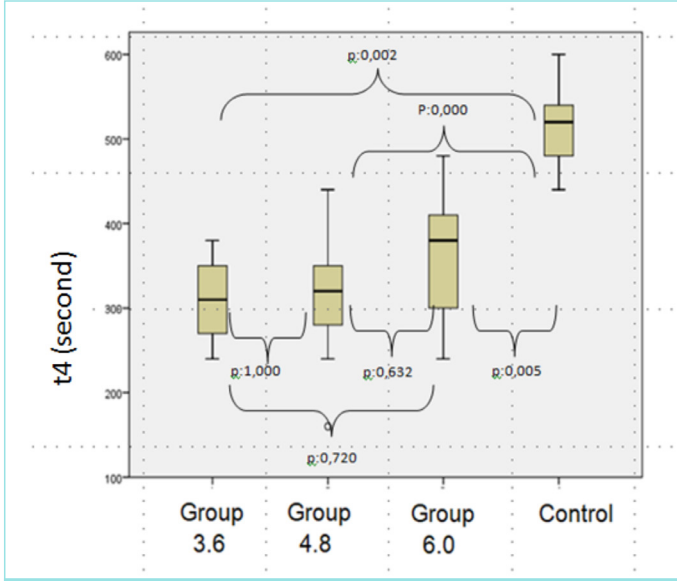


Figure 3. *t4* demonstrated as box plot.

Table 2. Comparison of t4 between groups					
	Control, (n=7)	Group 3.6, (n=4)	Group 4.8, (n=7)	Group 6.0, (n=7)	p
Mean ± SD	514.29±53.80	310.00±57.73	311.43±33.76	360.00±86.41	0.001*
SD: Standard deviation.					

reversed by 4 mg/kg of sugammadex. Emergency resurgery was required 30 min later, and adequate NMB was achieved at the 6th minute following 2 mg/kg of rocuronium.[10] In another case report, 0.6 mg/kg of rocuronium led to adequate NMB for intubation in 102 s following the reversal of rocuronium with 2 mg/kg sugammadex 6 h previously.[8] The authors reported that as the half-life of sugammadex is 2 h, a normal dose of rocuronium (0.6 mg/kg) would be adequate after 3 half lives or more had passed.[8] Case reports have reported varying times for administration of rocuronium following NMB reversal with sugammadex, with even more variation for dosages of 1.2 mg/kg and 3.4 mg/kg.[2,11,12] Although our study supports the hypotheses suggested by these case report examples, our goal is not only to determine the rocuronium dose after sugammadex in rats but also to project the rocuronium doses in humans.

In a study by de Boer et al.[13], body distribution and drug pharmacodynamics and pharmacokinetics were used to determine the dosage of sugammadex required to reverse an initial dose of 0.6 mg/kg rocuronium and the required repeat dose of rocuronium to achieve NMB after sugammadex. The investigators determined that adequate conditions for intubation would be achieved with 1 mg/kg rocuronium for 2 mg/kg sugammadex, 1.5 mg/kg rocuronium for 4 mg/kg sugammadex, and 2.25 mg/kg rocuronium for 8 mg/kg sugammadex. However, these findings do not correlate with previous case reports, and the onset time of rocuronium was not specified in this dose determination study.

Cammu et al.[5] reported a pilot study where the effect of 1.2 mg/kg of rocuronium following 4 mg/kg sugammadex was evaluated in 16 healthy volunteers. 1.2 mg/kg rocuronium was applied at different times after reversal of NMB and their time to reach T1: 0% and TOF rate 0.9 were determined. They were grouped according to the time when rocuronium was performed after sugammadex [5 min (n=6), 5-25 mins (n=6) and after 25 min (n=5)]. Average time to T1: 0% and time to TOF: 0.9 was 3.06 m and 25.3 m for the 5 m group, 3.09 m and 24.8 m for the 5-25 m group, and 1.73 m and 38.2 m for the over 25 m group, respectively. In a later group, NMB commencement in volunteers was found to be significantly shorter. The study reported that the differing time of effect for rocuronium after sugammadex was not predictable in all patients. Therefore, the authors concluded that rocuronium usage after reversal with sugammadex was not a safe and feasible option.[5]

To our knowledge, there are no animal studies regarding the re-use of rocuronium after sugammadex, therefore, our study is the first in the literature on this matter. When published case reports and volunteer-based studies are taken into consideration, the literature generally reports re-use of rocuronium 5 minutes after sugammadex, more often than not 30 minutes after.[8,11,12,14] However, a recently published systematic review reported that allergic reactions and respiratory complications occur within the first 3 min after sugammadex administration.[15] Instead of evaluating redose of rocuronium after 5 min, we therefore decided to evaluate the effect of various doses of rocuronium 2 min after reversal with sugammadex.

Some unforeseen complications have also been described after the widespread use of sugammadex in anesthesia practice. Cases of acute coronary syndrome resulting from a strong allergic/immune reaction to any drug or product, also called Kounis syndrome, have been associated with the use of sugammadex.[16] This newly described-allergic condition has been described in many clinical presentations, from atropine-resistant bradycardia to sudden cardiac arrest.[16-19] Both the aforementioned post-extubation respiratory complications and allergy-related coronary symptoms are conditions that develop in a short time, and the findings of our study may guide clinicians in dealing with these clinical scenarios in case of re-intubation. We demonstrated that the time for reversal of NMB of rocuronium after sugammadex reversal of initial NMB was statistically shorter for the control group than for the other groups. Clinicians should closely follow up for motor blockage in the event of such re-intubation.

