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galenos yayınevi

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Publisher Contact
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AIMS AND SCOPE

Namık Kemal Medical Journal is the scientific official journal of Tekirdağ Namık Kemal University School of Medicine. The journal is electronically publised tri-annualy (April, August, December). Articles accepted until one month before the month of publication are added to the relevant issue. The three issues form a volume.

Namık Kemal Medical Journal is an independent scientific medical journal that aims to reach its readers in electronic and open access free of charge. It is aimed to publish original articles, case reports and reviews that include the results of studies and research conducted in the fields of basic, internal and surgical medical sciences and medical nursing and passed through the peer review process.

The official publishing language of the Journal is both Turkish and English.

Namık Kemal Medical Journal (NKMJ) is indexed by **Turkish Index (TR DIZIN/ULAKBIM), EBSCO Host, Index Copernicus, TürkMedline, Ideal Online, J-Gate, Gale and CAB International (CABI).**

The Journal will not consider manuscripts any that have been published elsewhere, or manuscripts that are being considered for another publication, or are in press. Studies previously announced in the congresses are accepted if this condition is stated. If any part of a manuscript by the same author(s) contains any information that was previously published, a reprint or a copy of the previous article should be submitted to the Editorial Office with an explanation by the authors.

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Statements and suggestions published in manuscripts are the authors' responsibility and do not reflect the opinions of the Editor and the Associate Editorial Board members.

All scientific responsibility for the articles belongs to the authors. The name order of the submitted articles should be a joint decision. The responsible author is considered to accept the author order in the name of all authors by signing the " Copyright Transfer Form". Anyone who does not meet the criteria for authoring but has contributed to the study can be listed in "Acknowledgements". The authors should declare in writing that the article is an original paper that has not been published before and that they will not attempt to publish it somewhere else during the evaluation process.

The authors are considered to have accepted any changes and corrections made by the editor as long as the scientific content is not changed. The articles sent are not returned whether published or not, and only images and figures that are not published can be returned to the author upon request.

Editorial Policies and Peer Review Process

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

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

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

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"Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals (ICMJE Recommendations)" (2013, http://www.icmje.org/). The ICMJE recommends that authorship be based on the following 4 criteria:

1. Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work;

2. Drafting the work or revising it critically for important intellectual content;

3. Final approval of the version to be published;

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.

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

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Readers can download articles, case reports and reviews free of charge for academic / educational use. The journal is free for everyone at any time. In order to achieve this, the journal benefits from the financial resources of Tekirdağ Namık Kemal University and the ongoing voluntary efforts of the editors and referees.

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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, is given to authors to retain control over the integrity of their work and the right to be properly acknowledged and cited.

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Address: Molla Gürani Mah. Kaçamak Sk. No: 21, 34093 Fındıkzade-İstanbul-Turkey

Phone: +90 212 621 99 25 Fax: +90 212 621 99 27

E-mail: info@galenos.com.tr

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Namık Kemal Medical Journal is the official journal of Tekirdağ Namık Kemal University Faculty of Medicine. Original articles, case reports, and short communications related to the either basic or extended clinical experience in medical sciences (i.e general medicine, basic medical sciences, surgical sciences) will be considered for publication. Review articles will be accepted upon request of the editorial board. The journal does not accept unsolicited review articles for consideration.

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Namık Kemal Medical Journal is a scientific journal that aims to reach all relevant national and international medical institutions and persons in print and electronic form free of charge.

Namık Kemal Medical Journal gives priority to publishing original research articles since it has the mission to announce and share the research to the international scientific circles in Turkey and to contribute to the promotion of Turkey in this context.

Namık Kemal Medical Journal, the Publisher, and the Editors assume no responsibility for the statements in the articles; authors carry the scientific and legal responsibilties of their own articles. The manuscript which is submitted to the journal must not contain previously published material or material under consideration for publication elsewhere. Accepted manuscripts become the property of Namık Kemal Medical Journal and may not be republished. All manuscripts will undergo peer review. A final review and a subsequent decision relative to publication will then be made by the editorial board of Namık Kemal Medical Journal.

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Publication Language

Articles are published in Turkish or English. In Turkish articles, the integrity of the Turkish language should be preserved and the Spelling Guide and Turkish Dictionary of the Turkish Language Association should be taken as basis.

In the use of medical terms, care should be taken to use “Turkish Science Terms” as much as possible. For this, the “Medical Terms Guide” of the Turkish Language Institution is the main source book, but when this book cannot be obtained, the “Country Medical Terms Dictionary” can be used. The authors are deemed to have accepted the changes to be made by the editor in Turkish articles, provided that no changes are made in the content.

English texts should be clear and accurate. In case the publication is accepted, the article is reviewed by the language editor of the journal.

Article Submission

All manuscripts should be submitted via https://jag.journalagent.com/nkmj/ online submission system.

Manuscript Evaluation

As a first step, manuscripts received are evaluated with regards to the instructions to authors. The approved articles are delivered to Editorial Board. The Editorial Board evaluates the context of articles and sends them to two referees in related fields. A final review and a subsequent decision relative to publication will then be made by the editor. The articles which are accepted by the Editorial Board, come in the list of publication and the authors of these articles are informed.

This journal uses double-blind peer review, which means that both the reviewer and author identities are concealed from the reviewers, and vice versa, throughout the review process. To facilitate this, authors need to ensure that their manuscripts are prepared in a way that does not give away their identity.

Manuscript Layout

Manuscripts sent to the editor to be published in the journal must be written on one side of the A4 page, in Arial, 10 pt, double spacing and 2.5 cm margins each. The abbreviations used should be written clearly in parentheses at the first mention in the text, and no special abbreviations should be made. Numerical data between 1-10 in the text should be indicated in writing (in both treatment groups, second day .....), with 10 and above numbers. However, numbers between 1-10, which have a descriptive tag, should be written in writing (.... 1 year) and the numbers at the beginning of the sentence (a fifteen-year-old female patient .....).

The title page, abstract, text, figures, tables and references of the article should be given in the same WORD file.

All visual elements except the table should be named as “Figure”. Table and Figure numbering (1,2,3 ..) should be made. The description text for the table should be placed above the table, below the figure for the figure. The abbreviations used in the table should be written clearly under the table.

The use of resources in the text should be indicated at the end of the quote and as superscript.

Title Page: On the title page; The title of the article in Turkish and English, the names and duties (academic titles) of the authors, the institution from which it was sent, if any, the institution supporting the study should be written. If the article has been communicated before in any congress, its place and date should be specified. In addition, the name, surname, addresses, telephone and fax numbers, e-mail address of the author to be corresponded and Ethics Committee Approval must be clearly written on this page.

Author’s name (Only the first letter is capital) surname (all text is capital) should be written clearly,

Abstracts: It should be prepared on a separate page in Turkish and English. The abstract should reflect the article, significant results should be given and their interpretation should be made. Abbreviations that are not explained in the abstract should not be used, and the source should not be shown. Abstracts should not exceed 250 words.

In research articles; Turkish and English abstracts should be divided and structured as follows:

Aim/Amaç, Materials and Methods/Gereç ve Yöntem, Results/Bulgular, Conclusion/Sonuç.

Structured abstract should not be used in reviews and case reports.

INSTRUCTIONS TO AUTHORS

Keywords: Turkish and English keywords should be found in accordance with the standards of "Index Medicus: Medical Subject Headings (MeSH)". (http://www.nlm.nih.gov/mesh/authors.html). Keywords should be given at least 3 and at most 5 on the page under the Turkish and English abstracts in all kinds of articles.

Sections: Original research articles should include Introduction, Materials and Methods, selection and description of the cases, technical information, Statistical Analysis, Results, Discussion, Study Limitations and Conclusion sections. Case reports should include Introduction, Case Report and Discussion sections.