In our study, the first dose of rocuronium was administered at 1.2 mg/kg. The suggested induction dose of rocuronium in humans is 0.6 mg/kg. However, due to faster metabolism, this dose does not provide adequate NMB. Differing doses of rocuronium are used in rats, while in most studies a high dose of 3.5 mg/kg is used.[20,21] It has been reported that this dose corresponds to 0.6 mg/kg in humans. However, most studies have used lower doses of 1.2-1.5 mg/kg.[22,23] Our primary aim was not to determine the optimal human dosage but to evaluate differing doses according to total effect time and time to NMB. Therefore, we used the minimum accepted dosage of 1.2 mg/kg for rocuronium. We prevented mortality and complications by keeping experiment time and time on mechanical ventilation to minimum. Intravenous sugammadex demonstrated linear pharmacokinetic properties over the dose range of 1-16 mg/kg. On the other hand, when sugammadex is administered at high doses, the unbound sugammadex molecules will remain free, increasing the possibility of inducing toxic effects. Studies have shown that 1 mg of sugammadex is equivalent to 4 mg in rats.[23,24] Therefore, we used 4 mg/kg of sugammadex to correspond to the minimum dosage in humans. To our knowledge, there are no animal study, rat model, or a pilot study similar to ours in the literature. There is also no study similar to ours in which TOF usage in rats is demonstrated in detail.

In summary, the above-mentioned studies and case reports report that after reversal of NMB with sugammadex, high dosage (four times of normal) of rocuronium leads to NMB and an increase in rocuronium dosage after sugammadex lengthens the time for beginning of its effect. In our study we used 2-5 times more dosage to determine the effect of rocuronium after reversal with sugammadex. While 4-5 times higher dose led to NMB, there was no difference between the time of effect start between 4 and 5 times dosages.

Study Limitations

Our study has some limitations. Due to technical reasons, we were unable to monitor blood gases and other physiological responses in rats. We had to ignore the factors such as the metabolic rate that would affect the effect time and metabolism of drugs. To prevent any negative effects of mechanical ventilation, we kept the rocuronium doses low.

We were therefore unable to administer higher doses of rocuronium. Also, we did not perform any pathophysiological evaluation of the end organ effects of our experimental drugs.

CONCLUSION

Adequate NMB for intubation is possible when rocuronium is applied 2 min after sugammadex. However, the total dose of rocuronium, sugammadex and the time required for intubation after sugammadex should all be kept in mind. A non-steroidal non-depolarising NMB should be used in this case. Rocuronium can be used in 3-4 times of normal dose when other medications are not available. However, studies on the end-organ effect of these dosages of rocuronium must be evaluated.

ETHICS

Ethics Committee Approval: This experimental, randomized, controlled, and blinded animal study was approved by the Yeditepe University Local Animal Studies Ethical Board (approval number: 478, date: 19.08.2015).

Informed Consent: Patient approval has not been obtained as it is performed on animals.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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. de Boer HD. Sugammadex, a new reversal agent for rocuronium-induced neuromuscular block: a step forward in improving patient's safety. [Sl: sn]; 2008.
2. Iwasaki H, Sasakawa T, Takahoko K, Takagi S, Nakatsuka H, Suzuki T, et al. A case series of re-establishment of neuromuscular block with rocuronium after sugammadex reversal. J Anesth. 2016; 30(3): 534-7.
3. Bridion 100 mg/ml solution for injection (emc) [Internet]. [cited 2017 Apr 4]. Available from: https://www.medicines.org.uk/emc/product/6409
4. Williamson RM, Mallaiah S, Barclay P. Rocuronium and sugammadex for rapid sequence induction of obstetric general anaesthesia. Acta Anaesthesiol Scand. 2011; 55(6): 694-9.
5. Cammu G, de Kam PJ, De Graeve K, van den Heuvel M, Suy K, Morias K, et al. Repeat dosing of rocuronium 1.2 mg kg-1 after reversal of neuromuscular block by sugammadex 4.0 mg kg-1 in anaesthetized healthy volunteers: a modelling-based pilot study. Br J Anaesth. 2010; 105(4): 487-92.
6. Matsuki G, Takahata O, Iwasaki H. Repeat dosing of rocuronium after reversal of neuromuscular block by sugammadex. Can J Anaesth. 2011; 58(8): 769-70.
7. Kim YH. Repeat dosing of rocuronium-sugammadex: unpredictable. Korean J Anesthesiol. 2014; 67(1): 1-3.
8. Sasakawa T, Iwasaki H, Kurosawa A, Kikuchi C, Takahata O, Iwasaki H. Case report: a normal dose of rocuronium achieved the desired effect in a short time after the administration of sugammadex during reoperation. Masui. 2011; 60(5): 621-4.
9. National Research Council, Division on Earth and Life Studies, Institute for Laboratory Animal Research, Committee on Guidelines for the Use of Animals in Neuroscience and Behavioral Research. Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research. National Academies Press; 2003.p.224.
10. Nishi M, Fujii S, Nitta S. A two-year-old patient who received readministration of rocuronium for re-operation 30 minutes after sugammadex reversal. Masui. 2011; 60(10): 1189-91.
11. Sakai Y, Tsutsumi YM, Wakamatsu N, Soga T, Tanaka K, Oshita S. A case where rocuronium was unable to achieve neuromuscular block immediately after sugammadex administration. J Med Invest. 2011; 58(1-2): 163-5.
12. Real C, Silva J, Esteves S, Machado HS. The use of rocuronium 20 minutes after sugammadex administration - a case report. Glob Anesth Perioper Med. 2015; 1(4): 118-20.
13. de Boer HD, Driessen JJ, van Egmond J, Booij LH. Non-steroidal neuromuscular blocking agents to re-establish paralysis after reversal of rocuronium-induced neuromuscular block with sugammadex. Can J Anaesth. 2008; 55(2): 124-5; author reply 125-6.
14. Cammu G, de Kam PJ, De Graeve K, van den Heuvel M, Suy K, Morias K, et al. Repeat dosing of rocuronium 1.2 mg kg-1 after reversal of neuromuscular block by sugammadex 4.0 mg kg-1 in anaesthetized healthy volunteers: a modelling-based pilot study. Br J Anaesth. 2010; 105(4): 487-92.
15. Tsur A, Kalansky A. Hypersensitivity associated with sugammadex administration: a systematic review. Anaesthesia. 2014; 69(11): 1251-7.
16. Kounis NG, Koniari I, Soufras GD, Tsigkas G, Plotas P, Davlouros P, et al. Sugammadex-induced atropine-resistant bradycardia: clinical, pathophysiologic, and electrocardiographic considerations. JA Clin Rep. 2020; 6(1): 31.
17. Yanai M, Ariyoshi K. Two Cardiac Arrests that Occurred after the Administration of Sugammadex: A Case of Kounis Syndrome. Case Rep Emerg Med. 2020; 2020: 6590101.
18. Yoshida T, Sumi C, Uba T, Miyata H, Umegaki T, Kamibayashi T. A rare case of atropine-resistant bradycardia following sugammadex administration. JA Clin Rep. 2020; 6(1): 18.
19. Yang HS, Kim HJ, Koh W. Effects of sugammadex on the coronary circulation: direct effects on coronary vessels or hypersensitivity (Kounis syndrome)? Korean J Anesthesiol. 2017; 70(3): 363-4.
20. Et T, Topal A, Erol A, Tavlan A, Kılıçaslan A, Uzun ST. The Effects of Sugammadex on Progesterone Levels in Pregnant Rats. Balkan Med J. 2015; 32: 203-7.
21. Eikermann M, Zaremba S, Malhotra A, Jordan AS, Rosow C, Chamberlin NL. Neostigmine but not sugammadex impairs upper airway dilator muscle activity and breathing. Br J Anaesth. 2008; 101(3): 344-9.
22. Tomak Y, Yılmaz A, Bostan H, Tümkaya L, Altuner D, Kalkan Y, et al. Effects of sugammadex and rocuronium mast cell number and degranulation in rat liver. Anaesthesia. 2012; 67(10): 1101-4.
23. Platt PR, Sadleir PH, Clarke RC. Sugammadex, rocuronium and mast cell numbers in the rat liver. Anaesthesia. 2013; 68(2): 208-9.
24. Hoffmann U, Grosse-Sundrup M, Eikermann-Haerter K, Zaremba S, Ayata C, Zhang B, et al. Calabadion: A new agent to reverse the effects of benzylisoquinoline and steroidal neuromuscular-blocking agents. Anesthesiology. 2013; 119(2): 317-25.