After these chapters, "acknowledgements" can be written to those who contributed to the research or to the preparation of the article, if any. Acknowledgments are given at the end of the article before references. In this section, there are expressions of thanks for personal, technical and material assistance.

References

References should be numbered and listed according to the order in the text under the heading (References) at the end of the article. There should be no discrepancy between the list of references and the order they appear in the text.

References that have information obtained through another reference without the original appearance are not numbered, they are given in parentheses when necessary. Author (s) are responsible for the accuracy of the references. All references should be stated in the text.

The names of the journals are given in abbreviated form in accordance with Index Medicus. Journal names that are not included in the index are not abbreviated.

Examples for Writing Resources

For journals:

For the journal article in MEDLINE and abbreviated according to MEDLINE:

The first letters of the authors' surnames and names should be entered with a comma at the end without a full stop, and a period should be placed after the first name of the last author without passing to the title. If the authors are 6 authors and less, all of them should be written. If the authors are more than 6 authors, the first 6 should be written, then et al. should be used. Then the title of the article should be entered and a period should be placed at the end. The short name of the journal in MEDLINE is put at the end and a space is left after it is written; publication date semicolon; number in parenthesis, the starting number of the published pages are put in a colon, and the last page is written after the hyphen is inserted, but the numbers on the first page are not repeated on the last page.

Ozkan G, Ulusoy S, Guvercin B, Menteşe A, Karahan SC, Yavuz A. A new player in chronic kidney disease mineral and bone disorder: tenascin-C. Int J Artif Organs. 2015;38:481-7.

For books:

West JB: Respiratory Physiology (2nd ed). Baltimore: Williams and Wilkins, 1974; 72-5.

For chapters taken from the book:

Sagawa K: Analysis of the CNS ischemic feed back regulation of the circulation. In: Reeve EB, Guyton AC (eds), Physical Basis of Circulation Transport. Philadelphia: WB Saunders, 1967; 129-139.

For online articles:

Abood S: Quality improvement initiative in nursing homes: the ANA acts in an advisory role. Am J Nurs [Webcast]. 2002 Jun [citation 12.08.2002]; 102 (6). Access: http://www.nursingworld.org/AJN/2002/june/Wawatch.htm PMID: 12394070.

For the citations from the thesis:

Kulu A. Evaluation of Quality of Life After Surgical Interventions Applied to Patients with Bladder Tumors, Trakya University, Institute of Health Sciences, Department of Nursing. Master Thesis. 2010; Edirne.

For congress papers:

Felek S, Kılıç SS, Akbulut A, Yıldız M. A case of phylgellosis with visual hallucinations. XXVI. Turkish Microbiology Congress Abstract Book, 22-27 September 2000, Antalya, Mars Printing House, 1994, p.53-6.

In Turkish sources that are not included in Index Medicus, the above examples are followed, but journal names are written without abbreviation.

Tables and Figures

Tables and figures should be numbered according to the order of appearance in the text, and should be placed in the closest section with their explanations according to the place in the text. The information given in the table should not be repeated in the text, permission should be obtained for tables taken from other sources. Figures should be drawn professionally, photographed or presented as digital prints in photographic quality. The part of the photos and pictures to be printed should be at least 9x13 cm in size and 300 DPI resolution.

Symbols, arrows or letters should contrast with the background. The magnification rate and dyeing technique used should be specified in microscopic pictures.

If human photography is to be used, either they should not be identified from the photograph or written permission should be obtained (see Ethics section). Figures and pictures should be written at the bottom, together with (1, 2, 3, ..) numbers.

Publication Review Process

All articles (Original articles, Reviews, Case Reports and Letter to the Editor) are subject to the following process:

After the formal check, articles that comply with the journal's spelling rules are directed to the editor. All applications are evaluated by the responsible editor in terms of the journal's scope, purpose and compliance with the target audience. If the article sent to Namık Kemal Medical Journal is in conformity with the formal principles, the editor is directed to two independent reviewers by providing blindness between the reviewers and authors from Turkey and/or abroad.

If the reviewers deem necessary, they approve the publication after the changes requested in the article are made by the authors.

INSTRUCTIONS TO AUTHORS

Studies coming to the journal management system pass the pre-evaluation of the editor and the editorial board within 2 weeks at the latest;

Compliance with the scope and subject areas of the journal,

Compliance with the journal spelling rules (if any, article template, font, titles, submission and bibliography style, etc.)

Publication language (abstract, sufficiency of keywords, structured self-translation, etc.).

Studies that pass the pre-evaluation stage mentioned above by the editorial board are sent to at least 2 reviewers who are experts in their field, depending on the nature of the study.

Based on the nature of the work, the referees evaluate working on the standard evaluation form.

In addition, if the reviewers wish, they can submit notes on the full text, stating their suggestions and opinions, to the editorial board.

The deadline for reviewers evaluations is 6 weeks.

After the study is sent to the reviewer, it states to the editorial board whether it can be evaluated within 2 weeks at the latest. Rewievers who do not specify are removed from the study and a new reviewer assignment is made.

The reviewer opinions for the studies guide the editorial board to make the final decision. The final decision always belongs to the editorial board.

The reviewers can give opinions for the study in 4 ways;

- Publication is acceptable
- It can be accepted for publication after corrections (It is decided by the editorial board whether the corrections have been made or not)
- After revisions I would like to see it again (The work is taken into a second round evaluation after it is submitted by its authors)
- Unpublished (Rejection)

A 3rd or 4th reviewer can be appointed to work in line with the reviewer 's opinions, and this process works in the same way as the time specified above.

After the completion of the evaluation in line with the reviewer opinions, the reviewer opinions are reviewed by the editorial board (maximum 2 weeks). In this process, the aim is to adapt the articles of the authors to publication standards.

The editorial board makes the final decision in line with the reviewer opinions and suggestions and conveys the result to the study authors.

Accepted articles are sent to the correspondence author for proof control before the publication, and a reply must be given within 48 hours.

The responsibility of the ideas advocated in the articles belongs to the author.

The copyright of the published articles belongs to the journal and cannot be transferred, even partially, without the permission of the journal.

Plagiarism

Submitted articles are evaluated after plagiarism control. This evaluation contributes to the decision-making of the editorial group.

Final Checklist

1) Presentation page to editor
   a. Category of the article
   b. Information that it has not been sent to another journal
   c. Relationship with a sponsor or a commercial company (if any)
   d. Statistical control was made (for research articles)
   e. Checked in terms of English
2) Copyright transfer form
3) Permit certificate if previously printed material (text, picture, table) is used
4) Compliance with the principles of the Helsinki Declaration in the "material and method" section in the studies with human elements, the approval of the ethics committee from their institutions and the "informed consent" from the patients.
5) If the animal element is used, indicating its compliance with the principles of "Guide for the Care and Use of Laboratory Animals" in the "material and method" section.
6) Cover page
   a. The title of the article in Turkish and English (preferably one line each)
   b. Authors and their institutions
   c. Correspondence address, business phone number, GSM number, e-mail addresses of all authors
7) Abstracts: (Turkish and English)
8) Keywords: Between 3-5 (Turkish and English)
9) Acknowledgement
10) References

Ethic

Scientific Responsibility

The authors are responsible for the compliance of the articles with scientific rules.

All authors should have a direct academic and scientific contribution in the submitted article.

The name order of the authors should be a joint decision. All authors must indicate the author list on the copyright transfer form signed. The names of all authors should be included in the section under the title of the article.

All individuals who do not meet the sufficient criteria for authorship but contributed to the study can be listed in the "Acknowledgements/ Information" section. Written permission must be obtained from these individuals to be specified in the acknowledgment section.

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The authors are responsible for the compliance of the articles with the ethical rules.

Namık Kemal Medical Journal is a journal that has adopted the principle of complying with the ethical standards of the Human Experiments

INSTRUCTIONS TO AUTHORS

Committee (https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/), which was revised in 2013 in the 1975 Helsinki Declaration. Therefore, in the articles sent for publication, it must be stated that the ethical standards of the specified committee are complied with. In addition, approval letters from local or national ethics committees should be sent with the letter.

In the material and method section of the article, the authors must state that they carried out the study in accordance with these principles, and that they received "informed consent" from the ethics committees of their institutions and the people who participated in the study.

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Confidentiality and Confidentiality of Patients and Study Participants

Privacy cannot be impaired without permission from patients. Descriptive information such as patients' names, initials or hospital numbers, photographs and family tree information, etc. They are not published unless absolutely necessary for scientific purposes and the patient (or parent, or guardian) gives written informed consent.

The informed consent should also be stated in the article.

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The scanned form is loaded on the system during the online article submission steps, in the additional file upload step,

The works of the authors who do not submit the Copyright Transfer Form will definitely not be published.

International Study Design Guidelines

Preparation of research articles, systematic reviews and meta-analyses must comply with study design guidelines:

CONSORT statement for randomized controlled trials (Moher D, Schultz KF, Altman D, for the CONSORT Group. The CONSORT statement revised recommendations for improving the quality of reports of parallel-group randomized trials. JAMA 2001; 285: 1987-91) (http://www.consort-statement.org/);

PRISMA statement of preferred reporting items for systematic reviews and meta-analyses (Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 2009; 6(7): e1000097.) (http://www.prisma-statement.org/);

STARD checklist for the reporting of studies of diagnostic accuracy (Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig LM, et al., for the STARD Group. Towards complete and accurate reporting of studies of diagnostic accuracy: the STARD initiative. Ann Intern Med 2003;138:40-4.) (http://www.stard-statement.org/);

STROBE statement, a checklist of items that should be included in reports of observational studies (http://www.strobe-statement.org/);

MOOSE guidelines for meta-analysis and systemic reviews of observational studies (Stroup DF, Berlin JA, Morton SC, et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting Meta-analysis of observational Studies in Epidemiology (MOOSE) group. JAMA 2000; 283: 2008-12).

Correspondence Address:

Erdoğan Selçuk ŞEBER, MD

Tekirdağ Namık Kemal University Faculty of Medicine, Division of Medical Oncology, Tekirdağ, Turkey

Phone: +90 282 250 50 00 E-mail: nkmj@nku.edu.tr

NAMIK KEMAL MEDICAL JOURNAL

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ORIGINAL ARTICLES/ÖZGÜN ARAŞTIRMALAR

NAMIK KEMAL MEDICAL JOURNAL

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ORIGINAL ARTICLE

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Comparison of Biopsy, Computed Tomography and Magnetic Resonance Imaging in the Detection of Hepatosteatosis in Live Liver Donor Candidates

Canlı Karaciğer Donör Adaylarında Hepatosteatozun Saptanmasında Biyopsi, Bilgisayarlı Tomografi ve Manyetik Rezonans Görüntülemenin Karşılaştırılması

Bedriye KOYUNCU SÖKMEN[1], Tolga ŞAHİN[2], Alihan ORAL[3], Erdem KOÇAK[4]

[1]*Demiroğlu Bilim University, Gayrettepe Florence Nightingale Hospital, Clinic of Radiology, İstanbul, Turkey*
[2]*Gayrettepe Florence Nightingale Hospital, Clinic of Gastroenterology, İstanbul, Turkey*
[3]*Fenerbahçe University, Medicana Bahçelievler Hospital, Clinic of Internal Medicine, İstanbul, Turkey*
[4]*Kadıköy Florence Nightingale Hospital, Clinic of Gastroenterology, İstanbul, Turkey*

ABSTRACT

Aim: The presence of hepatosteatosis (HS) in the donor has negative effects on the results of liver transplantation (LT). Therefore, the detection of donor HS is vital during the pre-transplant period. The aim of this study was to compare the efficacy of liver biopsy and radiological methods in the detection of HS in live liver donor candidates.

Materials and Methods: Two hundred twenty-six healthy individuals who were admitted to Demiroğlu Bilim University as donor candidates for LT were included in the study. Demographic, histopathological, laboratory and imaging findings of the donors were retrospectively reviewed. Computed tomography (CT) and magnetic resonance imaging (MRI) scans of the donors were retrospectively reevaluated and liver fat measurements were recorded.

Results: 39% (88) of the patients were female and 61% (138) were male. In the study population, the mean age was 34.3±8.7 years, the mean weight was 78.0±12.6 kg, the mean height was 169.1±9.6 cm, and the mean body mass index was 27.2±4.0. 42% of donors had <5% HS, and 58% of donors had >5% HS in liver biopsy. Both CT and MRI showed significant correlations with biopsy in HS detection ($p<0.05$).

Conclusion: In our study, it was found that MRI correlated with biopsy as much as CT and could be used easily in the detection of HS. The use of MRI in liver donors may be more appropriate for donor health prior to transplantation.

Keywords: Liver transplantation, donor steatosis, hepatosteatoz, computed tomography, magnetic resonance imaging

ÖZ

Amaç: Karaciğer donöründe hepatosteatoz (HS) varlığı, karaciğer transplantasyonu sonuçları üzerinde olumsuz etkilere sahiptir. Bu nedenle, donörde HS'nin tespiti, nakil öncesi dönemde hayati önem taşımaktadır. Bu çalışmanın amacı, canlı karaciğer donör adaylarında HS'nin saptanmasında karaciğer biyopsisi ve radyolojik yöntemlerin etkinliğini karşılaştırmaktır.

Gereç ve Yöntem: Demiroğlu Bilim Üniversitesi'ne karaciğer transplantasyonu için donör adayı olarak kabul edilen 226 sağlıklı birey çalışmaya dahil edildi. Donörlerin demografik, histopatolojik, laboratuvar ve görüntüleme bulguları retrospektif olarak incelendi. Donörlerin bilgisayarlı tomografi (BT) ve manyetik rezonans görüntüleme (MRG) taramaları geriye dönük olarak yeniden değerlendirildi ve karaciğer yağ ölçümleri kaydedildi.

Bulgular: Hastaların %39'u (88) kadın, %61'i (138) erkekti. Çalışma popülasyonunda ortalama yaş 34,3±8,7 yıl, ortalama ağırlık 78,0±12,6 kg, ortalama boy 169,1±9,6 cm ve ortalama vücut kitle indeksi 27,2±4,0 idi. Karaciğer biyopsisinde donörlerin %42'sinde <%5 HS vardı ve donörlerin %58'inde >%5 HS vardı. Hem BT hem de MRG, HS saptamada biyopsi ile anlamlı korelasyon gösterdi ($p<0,05$).

Address for Correspondence: Bedriye KOYUNCU SÖKMEN MD, Demiroğlu Bilim University, Gayrettepe Florence Nightingale Hospital, Clinic of Radiology, İstanbul, Turkey
Phone: +90 505 714 83 55 **E-mail:** bedriyekoyuncu@yahoo.com **ORCID ID:** orcid.org/0000-0002-1470-803X
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Sonuç: Çalışmamızda MRG'nin BT kadar biyopsi ile ilişkili olduğu ve HS'nin saptanmasında rahatlıkla kullanılabileceği bulunmuştur. Karaciğer donörlerinde MRG kullanımı, iyonizan radyasyon içermemesinden dolayı nakil öncesi donör için daha uygun bir yöntem olabilir.