CASE REPORT

CYPRUS
JOURNAL OF MEDICAL SCIENCES

DOI: 10.4274/cjms.2020.2734
Cyprus J Med Sci 2023;8(3):234-236

mDIXON Quant MRI Findings of Breast Myofibroblastoma

Hakan Abdullah Özgül[1], Işıl Başara Akın[1], Canan Altay[1], Merih Güray Durak[2], Pınar Balcı[1]

[1]Department of Radiology, Dokuz Eylül University Faculty of Medicine, İzmir, Türkiye
[2]Department of Pathology, Dokuz Eylül University Faculty of Medicine, İzmir, Türkiye

Abstract

The mDIXON quant sequence is a chemical shift encoding based on the proton density fat fraction, which can assess fat content quantitatively. Myofibroblastoma (MFB) is a benign mesenchymal entity, and its prevalence is less than 1% of all breast tumors. Since MFB includes variable amounts of fat-containing fascicles, the mDIXON quant sequence provides a prominent contribution in evaluating fat content in the lesion.

Keywords: Breast, myofibroblastoma, mDIXON quant, magnetic resonance imaging

INTRODUCTION

Myofibroblastoma (MFB) is a benign mesenchymal entity. It is a rare tumor with a prevalence of less than 1% of all breast tumors.[1] It is more common in postmenopausal women and middle- to older men. Although its etiology has not been fully understood, cases with a history of steroid hormone use, gynecomastia, chest wall trauma, and surgical scars have been reported in the literature.[2] Although MFB is a benign entity, in radiological evaluation, there is no specific diagnostic feature[3]. The mDIXON quant sequence is a chemical shift encoding based on proton density fat fraction, which can assess fat content quantitatively with fat fraction value.[4] In this case report, we present the mDIXON quant magnetic resonance imaging (MRI) findings of MFB. To the best of our knowledge, this is the first case presenting mDIXON quant MRI findings of MFB in the published literature.

CASE PRESENTATION

A 55-year-old male patient with a complaint of abdominal pain underwent abdominal MRI. Although he had no history of cancer in his own and family history, he had a subcutaneous lipoma at the lower thoracic levels on the right anterior axillary line for a long time. An abdominal MRI examination included upper extraabdominal sites up to the retroaerolar region. In this examination, there were solid lesions in both retroareolar areas. The most significant lesion was located at the left retroareolar localization (Figure 1A, B). In our institute, routine abdominal MRI protocol includes another sequence called mDIXON quant, showing macroscopic-microscopic fat and water content (Figure 2). In the macroscopic fat content measurements with the lesion's largest diameter, the lesion contained 22.51% fat, and the lesion T2

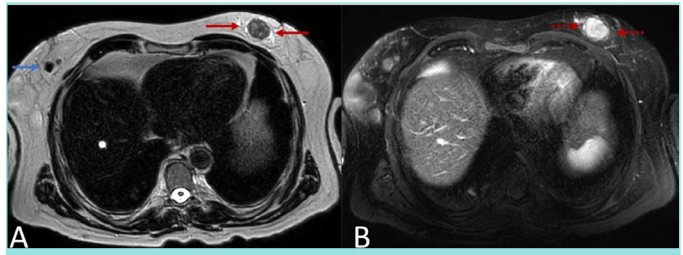


Figure 1. Solid lesion located at the left retro areolar localization was inhomogeneous; on T2-weighted images (A), it was hypointense, and there were hyperintense areas in the lesion (red arrows). Also, smaller solid lesions were detected in the right retro areolar area (blue arrows). On fat-saturated T2-weighted (spectral presaturation with inversion recovery) sequence (B), the lesion was hyperintense. There was no signal suppression due to any fat content (dashed red arrows).