Anahtar Kelimeler: Karaciğer transplantasyon, donör steatoz, hepatosteatoz, bilgisayarlı tomografi, manyetik rezonans görüntüleme

INTRODUCTION

Liver transplantation (LT) is accepted as a revolutionary treatment option for end stage liver disease (ESLD). Donor hepatosteatosis (HS) is one of the major risk factors that adversely affect post-transplant outcomes. HS in donors is common in both deceased and living donor liver transplantation (LDLT). Many transplant centers generally accept cadaveric liver donors with HS up to 30%[1]. Similar criteria are used for LT from living donors. Nowadays, 10-30% HS levels are acceptable for many transplant centers in LDLT[2]. However, >60% liver HS is closely related to primary liver non-function (PNF) at recipients after LT[3,4] and prolongs the donor healing process after LT. Therefore, the detection of donor HS is one of the most important points for LDLT[5].

Currently, invasive liver biopsy is accepted as the gold standard method for the detection of HS but it has some limitations such as complications, high cost, and sampling errors. A non-invasive method is desirable in the diagnosis of HS in order to avoid the risk of liver biopsy. Moreover, histopathological evaluation may show significant differences among pathologists[6]. Many radiological methods have been used for the non-invasive detection of HS, such as ultrasound (US), computed tomography (CT), and magnetic resonance imaging (MRI). US is an easy, cheap, non-invasive and simple method for this purpose. Although the presence of HS can be detected with US, the rate of fatty accumulation in the liver cannot be measured quantitively. US has also some limitations depending on operator. It also has limited sensitivity, specificity and reliability in obese patients and relatively low levels of HS[7,8].

CT fat quantification has undesired radiation exposure for healthy donor candidates. This method is based on the reverse correlation between liver fat content and liver attenuation which is measured by reduced parenchymal attenuation values in Hounsfield units (HU). Many studies have evaluated the accuracy and sufficiency of CT scan in the detection of HS in living liver donor candidates[9-12].

MRI with different techniques was used to detect HS with high sensitivity and specificity. It is considered by many researchers as one of the most adequate methods for the noninvasive measurement of HS. However, MRI is an expensive and time-consuming radiological method and these disadvantages limit its usefulness[13,14].

The aim of this study was to compare the biopsy, CT and MRI findings in the detection and quantification of HS in live liver donor candidates.

MATERIALS AND METHODS

This study was approved by the institutional review board and protocol review committee of Demiroğlu Bilim University (2019-16-03). Two hundred and twenty-six live liver donor candidates with varying degrees of HS confirmed by biopsy between January 2004 and January 2019 were included in this retrospective study. Patients who had acute and/or chronic viral hepatitis (hepatitis A, B or C), autoimmune, drug-induced or metabolic liver disease, and whose CT and MRI were inadequate for measurements were excluded from the study. The demographic, laboratory, CT and MRI findings of the patients were retrospectively reviewed and recorded from the hospital central information system. All donor candidates underwent a non-contrast upper abdomen and post-contrast triphasic CT imaging protocol with 16-detector MDCT (multidetector CT). (Somatom Sensation - Siemens Medical Systems, Forchheim, Germany). HS evaluation was made from unenhanced CT sections. The median time interval between liver biopsy and imaging (MRI and CT) was 9 days (range, 0-128 days). MRI and CT scans of the patients were performed on the same day.

While <5% HS levels were accepted normal, >5% HS levels were accepted as fatty liver according to biopsy results. Liver attenuation index (LAI) was used to calculate the degree of HS. Density measurements were performed on average 20 region of interest (ROI) in the liver and 10 ROI in the spleen for the evaluation of HS. Areas away from the vascular structures were selected for density measurements in both organs. LAI was calculated by subtracting mean splenic density from mean hepatic density. LAI >5 was accepted as steatosis <5%, 5>LAI>-10 was accepted as steatosis between 5% and 30%, and LAI<-10 was accepted as steatosis >30% (Figure 1)[15].

MR cholangiopancreatography (MRCP) was performed to evaluate biliary anatomy and variations of all donors in preoperative period. MR images were obtained on a 1.5 Tesla MR device (Siemens Magnetom Symphony, 1.5 T MRI System, Erlangen, Germany) using a 4-channel abdominal coil. In phase (IP) and out of phase (OP) images were taken in order to evaluate liver fatty tissue during the MRCP examination.

Chemical shift imaging (CSI) protocol: IP and OP MR images were obtained in sagittal projection (IP time to repeat/time to echo (TR/TE): 118/5.27, OP TR/TE: 118/2.35). In CSI, the matrix selected was 270×512 mm, number of acquisition=1 and field of view=256×256 mm, the cross-sectional thickness was 5 mm,

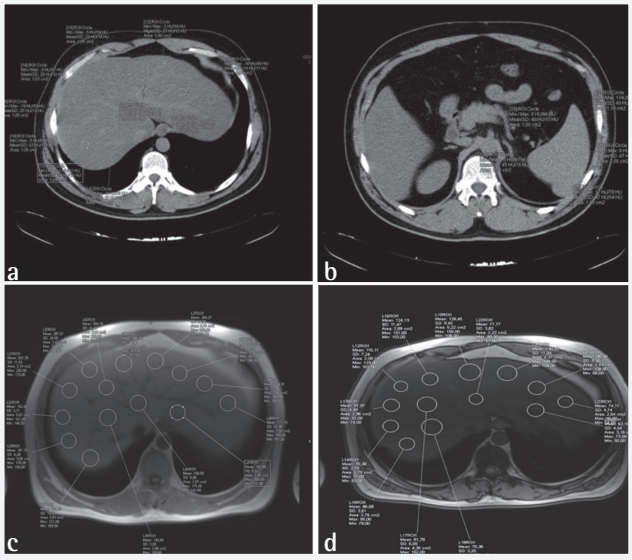

Figure 1. Images of a 35-year-old male who is a candidate for liver donor with hepatostaetosis. (a, b) Unenhanced transverse CT images are shown. HU was measured with ROI from the liver and spleen. The mean pancreatic and splenic CT attenuation was 37 and 47 HU, respectively. The liver attenuation index was found <-10 and was accepted as steatosis >30%. Check the mean SI for the mean measurements made on the MRI in-phase and out of-phase sequences (c, d). The percentage of hepatosteatosis found to be mean 165 SI (IP) and mean 75 SI (OP) was calculated to be 27%.

CT: Computed tomography, HU: Hounsfield unit, ROI: Region of interest, MRI: Magnetic resonance imaging, IP: In phase, OP: Out of phase, SI: Signal intensity

and the cross-sectional range was 0.5 mm. No contrast agents were applied during the examination.

The ROI was determined from IP and OP images and signal intensity (SI) measurements were performed quantitatively. ROIs were inserted avoiding from major intrahepatic vascular structures. An average of 20 measurements were taken from the liver parenchyma on IP and OP images. Then, liver fat ratio was calculated according to the following formula: Fat ratio=(IP-OP/2xIP)x100[16].

Statistical Analysis

SPSS 21.00 for Windows program was used for statistical analysis. As descriptive statistics, the number, percentage for categorical variables, and mean, standard deviation were given. Correlation analysis was performed using the Spearman correlation test. Data with normal distribution were calculated with the Student's t-test and data without normal distribution were calculated using the Mann-Whitney U test. Categorical data were calculated using the chi-square test. Significance level was accepted as $p<0.05$.

RESULTS

Two hundred and twenty-six donors were included in the study. Eighty-eight donors were female (39%) and one hundred and thirty-eight donors were male (61%). The mean age was 34.3±8.7 years, the mean height was 169.1±9.6 cm, and the mean weight was 78.0±12.6 kg. The mean body mass index (BMI) was 27.2±4.0 (Table 1). Complete blood count and laboratory findings of study population were shown in Table 2. The donors

Table 1. General demographic characteristics of the patients

	Mean±SD	Min-Max
Age	34.3±8.7	19-57
Gender		
Female	88 (39.0)	
Male	138 (61.0)	
Height (cm)	169.1±9.6	140-197
Weight (kg)	78.0±12.6	44-112
BMI	27.2±4.0	18-37.8

SD: Standard deviation, Min: Minimum, Max: Maximum, BMI: Body mass index, cm: Centimeter, kg: Kilogram

Table 2. Average laboratory findings of study population

	Mean±SD	Min-Max
Hb	14.4±1.6	9.9-18.1
WBC	7.15±1.91	3.36-12
PLT	248.6±63.4	135-622
INR	1.04±0.09	0.8-1.6
AST	18.5±5.7	10-41
ALT	22.8±14.1	3-103
ALP	72.8±24.4	6-242
GGT	20.8±15.7	3-111
Albumin	4.68±0.32	3.7-5.5
Total bilirubin	0.59±0.31	0.1-2.5
Bun	12.2±3.1	5-25
Creatinine	0.79±0.16	0.4-1.3
Na	140.1±2.2	135-146
K	4.4±0.3	3.5-5.5
FPG	95.2±9.5	71-168
Insulin	11.1±6.2	1.21-45.9
HbA1c	5.3±0.5	2.7-10
HOMA-IR	2.60±1.61	0.25-13.6
Total cholesterol	186.0±41.4	90-304
TGL	118.0±66.1	10-487
TSH	2.00±1.21	0.23-8.65
FT3	4.83±1.56	1.2-23
FT4	13.7±10.3	0.99-138

SD: Standard deviation, Min: Minimum, Max: Maximum, Hb: Hemoglobin, WBC: White blood cell, PLT: Platelet, INR: International normalized ratio, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, GGT: Gama glutamyl transpeptidase, ALP: Alkaline phosphatase, Na: Sodium, K: Potassium, FPG: Fasting plasma glucose, HbA1c: Hemoglobin A1c, HOMA-IR: Insulin resistance, TGL: Triglyceride, TSH: Thyroid stimulating hormone, FT3: Free T3, FT4: Free T4

were divided into groups according to HS levels according to the liver biopsy results. Considering histopathological results, 42% of the donors had <5% HS, 58% of donors had ≥5% HS. According to the LAI calculated from CT images, 83 (36.7%) donors had <5% HS and 143 (63.3%) donors had ≥5% HS. MRI showed ≥5% HS in 203 (89.8%) donors and <5% HS in 23 (10.2%) donors (Table 3). Biopsy sensitivities for HS were 63.3% for CT and 89.8% for MRI, respectively (Table 3).

Table 3. Liver steatosis levels in invasive and non-invasive methods for study population

		n	%
Liver steatosis level at biopsy	<5%	95	42.0
	5-10%	53	23.5
	10-15%	37	16.4
	15-20%	17	7.5
	20-25%	5	2.2
	25-30%	8	3.5
	30-35%	6	2.7
	>35%	5	2.2
Liver steatosis on CT	No (<5%)	83	36.7
	Yes (≥5%)	143	63.3
Liver steatosis on MRI	No (<5%)	23	10.2
	Yes (≥5%)	203	89.8

CT: Computed tomography, MRI: Magnetic resonance imaging

Then, CT and MRI were compared to invasive liver biopsy, which is accepted as the gold standard method for the detection of HS level. Both CT and MRI showed a strong correlation with biopsy both in the detection of fatty liver and in the calculation of the amount of HS (p<0.001 for CT and p=0.003 for MRI) (Table 4).

Correlations were found between liver biopsy and CT, between liver biopsy and MRI, and between CT and MRI in the Spearman correlation analysis (r=0.452, r=0.438 and r=0.614, respectively, p<0.001) (Table 5).

Table 4. Comparison of radiological methods and liver biopsy for the detection of liver steatosis level in study population

		Steatosis in CT				p
		No		Yes		
		n	%	n	%	
Steatosis level at biopsy	<5%	50	60.2	45	31.5	<0.001
	5-10%	19	22.9	34	23.8	
	10-15%	8	9.6	29	20.3	
	15-20%	3	3.6	14	9.8	
	20-25%	1	1.2	4	2.8	
	25-30%	1	1.2	7	4.9	
	30-35%	1	1.2	5	3.5	
	>35%	0	0.0	5	3.5	
		Steatosis in MRI				p
		No		Yes		
		n	%	n	%	
Steatosis level at biopsy	<5%	17	73.9	78	38.4	0.003
	5-10%	5	21.7	48	23.6	
	10-15%	0	0.0	37	18.2	
	15-20%	1	4.3	16	7.9	
	20-25%	0	0.0	5	2.5	
	25-30%	0	0.0	8	3.9	
	30-35%	0	0.0	6	3.0	
	>35%	0	0.0	5	2.5	

CT: Computed tomography, MRI: Magnetic resonance imaging

Table 5. Correlation analysis of biopsy, computed tomography and magnetic resonance imaging in the detection of fatty liver

	Steatosis at biopsy		Steatosis at CT %	
	Rho	p	Rho	p
Steatosis at CT %	0.452	<0.001		
Steatosis at MRI %	0.438	<0.001	0.614	<0.001

CT: Computed tomography, MRI: Magnetic resonance imaging

DISCUSSION

LT is still accepted as the best treatment modality for ESLD. Due to insufficient number of cadaveric donors, LDLT has become the primary treatment option in worldwide. Donor HS is one of the most important limiting factors affecting the outcomes of LDLT. Using fatty graft is closely associated with the increased risk of post-operative complications in both donor and recipient. Severe HS is found to be associated with the delayed hepatic regeneration and increased risk of PNF. Therefore, accurate detection of HS in donors is one of the key points for the LDLT process[1,2].

Liver biopsy is still accepted as the gold standard method to evaluate HS but it has also many disadvantages[6]. CT has been used at many critical points such as volume and remnant calculation, evaluation of vascular structures and determination of HS in live liver donor candidates. Several studies have shown a strong correlation between liver biopsy and CT scan in the detection of HS in liver donor candidates[9,10]. Nowadays, CT scanning is accepted as a reliable alternative method to biopsy in many transplant centers in live liver donors.

In our study, CT showed a high correlation with both biopsy and MRI in the determination of liver fat in live liver donor candidates, consistent with the literature. Nevertheless, CT also has its own disadvantages. Notably, high dose radiation exposure is a serious limiting factor for both adults and children. HS measurement at CT depends on hepatic and

splenic attenuations. Hepatic attenuation may be adversely affected by the presence of copper, glycogen, iron, edema, or fibrosis in liver parenchyma. In addition, iron overload may mask the real HS rate and lead to misdiagnosis of HS. Because iron overload may increase the attenuation in liver parenchyma, coexistence of fatty infiltration and iron overload may lead nearly normal attenuation in the liver. Although CT is closely related to biopsy on the detection of liver fat, histological evaluation of the liver is not possible with CT scan. The detection of steatohepatitis requires histopathological evaluation of the liver by an experienced pathologist and this can be a disadvantage for the detection of potential liver damage at donor candidates. CT attenuation values also vary among the different manufacturers, CT scanners, and CT generations. Finally, different CT scan parameters (such as tube current, voltage, step) and patient-dependent parameters (such as BMI, length) can be listed as the other limiting factors for HS assessment with CT[11].