To cite this article: Özgül HA, Başara Akın I, Altay C, Durak MG, Balcı P. mDIXON Quant MRI Findings of Breast Myofibroblastoma. Cyprus J Med Sci 2023;8(3):234-236

ORCID IDs of the authors: H.A.Ö. 0000-0001-6123-0668; I.B.A. 0000-0002-0786-1490; C.A. 0000-0003-0417-7770; M.G.D. 0000-0003-3516-952X; P.B. 0000-0002-2425-7631.

Address for Correspondence: Hakan Abdullah Özgül
E-mail: haozgul@hotmail.com
ORCID ID: orcid.org/0000-0001-6123-0668

Received: 29.08.2020
Accepted: 21.11.2020

relaxation time was measured as 82.03 ms (Figure 3A, B). In the coloured maps created in the section that passed through the lesion level, the percentage of fat contained in the lesion was shown in the appropriate color (Figure 3C, D). Mammography and breast ultrasonography were recommended for further examination. The left retroareolar solid lesion was excised because of breast evaluation. Histopathological diagnosis of the lesion has been reported as (MFB) (Figure 4).

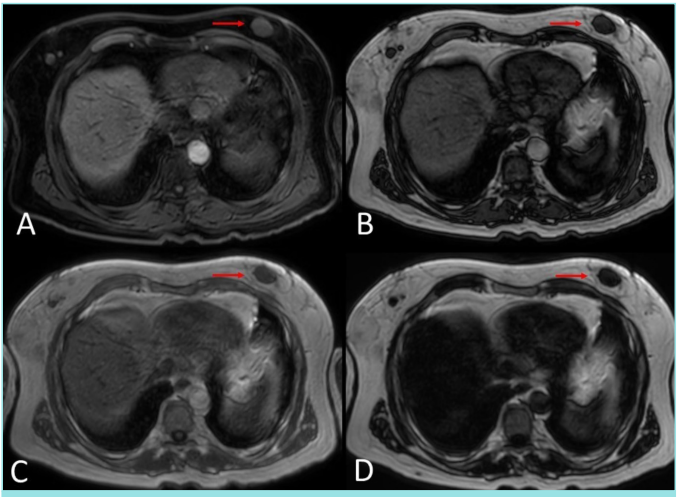


Figure 2. In the mDIXON Quant sequence, the lesion (red arrow) was hyperintense in the water only images (A) and hypointense in in-phase (C) and out-phase (B) images. In fat only images (D), hypointense and hyperintense areas show macroscopic fat content in the lesion.

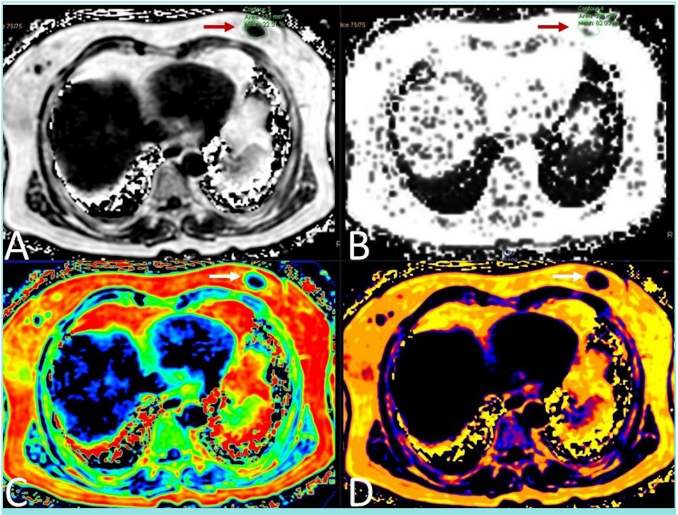


Figure 3. Quantification of fat with the largest diameter of the lesion (red arrow), the lesion contains 22.51% fat (A), and lesion T2 relaxation time was measured as 82.03 ms (B). In the measurements made at the workstation on the mDixon quant sequence images, the quantification of fat can be made numerically. The fat content can be visually understood by looking at the scale consisting of the colors assigned to the percentage ranges. Here, as in the C and D figures, two different color scales can be used. In (C), fat-rich areas are shown in red, poor areas in dark blue. In (D), fat-rich areas are bright yellow, while areas poor in oil are identified in black. The lesion was marked with white arrows, which corresponds to 22.51% fat content; Green and blue in (C) and purple and black in (D) were assigned to the lesion by the program.

DISCUSSION

MFB belongs to the family of CD34-positive tumors and is characterized by spindle-shaped cells growing in collagen bands and variable numbers of fat-containing fascicles.[3]

The mDIXON quant sequence is a proton-density-based chemical shift encoding that uses a fat fraction value to quantitatively analyze fat content.[4] This sequence consists of four separate series: in-phase, out-phase, water only, and fat only, using mathematical calculations taking advantage of the T2* relaxation time difference of fat and water. In this way, it is possible to make fat rate measurements that give meaningful results in different organs and lesions using this sequence, which is generally used in the abdominal region.[5]

Because MFB is a rare disease and includes variable amounts of fat-containing fascicles, the mDIXON quant sequence provides a prominent contribution in evaluating fat content in the lesion. By using this sequence, quantification of fat can be made. Additionally, this sequence provides us with better management in the evaluation and treatment of similar lesions. Written informed consent was obtained from the patient who participated in this study.