There are different MRI techniques that have been shown to be effective to detect HS in many studies[14,17,18]. MRI includes multiple measurement techniques such as MR spectroscopy (MRS), MR elastography (MRE), and chemical shift MRI for HS assessment. In a recent meta-analysis by Zheng et al.[19] the strength and accuracy of these three different MRI techniques in the detection of HS have been demonstrated. Chemical shift MRI is a measurement method based on the decomposition of liver signals into water and fat signals. It allows the evaluation of whole liver parenchyma. Thus, it is accepted as the most accurate MRI method for the evaluation of HS in many studies[19-22]. With this assumption, in our study, the formula specified in the material and method section, which correlates well with MRS in the determination of HS, was used to calculate the percentage of fatty liver tissue[17,18]. In the literature, some studies with MRS have found low accuracy in the determination of HS[23]. In addition, MRS requires the installation of additional technical sequences and specific and expensive softwares that extend the MRI processing time. In some studies, it has been shown that MRE has low sensitivity in the detection of HS[24]. Therefore, chemical shift MRI seems to be the most accurate and feasible MRI method for the assessment of HS in current literature. In our study, the MRI images of donor candidates were retrospectively scanned, and their HS levels were determined by chemical shift MRI method. HS levels calculated with chemical shift MRI method were highly correlated with both invasive biopsy and CT scan. Our study confirmed the accuracy and sensitivity of chemical shift MRI method in the detection of HS in donor candidates.

HS can be seen in approximately 25% of live liver donor candidates[19]. Donor candidates with significant HS should be prepared for operation using dietary changes, physical exercises and medical treatments prior to transplantation. It is important to re-evaluate donor candidates who can lose weight through diet and other ancillary methods in terms of HS before transplantation and CT is the most common non-invasive method used in many transplant centers for this purpose. The use of recurrent CT scans in donor candidates also means giving high-dose and redundant radiation to healthy individuals. Future medical problems that can be caused by recurrent CT scans in donor candidates are uncertain. Therefore, MRI can be a useful alternative method with the same efficacy as CT, especially in donors requiring post-dietary liver fat control.

Calculation of proton density fat fraction (PDFF) is a recently described chemical shift-based water and fat separation technique that can be performed by magnitude and complex based techniques. The complex-based technique uses both magnitude and phase images, and magnitude-based techniques use only magnitude images for PDFF calculation. This is a promising method that can be completed in a breath hold and allows for the simple calculation of fat fraction in any segment of the liver. The advantage of this technique versus older MR imaging techniques (Dixon and fat saturation methods) is that this technique provides the correction of factors that influence MR SI, such as T1 bias, T2* decay, spectral complexity of fat, noise bias, and Eddy currents. This technique has been shown to provide accurate quantification of hepatic fat content compared to MRS[21,25-28]. Idilman et al.[29] found a good correlation in the comparison of PDFF measurements with biopsy results in their study. PDFF distinguished moderate or severe steatosis from mild or no steatosis with 93.0% sensitivity and 85.0% specificity.

In the meta-analysis performed by Gu et al.[30], the degree of hepatic steatosis corresponding to <5%, 5-33%, 33-66% and >66% steatosis was defined as 0, 1, 2 and 3 according to the Non-alcoholic Steatohepatitis Clinical Research Network histological scoring system for non-alcoholic fatty liver disease (NAFLD). This meta-analysis contains 6 original articles (635 patients) and has sufficient data to investigate the diagnostic performance of MRI-PDFF in steatosis classification. In this study, the summary AUROC values of MRI-PDFF in steatosis grades 0 versus 1-3, 0-1 versus 2-3, and 0-2 versus 3 were significantly higher, similar to previous studies. In addition, they found that with increasing liver fat content, overall sensitivity and specificity decreased, indicating lower accuracy of MRI-PDFF in patients with severe hepatic steatosis. In summary, this meta-analysis shows that MRI-PDFF is a sensitive and non-invasive diagnostic method for classifying the degree of hepatic steatosis in patients with NAFLD.

Study Limitations

Limitations of this study include possible population bias toward a cohort of individuals considering living related liver donation, its retrospective design and small sample size, and

wide range of time between liver biopsy and CT scanning. Furthermore, the liver biopsy specimens were acquired only from the right hepatic lobe, whereas the mean hepatic attenuation and SI were acquired from 10 ROIs of both hepatic lobes. Therefore, it is thought that there may be some degree of sampling error in hepatic needle biopsy.

CONCLUSION

Donor HS is a limiting factor for LDLT. CT is still an inevitable option for liver volume calculation and evaluation of vascular structures during the pre-transplant period. CT is also highly correlated with biopsy in the detection of HS. Donor safety is the most important ethical problem in LDLT. Recurrent CT scans for the evaluation of HS can lead to damage in donor bodies because of unnecessary radiation exposure in the following years. MRI showed its own strength and accuracy in the detection of HS in donor candidates in our study. Our study demonstrated that MRI might be a powerful alternative to CT for donor candidates with HS in the pre-transplant period, who needed to control HS after diet and other treatments. Future studies can provide new and beneficial findings in this issue.

Ethics

Ethics Committee Approval: This study was approved by Demiroğlu Bilim University institutional review committee and protocol review committee (2019-16-03).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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Survival Outcomes and Prognostic Factors in Salivary Gland Cancers Treated by Surgery and Adjuvant Radiotherapy

Cerrahi Sonrası Adjuvan Radyoterapi Gören Tükürük Bezi Tümörü Tanılı Hastalarda Prognostik Faktörlerin Değerlendirilmesi ve Sağkalım Sonuçları

Mürsel DÜZOVA[1], Tahir Sedat TURKAN[2]

[1]*Selçuk University Faculty of Medicine, Department of Radiation Oncology, Konya, Turkey*
[2]*Kadıköy Florence Nightingale Hospital, Clinic of Radiation Oncology, İstanbul, Turkey*

ABSTRACT

Aim: To evaluate the variables affecting prognostic factors in patients with salivary gland cancer who have received surgery followed by radiotherapy (RT).

Materials and Methods: Fifty-three patients with major and minor salivary gland cancer were treated with curative surgery and postoperative RT between 1993 and 2011. We evaluated patients with regard to overall survival, locoregional recurrence-free survival, disease free survival, and distant metastasis-free survival.

Results: The median follow-up period was 40 (8-174) months. The rates of local control, distant metastasis-free, disease-free survival and overall survival after 10 years were 65%, 57,7%, 38,8%, and 48,2%, respectively. In univariate analysis, histological subtype, histologic grade, extraglandular extension and delivered dose of RT were found to be as prognostic factors affecting locoregional recurrence-free survival; gender, histological subtype, extraglandular extension influenced disease-free survival; overall survival was only affected by age. In multivariate analysis, locoregional recurrence-free survival was affected by histologic grade and dose of RT (60 Gy< better prognosis); distant metastasis-free survival was affected by histological subtype; disease-free survival was affected by histological subtype, histologic grade, and lymph node status; overall survival was affected by lymph node status, extraglandular extension, and dose of RT (60 Gy< better prognosis).

Conclusion: Several prognostic factors affecting local control, distant metastases, and overall survival were found. Postoperative RT is an effective treatment modality that increases local control and overall survival in patients with Salivary Gland Carcinoma at doses over 60 Gy.

Keywords: Salivary gland cancer, radiotherapy, prognostic factor

ÖZ

Amaç: Postoperatif radyoterapi (RT) almış tükürük bezi tümörü tanılı hastalarda prognostik faktörleri etkileyen değişkenleri değerlendirmek amaçlandı.

Gereç ve Yöntem: 1993-2011 yılları arasında küratif cerrahi ve postoperatif RT ile tedavi edilen majör ve minör tükürük bezi kanserli elli üç hasta çalışmaya dahil edildi. Hastalar; genel sağkalım, lokorejyonel rekürrenssiz sağkalım, hastalıksız sağkalım ve uzak metastazsız sağkalım açısından değerlendirildi.