MAIN POINTS

- The mDIXON quant sequence is a chemical shift encoding based on proton density fat fraction, which can assess fat content quantitatively with fat fraction value.
- It is possible to make fat rate measurements that give meaningful results in different organs and lesions using the mDIXON sequence, which is generally used in the abdominal region.
- Because MFB is a rare disease and includes variable amounts of fat-containing fascicles, the mDIXON quant sequence provides a prominent contribution in evaluating fat content in the lesion.

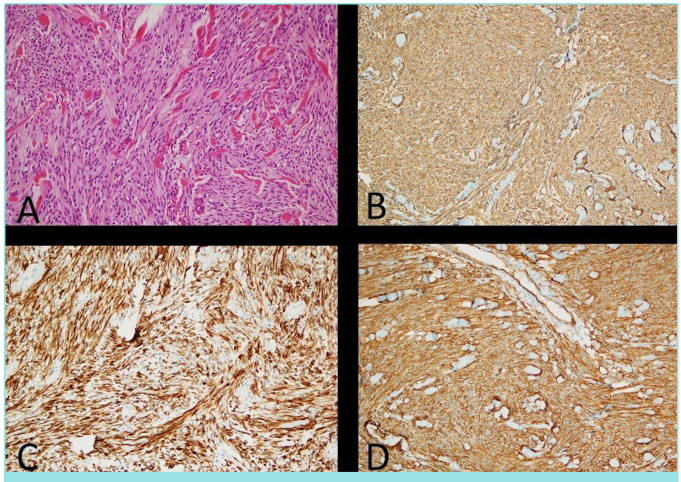


Figure 4. The tumor shows uniform spindle cells growing in fascicles with intervening bands of ropey collagen (A) (hematoxylin and eosin, x200). In immunohistochemical analysis, the tumor cells show diffuse and robust positive staining [(B) vimentin, (C) desmin, and (D) CD34 (x200)].

- The mDIXON sequence provides us with better management in the evaluation and treatment of similar lesions.

ETHICS

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

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: H.A.Ö., Design: I.B.A., C.A., M.G.D., Supervision: I.B.A., P.B., Fundings: H.A.Ö., M.G.D., Materials: I.B.A., M.G.D., Data Collection and/or Processing: H.A.Ö., M.G.D., Analysis and/or Interpretation: I.B.A., Literature Search: C.A., P.B., Writing: H.A.Ö., C.A., P.B., Critical Review: C.A., P.B.

DISCLOSURES

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

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

REFERENCES

1. Schickman R, Leibman AJ, Handa P, Kornmehl A, Abadi M. Mesenchymal breast lesions. Clin Radiol. 2015; 70(6): 567-75.
2. Howitt BE, Fletcher CD. Mammary-type Myofibroblastoma: Clinicopathologic Characterization in a Series of 143 Cases. Am J Surg Pathol. 2016; 40(3): 361-7.
3. Wargotz ES, Weiss SW, Norris HJ. Myofibroblastoma of the breast. Sixteen cases of a distinctive benign mesenchymal tumor. Am J Surg Pathol. 1987; 11(7): 493-502.
4. Takatsu Y, Kyotani K, Ueyama T, Miyati T, Yamamura K, Andou A. Assessment of the Quality of Breast MR Imaging Using the Modified Dixon Method and Frequency-Selective Fat Suppression: A Phantom Study. Magn Reson Med Sci. 2018; 17(4): 350-5.
5. Zhang Y, Zhou Z, Wang C, Cheng X, Wang L, Duanmu Y, et al. Reliability of measuring the fat content of the lumbar vertebral marrow and paraspinal muscles using MRI mDIXON-Quant sequence. Diagn Interv Radiol. 2018; 24(5): 302-7.

CASE REPORT

CYPRUS
JOURNAL OF MEDICAL SCIENCES

DOI: 10.4274/cjms.2023.2022-63
Cyprus J Med Sci 2023;8(3):237-240

Multidrug-Resistant *Elizabethkingia anophelis*, A Rare Causative Agent of Bacteremia in a Hemodialysis Patient Hospitalized in the Intensive Care Unit: First Case in North Cyprus

Emrah Güler[1], Ulaş Hürdoğanoğlu[2], Nedim Çakır[3], Özgen Alpay Özbek[4], Gülay Eren[5], Kaya Süer[6]

[1]Department of Molecular Biology and Genetics, European University of Lefke Faculty of Arts and Sciences, Lefke, North Cyprus
[2]Near East University Vocational School of Health Services, Nicosia, North Cyprus
[3]Department of Medical Microbiology and Clinical Microbiology, Near East University Faculty of Medicine, Nicosia, North Cyprus
[4]Department of Medical Microbiology, Dokuz Eylül University Faculty of Medicine, İzmir, Türkiye
[5]Unit of Intensive Care, Near East University Hospital, Nicosia, North Cyprus
[6]Department of Infectious Diseases and Clinical Microbiology, Near East University Faculty of Medicine, Nicosia, North Cyprus

Abstract

Elizabethkingia spp. bacteria are found in living and non-living things. *Elizabethkingia meningoseptica (E. meningoseptica)* and *Elizabethkingia anophelis (E. anophelis)* are the leading species that can cause diseases in humans. Most of the infections (80-87.5%) caused by *E. anophelis* are hospital-acquired. This bacterium is generally identified as *E. meningoseptica* by automated systems. In this study, a case of bacteremia due to *E. anophelis* in a hemodialysis patient is presented. This patient is a 72 years old female who is hospitalized in the intensive care unit. She was referred to the Near East University Hospital from the state hospital with pneumonia. *E. anophelis* was isolated from the patient's blood culture by the VITEK-2 automated system. The isolated bacteria were stored at -80 ºC and detected as *E. anophelis* by matrix-assisted laser desorption ionization time of flight. Success was achieved by adding levofloxacin to the ongoing meropenem treatment in our patient. Although the transmission route of our case with multidrug resistance is not exactly known, it is assumed that the transmission originated from the hospital. In this regard, increasing control measures in hospitals, mainly in water systems, will prevent such infections and deaths. In addition, it is concluded that for treating infections caused by *Elizabethkingia*, the use of fluoroquinolones should be the first choice.