Bulgular: Medyan takip süresi 40 (8-174) aydı. On yıl sonra lokal kontrol, uzak metastazsız, hastalıksız sağkalım ve genel sağkalım oranları sırasıyla %65, %57,7, %38,8 ve %48,2 idi. Tek değişkenli analizde, histolojik alt tip, histolojik grade, ekstraglandüler yayılım ve RT dozu lokorejyonel rekürrenssiz sağkalımı etkileyen prognostik faktörler olarak bulundu. Cinsiyet, histolojik alt tip, ekstraglandüler yayılım hastalıksız sağkalımı etkileyen

Address for Correspondence: Mürsel DÜZOVA MD, Selçuk University Faculty of Medicine, Department of Radiation Oncology, Konya, Turkey
Phone: +90 530 567 75 60 **E-mail:** mursel_duzova@hotmail.com **ORCID ID:** orcid.org/0000-0003-1036-2593
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faktörler olarak bulunurken, genel sağkalım sadece yaştan etkilendi. Çok değişkenli analizde, lokorejyonel rekürrenssiz sağkalım histolojik grade ve RT dozundan (>60 Gy, daha iyi prognoz); uzak metastazsız sağkalım, histolojik alt tipten; hastalıksız sağkalım, histolojik alt tip, histolojik grade, lenf nodu durumundan; genel sağkalım, lenf nodu durumu, ekstraglandüler yayılma ve RT dozundan (>60 Gy, daha iyi prognoz) etkilenmiştir.

Sonuç: Lokal kontrolü, uzak metastaz gelişimini ve genel sağkalımı etkileyen çeşitli prognostik faktörler bulundu. Postoperatif RT, 60 Gy'nin üzerindeki dozlarda tükürük bezi karsinomu olan hastalarda lokal kontrolü ve genel sağkalımı artıran etkili bir tedavi yöntemidir.

Anahtar Kelimeler: Tükürük bezi kanseri, radyoterapi, prognostik faktör

INTRODUCTION

Salivary gland cancers (SGCs) are very rare cancer group. In the western countries 2.5-3 cases are diagnosed in approximately 100,000 per year. SGCs account for approximately 0.5% of all malignant tumors and 3-5% of all head and neck cancers[1]. And also, it accounts for approximately 11% of oropharyngeal cancers[2]. Most SGCs are determined in 6-7th decades[3].

SGCs are diverse according to origin and pathology. They are classified according to the 2017 World Health Organization, which lists 20 subtypes of benign and 10 subtypes of malignant histopathologic types[4]. The most common histopathologic types are mucoepidermoid carcinoma (MEC), adenoid cystic carcinoma (ACC), adenocarcinoma not otherwise specified and salivary duct carcinoma. Generally, they are divided into two groups as those originating from the major and minor salivary glands. Parotid glands are the most common site of major SGCs, followed by submandibular glands and sublingual glands. Also, minor salivary glands are the source of SGCs, representing for 9-23% of all salivary gland tumors. The most common location of minor salivary glands is within the mucosa of the hard palate[5-7]. However, parotid gland tumors are less likely to be malignant compared to other major and minor salivary gland tumors[1].

Among the treatment options, surgical resection with adequate free margin is the most important option. Elective nodal treatment of the N0 neck is still controversial. Postoperative radiotherapy (PORT) is an effective treatment modality in patients with high-risk factors such as high grade, poor prognosis histological subtype, advanced stage, etc. Little is known about chemotherapy in treatment strategy due to the small number of cases.

MATERIALS AND METHODS

Ethical Approval

The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and approval from a local committee on human investigation was obtained. The study protocol was reviewed and approved by Clinical Research Ethics Committee, with decision date no of 06/11/2014-235913. Written informed consent forms were read by each patient and signed approvals were obtained before their treatment.

Study Population

Between the years of 1993 and 2011, a total of 211 cases with SGC of any prior treatment status and stage were identified in our databases. Due to the fact that SGCs are rare in the population, histopathological subtypes vary widely, and the treatment regimens are varied due to the lack of standard approaches in their treatment, an attempt was made to obtain a homogeneous group as much as possible to minimize confusion. Therefore, patients who did not have metastasis at the time of diagnosis, who had curative surgery with no macroscopic residue, who did not receive neoadjuvant, adjuvant or concurrent chemotherapy, and who received definitive dose of RT postoperatively were selected and included in the study. Fifty-three of these 211 cases suited with inclusion criteria and they were retrospectively reviewed.

Inclusion and Exclusion Criteria

Inclusion criteria:

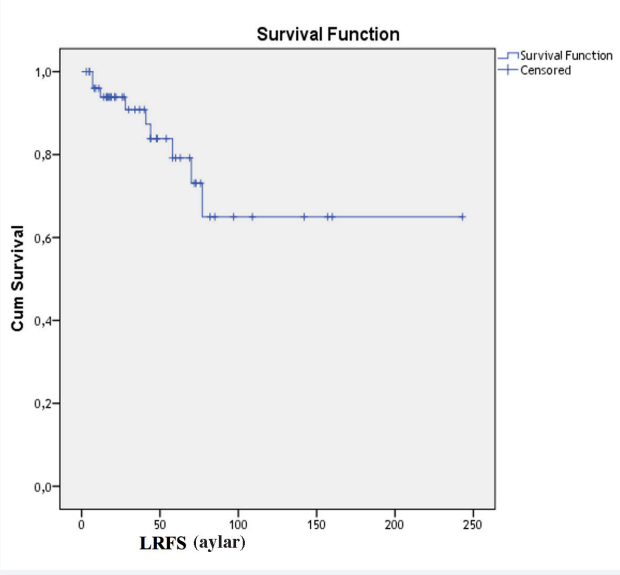
- Patients who were diagnosed with major or minor SGC,
- Patients who underwent surgery,
- Patients who received PORT,
- Patients without macroscopic residual mass after surgery,
- Patients who did not receive neoadjuvant, adjuvant or concurrent chemotherapy.

Exclusion criteria:

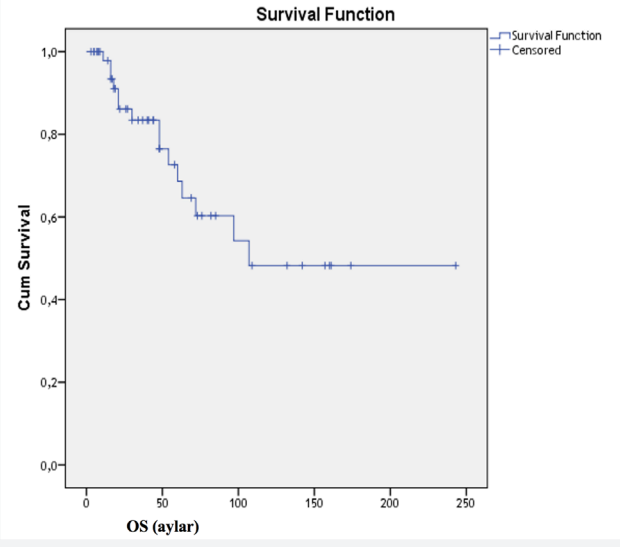
- Patients with relapse before RT,
- Patients who did not undergo surgery before RT,
- Patients who had a history of malignant disease priorly,
- Patients who developed second primer malignancy during follow-up,
- Patients who had metastasis before RT,
- Patients with macroscopic residual mass after surgery (R2 resection),
- Patients with immunosuppressive disease.

Statistical Analysis

Study data were analyzed using the statistical package program Statistical Package for the Social Sciences version 13.0. As descriptive statistics, numerics, percentage, standard deviation, average, and minimum and maximum values were used. Locoregional recurrence-free survival (LRFS), disease free survival (DFS), distant metastasis-free survival (DMFS), and overall survival (OS) were estimated using the Kaplan-Meier method (Graphic 1, 2). To identify prognostic factors that might influence survival, log rank tests were performed to examine the univariate associations between survival and parameters of interest and Cox regression analysis was performed to examine the multivariate associations. The value of $p<0.05$ was considered statistically significant.