Keywords: *Elizabethkingia anophelis*, bacteremia, hemodialysis, rare, North Cyprus

INTRODUCTION

Bacteria in the genus *Elizabethkingia* spp. are Gram-negative, aerobic, pale, yellow pigmented, non-motile, non-glucose fermenting, and oxidase positive. They are ubiquitously distributed in nature, including water, soil, sediment, plants, fish, frogs, insects, and some animal digestive tracts. *Elizabethkingia meningoseptica*, *Elizabethkingia anophelis*, *Elizabethkingia miricola*, *Elizabethkingia bruuniana*, *Elizabethkingia ursingii*, and *Elizabethkingia occulta* are known species of bacteria. The leading species that cause diseases in humans are

To cite this article: Güler E, Hürdoğanoğlu U, Çakır N, Özbek ÖA, Eren G, Süer K. Multidrug-Resistant *Elizabethkingia Anophelis*, A Rare Causative Agent of Bacteremia in a Hemodialysis Patient Hospitalized in the Intensive Care Unit: First Case in North Cyprus. Cyprus J Med Sci 2023;8(3):237-240

ORCID IDs of the authors: E.G. 0000-0002-1635-0051; U.H. 0000-0002-0182-674X; N.Ç. 0000-0002-3632-5187; Ö.A.Ö. 0000-0003-4415-7205; G.E. 0000-0002-5365-3641; K.S. 0000-0002-2565-3425.

Address for Correspondence: Emrah Güler
E-mail: eguler@eul.edu.tr
ORCID ID: orcid.org/0000-0002-1635-0051

Received: 05.12.2022
Accepted: 19.04.2023

Elizabethkingia meningoseptica (*E. meningoseptica*) and *Elizabethkingia anophelis* (*E. anophelis*).[1,2]

E. anophelis is a newly discovered bacterium isolated from the gut of *Anopheles gambiae* mosquito.[3] It is reported as an opportunistic bacterial pathogen that causes bacteremia in immunocompromised elderly patients and newborns.[4] Moreover, this bacterium can cause meningitis, pneumonia, septic arthritis, osteomyelitis, endocarditis, conjunctivitis, and cholangitis.[1] Most of the infections caused by *E. anophelis* (80%-87.5) are hospital-acquired. These bacteria, which can colonize hospital environments, are resistant to applied decontamination processes. In addition, hospitals are considered to be reservoirs of these bacteria since they can contaminate water systems, solutions, and hospital equipment.[1,5]

In recent years, an increase in *E. anophelis* infections that threaten human life has been reported in clinics.[6] In society, *E. anophelis* infection in humans was first observed in 2012 at a hospital in Singapore, when *E. anophelis* was isolated from five patients with a nosocomial infection. In two of these patients, infection resulted in death due to sepsis.[7] According to the literature, several epidemics were seen in Hong Kong, Taiwan, and the United States in the following years.[5]

Routine phenotypic and biochemical tests often fail to distinguish *E. anophelis* from other bacteria. At the same time, this bacterium is usually defined as *E. meningoseptica* by automated systems.[5] Additionally, the multi drug resistance (MDR) of the bacterium and the lack of a standard antibiotic susceptibility profile make empirical treatment almost impossible.[4] The mortality rate in *E. anophelis* infections due to MDR is estimated to vary between 24% and 30%.[2]

Early and accurate determination of appropriate antibiotic susceptibility results is critical and crucial to reduce morbidity and mortality in patients infected with *E. anophelis*. In our study, a case of bacteremia due to *E. anophelis* in a hemodialysis patient hospitalized in an intensive care unit (ICU) is presented. In the literature, there is no reported case of *E. anophelis* that has been found in North Cyprus, and hence this infection is the first *Elizabethkingia* infection presented in our country.

CASE PRESENTATION

A 72-year-old Ukrainian woman residing in North Cyprus applied to the Near East University Hospital on April 01, 2022, as a referral from Dr. Burhan Nalbantoğlu State Hospital, Nicosia due to respiratory distress. The patient had no fever, was conscious and cooperative. She was also receiving meropenem (3x500 mg) treatment at the hospital she came to. The patient had chronic renal failure and was on regular hemodialysis. First, severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) antigen and SARS-CoV-2 RNA polymerase chain reaction test (PCR) were performed on the patient, and she was hospitalized in the general ICU with negative results. Meanwhile, biochemical test were performed from the taken blood and the results were: uric acid (1.7 mg/dL), albumin (2.8 g/dL), amylase (15 U/L), lipase (<4 U/L), and calcium (8.2 mg/dL) were found to be low. In addition, creatinine (2.63 mg/dL), aspartate aminotransferase (AST) (52 U/L), procalcitonin (PCT) (12.26 ng/mL), and C-reactive protein (CRP) (28.63 mg/dL) elevations were noteworthy. In the complete blood count, hemoglobin (12.1 g/dL), and WBCs ($4.4x10^3$/µL) levels were found to be normal, but thrombocytopenia ($47x10^3$/µL) was detected in the patient.

Considering all these results and respiratory failure, thoracic computed tomography (CT) was performed on April 02, 2022. According to the CT results, massive pleural effusion in the right hemithorax and almost complete loss of aeration in the lower lobe of the right lung were observed. In addition, 14 mm thick pleural fluid was detected in the left hemithorax.

Samples for sputum and blood culture were taken from the patient on the day of hospitalization. The sputum sample was inoculated on blood agar and eosin methylene blue (EMB) agar and incubated for 24-48 hours at 35 ºC. Blood samples were taken from the patient's right and left arm at an interval of 15 min. The samples (8-10 mL) taken for blood culture were transferred to BD BACTEC Plus Aerobic/F bottles and loaded into the BACTEC 9120 (Becton, Dickinson and Company Sparks, USA) device. Two days later, when positive signals were received from the device, the blood sample was drawn with a sterile syringe from the blood culture bottles and inoculated on blood and EMB agars; the agars were incubated 35 °C for 24-48 hours. With the observation of growth in both sputum and blood cultures, suspensions were prepared from the colonies that grew in line with the manufacturer's VITEK-2 (BioMerieux, Inc. Durham, USA) compact automated system. In addition, smears were prepared from both colonies and stained with Gram-stain. Structures of *Candida* species were seen in the Gram-stain of colonies grown in sputum culture and *Candida albicans* was obtained as a result of VITEK-2. Gram-negative bacilli were observed in smear preparations made from colonies grown in both blood cultures. Upon this, bacteria identification (VITEK-2 GN ID card) and antibiotic susceptibility test (AST) (VITEK-2 AST-N326 card) were performed. AST was evaluated according to the European Committee on Antimicrobial Susceptibility Testing criteria. The result of *Elizabethkingia meningoseptica* was obtained by the automated system. According to the results of the antibiotic tests, only ciprofloxacin, levofloxacin, and trimethoprim/sulfamethoxazole (SXT) were susceptible. The AST results given by the VITEK-2 automated system of the bacteria are presented in Table 1. Considering the antibiogram, levofloxacin (1x500 mg) was added to the patient's ongoing meropenem (3x500 mg) treatment. Furthermore, 72 h later, a

Table 1. AST results of *Elizabethkingia anophelis* obtained from VITEK-2 automated system

Antibiotic	MIC (µg/mL)	Result
Piperacillin	64	I
Piperacillin/tazobactam	>=128	R
Ceftazidime	>=64	R
Cefepime	>=32	R
Aztreonam	>=64	R
Imipenem	>=16	R
Meropenem	>=16	R
Amikacin	>=64	R
Gentamicin	>=16	R
Netilmicin	>=32	R
Tobramycin	>=16	R
Ciprofloxacin	1	S
Levofloxacin	1	S
Tetracycline	8	I
Trimethoprim/sulfamethoxazole	40	S

MIC: Minimum inhibitory concentration; S: Sensitive; R: Resistant; I: Intermediate, AST: Aspartate aminotransferase.

decrease was observed in our patient's CRP and PCT levels (13.20 mg/dL and 6.18 ng/mL, respectively). At the end of seventh day, CRP and PCT values were observed as 8.33 mg/dL and 3.31 ng/mL respectively. Our patient had undergone dialysis twice within a week during her stay in the hospital. At the end of a week, the patient returned to the State Hospital again. After that, no information could be obtained from the patient.

E. meningoseptica strains isolated from blood cultures were stocked using bacteria stock tubes (OR-BAK, Ankara, Türkiye) and stored at -80 ºC. Since it is a rare bacterium, the sample was sent to Dokuz Eylül University under the appropriate conditions and the bacterium was detected as *E. anophelis* by matrix-assisted laser desorption ionization time of flight (MALDI-TOF) (Bruker Daltonics, Bremen, Germany).

An informed consent form was filled by the patient and her consent was obtained, which allowed us to present our case.

DISCUSSION

The case presented in this study is the first reported case of *E. anophelis* infection detected in Cyprus. Because the patient came to the Near East University Hospital as a referral from a state hospital, the source of the infection is not known exactly. Even though the predominance of this bacterium in the gut microbiota of *Anopheles gambiae* mosquitoes indicates that mosquitoes can be vectors for transmission, there is no clinical evidence to support this view.[2] However, considering that the patient is hospitalized in the ICU of the state hospital and regularly undergoes dialysis, it is possible to think that the infection originates either from the ICU or hemodialysis units. Recent studies suggest that hospital environments act as reservoirs for *Elizabethkingia* species. In fact, despite the control measures taken in hospitals, it has been proven that the bacterium continues to exist in the water resources of hospitals.[8,9] In a study by Kyritsi et al.[10], two different *Elizabethkingia* strains were detected in the water system of the same hospital, three months apart. In view of the data obtained from our study, it is concluded that this bacterium was transmitted to our patient from the hospital environment.

E. anophelis is the dominant strain of the septicemia-causing *Elizabethkingia* genus and is associated with fulminant complications such as acute pulmonary edema, congestive heart failure, septic shock, and death. *E. anophelis* infections should always be considered as clinically significant unless proven otherwise. In addition, there exist studies in the literature showing that automated systems such as VITEK-2 misidentify *E. anophelis* isolates as *E. meningoseptica*.[11] MALDI-TOF systems are extensively used for microbial identification in clinical microbiology laboratories. Unfortunately, systems that are most frequently used for microbial identification like VITEK-2 cannot distinguish *Elizabethkingia* species. VITEK-2 automated system can detect only *E. meningoseptica* with its reference database. The lack of species information in the reference databases prevents these platforms from correct recognition of the species of *Elizabethkingia*. Although MALDI-TOF systems with a large database can reliably identify *E. anophelis* and *E. meningoseptica*, these platforms cannot distinguish between the remaining species of the genus *Elizabethkingia*.[12] Moreover, according to the most recent studies, it is emphasized that MALDI-TOF systems are no longer able to accurately identify *Elizabethkingia* species and therefore 16S rRNA or full genome sequence analyzes are required for accurate identification.[13] The strain isolated in this study could not be performed using 16S rRNA or full genome sequence analysis, which can be a limitation of our study. However, showing that this strain exists in Cyprus contributes to clinicians, scientists, and the literature.

E. anophelis has an extensive antibiotic resistance profile, including most penicillins, cefalosporines, carbapenems, aminoglycosides, and macrolide antibiotics. Fluoroquinolones, SXT, and piperacillin/tazobactam are used as first-line therapy for *Elizabethkingia* infections, but resistance recently complicates treatment. Teng et al. draw attention to SXT resistance in *Elizabethkingia* species.[14] The combination treatment of vancomycin, piperacillin/tazobactam, and ciprofloxacin gave positive results in a newborn case with meningitis and septicemia determined by Baruah et al.[11] Nielsen et al.[13] isolated *E. anophelis* from a 76-year-old male patient and applied moxifloxacin and rifampicin treatment for two weeks, which resulted in success. Because they can cross the blood-brain barrier more easily, fluoroquinolones, especially ciprofloxacin, are a good choice for these infections.[11] In this study, levofloxacin was combined with meropenem treatment in our patient who presented with septicemia, and as a result, success was achieved.

In conclusion, although the transmission route of our case with multidrug resistance (Table 1) is not known for certain, it is thought that it was caused by the hospital. In this regard, increasing control measures in hospitals, mainly in water systems, will prevent such infections and deaths. For *Elizabethkingia* typing, if the facilities are sufficient, sequence analysis must be performed. Furthermore, it is concluded that the use of fluoroquinolones should be the first choice for treating infections caused by *Elizabethkingia*.

MAIN POINTS

- *Elizabethkingia anophelis* is a rare bacterium and was first isolated in North Cyprus.
- Since it is multiple drug-resistant bacterium, treatment options should be determined correctly.
- It is critical to detect and eliminate the source of contamination, especially in units where immunosuppressed patients are treated, such as intensive care and hemodialysis.

ETHICS

Informed Consent: An informed consent form was filled by the patient and her consent was obtained, which allowed us to present our case.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: N.Ç., G.E., K.S., Concept: E.G., N.Ç., K.S., Design: E.G., K.S., Data Collection and/or Processing: E.G., U.H., Ö.A.Ö., Analysis and/or Interpretation: U.H., N.Ç., Ö.A.Ö., G.E., K.S., Literature Search: E.G., Writing: E.G.

DISCLOSURES

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

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

REFERENCES

1. Nievas J, Ibanez ML, Allende L, Altclas J, Antezana G, Campos J, et al. Emergence of Elizabethkingia anophelis. First case of E. anophelis outbreak in a neonatal unit of a hospital in Argentina. Clinical Infection in Practice. 2022; 13: 100122.
2. Wang B, Cheng R, Feng Y, Guo Y, Kan Q, Qian A, et al. Elizabethkingia anophelis: An Important Emerging Cause of Neonatal Sepsis and Meningitis in China. Pediatr Infect Dis J. 2022; 41(5): e228-32.
3. Kämpfer P, Matthews H, Glaeser SP, Martin K, Lodders N, Faye I. Elizabethkingia anophelis sp. nov., isolated from the midgut of the mosquito Anopheles gambiae. Int J Syst Evol Microbiol. 2011; 61(Pt 11): 2670-5. Erratum in: Int J Syst Evol Microbiol. 2012; 62(Pt 4): 1016.
4. Sahoo RK, Sahoo S, Das A, Gaur M, Bhanjadeo D, Panda P, et al. A phylogenetic study of Elizabethkingia anophelis bloodstream isolates obtained from inpatients at a single medical center. Infect Control Hosp Epidemiol. 2019; 40(10): 1202-4.
5. Aydemir O, Sahin Ozozen E, Elmas B, Caha V. A Rare Infectious Agent: Elizabethkingia anophelis; Second Case Reported from Turkey. Konuralp Medical Journal. 2022; 14(1): 168-71.
6. Xu L, Peng B, He Y, Cui Y, Hu Q, Wu Y, et al. Isolation of Elizabethkingia anophelis From COVID-19 Swab Kits. Front Microbiol. 2022; 12: 799150.
7. Teo J, Tan SY, Tay M, Ding Y, Kjelleberg S, Givskov M, et al. First case of E anophelis outbreak in an intensive-care unit. Lancet. 2013; 382(9895): 855-6.
8. Breurec S, Criscuolo A, Diancourt L, Rendueles O, Vandenbogaert M, Passet V, et al. Genomic epidemiology and global diversity of the emerging bacterial pathogen Elizabethkingia anophelis. Sci Rep. 2016; 6: 30379.
9. Moore LS, Owens DS, Jepson A, Turton JF, Ashworth S, Donaldson H, Holmes AH. Waterborne Elizabethkingia meningoseptica in Adult Critical Care. Emerg Infect Dis. 2016; 22(1): 9-17.
10. Kyritsi MA, Mouchtouri VA, Pournaras S, Hadjichristodoulou C. First reported isolation of an emerging opportunistic pathogen (Elizabethkingia anophelis) from hospital water systems in Greece. J Water Health. 2018; 16(1): 164-70.
11. Baruah FK, Borkakoty B, Ahmed A, Bora P. Neonatal Meningitis and Septicemia Caused by Multidrug-Resistant Elizabethkingia anophelis Identified by 16s Ribosomal RNA: An Emerging Threat. J Glob Infect Dis. 2020; 12(4): 225-7.
12. Lin JN, Lai CH, Yang CH, Huang YH. Elizabethkingia Infections in Humans: From Genomics to Clinics. Microorganisms. 2019; 7(9): 295.
13. Nielsen HL, Tarpgaard IH, Fuglsang-Damgaard D, Thomsen PK, Brisse S, Dalager-Pedersen M. Rare Elizabethkingia anophelis meningitis case in a Danish male. JMM Case Rep. 2018; 5(8): e005163.
14. Teng LC, Wang JM, Lu HY, Mao YC, Lai KL, Tseng CH, et al. Elizabethkingia Intra-Abdominal Infection and Related Trimethoprim-Sulfamethoxazole Resistance: A Clinical-Genomic Study. Antibiotics (Basel). 2021; 10(2): 173.