Graphic 1. Locoregional recurrence-free survival



Graphic 2. Overall survival

RESULTS

Patient Characteristics

Of 53 patients, 40 (75.5%) had parotid, 6 (11.3%) had submandibular, 7 (13.2%) had minor salivary gland located tumors. Facial paralysis was presented in 16 (30.2%) patients with parotid gland tumors at the time of first presentation. The median age was 52 years (range 16-83 years). Twenty-eight (52.8%) patients were male and 25 (47.2%) were female. Histopathological distribution was like this; 13 (24.5%) patients had ACC, 10 (18.9%) patients had MEC, 9 (17%) patients had squamous cell carcinoma (SCC), 7 (13.2%) patients had adenocarcinoma, 5 (9%, 4) patients had acinic cell carcinoma, 3 (5.6%) patients had malignant mixed tumor, 2 (3.7%) patients had carcinoma ex pleomorphic adenoma, 1 (1.8%) patient had clear cell carcinoma, 1 (1.8%) patient had lymphoepithelioma, 1 (1.8%) patient had malignant eccrine poroma, 1 (1.8%) patient had malignant lymphoepithelial lesion, respectively (Table 1).

Treatment Outcome and Survival

We analyzed general results and investigated the potential prognostic variables of age, gender, tumor size and lymph node status, anatomic site, facial weakness, histological types, histological grade, extraglandular extension, resection margins, perineural invasion, and radiotherapy (RT) dose. The date of diagnosis was accepted as the date of histological diagnosis of SGC. The last follow-up date was the date of the last consultation. All tumors were classified by tumor (T), lymph node (N), and metastasis staging system, seventh edition (International Union Against Cancer, 2009). All patients underwent surgery. In forty of 53 patients, tumors were located in the parotid gland. Among 40 patients with parotid gland tumors, total parotidectomy was performed for 14 (26.4%), partial parotidectomy for 3 (5.6%), superficial parotidectomy for 3 (5.6%), total parotidectomy with neck dissection for 19 (35.8%), and superficial parotidectomy with neck dissection for 1 (1.8%) patient. In cases of submandibular gland tumors, wide excision was performed for 4 (7.5%) patients and wide excision with neck dissection was performed for 2 (3.7%) patients. In cases of minor salivary gland tumors, mass excision was performed for 5 (9.4%) patients, mass excision with neck dissection was performed for 1 (1.8%) patient. Totally, neck dissection was performed for 24 (45.2%) patients. Fourteen (26.4%) of the cases were with node involvement.

Only patients who received definitive PORT were included in the study. RT indication was decided according to whether the patients were at high risk or not. Patients having T stage

T3-T4 tumor, and/or lymph node positivity, and/or perineural or lymphovascular invasion, and/or positive surgical margins, and/or high-grade tumor were considered high-risk patients, and those who had one or more of these features were treated with PORT. RT period was median 42 days (range 33-61). In 35 (66%) patients, RT was performed only to the postoperative tumor bed. In 18 patients, neck region was also included in RT treatment site. The median 50 Gy (46-66 Gy) was delivered to neck region and 60 Gy (50-70 Gy) to postoperative tumor bed.

None of the patients received concurrent, adjuvant or neoadjuvant chemotherapy. The median follow-up was 40 months (range 8-174). Patients were followed up after 4-6 weeks following the completion of treatment and then every 3 months for 2 years. The patients were then followed up every 6 months. Physical examination and head and neck/thorax computed tomography scan was performed during follow-up visit when necessary.

The 5-year and 10-year LRFS, DMFS, and DFS rates were 79.2% and 65%, 77% and 57.7%, 61.6% and 38.8%, respectively.

Table 1. Patients characteristics

Parameter	Number of patients (%)
Age	
60 y>	31 (58.5)
60 y≤	22 (41.5)
Gender	
Male	28 (52.8)
Female	25 (47.2)
Facial paralysis	16 (32.2)
Location	
Parotid gland	40 (75.5)
Submandibular gland	6 (11.3)
Minor salivary gland	7 (13.2)
Histological subtype	
Adenoid cystic Ca	13 (24.5)
Mucoepidermoid Ca	10 (18.9)
Squamous cell Ca	9 (17)
Adeno Ca	7 (13.2)
Acinic cell Ca	5 (9.4)
Other subtypes	9 (17)
Tumor diameter	
4 cm>	27 (51)
4 cm≤	26 (49)
Lymph node involvement	14 (26.4)
Extraglandular extension	10 (18.9)
Dose of RT	
60 Gy	32 (60.4)
60 Gy>	14 (26.4)
60 Gy<	7 (13.2)

RT: Radiotheraphy

The 5-year and 10-year OS rates were 68.6% and 48.2%, respectively. Twenty patients (37.7%) had recurrence (only locoregional failures in 7, only distant failures in 11, and both locoregional and distant failures in 2 patients). The sites of distant metastases included the lungs (n=9), bone (n=3) and brain (n=1). Of nine patients who developed locoregional recurrence, 4 had MEC, 2 had ACC, 2 had adenocarcinoma, and 1 had SCC histological subtype.

Prognostic Factors

Univariate analysis revealed that extraglandular extension (p<0.007), dose of RT (p<0.006), histological subtype (p<0.019) and histological grade (p<0.005) were significant prognostic factors on LRFS. And also, histological grade (p<0.038) and dose of RT (p<0.019) were significant prognostic factors on LRFS in multivariate analysis (Table 2, 3).

The factors that affecting OS were age (60 y≤, poor prognosis) (p<0.014) in univariate analysis and extraglandular extension (p<0.033), dose of RT (p<0,04), and lymph node involvement (p<0.024) in multivariate analysis. In addition, histological

Table 2. Significant prognostic factors in univariate analysis

Overall survival	Age (60 y≤, poor prognosis)	p<0.014
Locoregional recurrence-free survival	Histological subtype (MEC, poor prognosis)	p<0.019
	Histological grade	p<0.005
	Extraglandular extension	p<0.007
	Dose of RT (60 Gy<, better prognosis)	p<0.006
Distant metastasis-free survival	No	No
Disease-free survival	Gender (male, worse)	p<0.037
	Histological subtype (MEC, worse)	p<0.032
	Extraglandular extension	p<0.004

MEC: Mucoepidermoid carcinoma

Table 3. Significant prognostic factors in multivariate analysis

Overall survival	Lymph node status	p<0.024
	Extraglandular extension	p<0.033
	Dose of RT (60 Gy<, better prognosis)	p<0.040
Locoregional recurrence-free survival	Histological grade	p<0.038
	Dose of RT (60 Gy<, better prognosis)	p<0.019
Distant metastasis-free survival	Histological subtype (MEC, poor prognosis)	p<0.028
Disease-free survival	Histological subtype (MEC, poor prognosis)	p<0.031
	Histological grade	p<0.015
	Lymph node status	p<0.013

MEC: Mucoepidermoid carcinoma

grade (p<0.057) showed a trend to be significant in multivariate analysis (Table 2, 3).

Histological subtype was the only factor affecting DMFS in multivariate analysis (p<0.028). The factors affecting DFS were gender (p<0.037, male poor prognosis), histological subtype (p<0.032), and extraglandular extension (p<0.004) in univariate analysis; histological subtype (p<0.031), histological grade (p<0.015), and lymph node involvement (p<0.013) were found statistically significant in multivariate analysis (Table 2, 3).

DISCUSSION

In the past, surgery was the primary treatment for SGCs, and the concept of PORT was controversial. The reason for this was the lack of studies comparing only surgery and surgery with PORT. Although there is currently no randomized study on this subject, it has been shown that local control has increased with the addition of RT to surgery as a result of many retrospective studies in recent year[8-12]. Still today, there are no published prospective studies evaluating prognostic factors in SGCs. There are several studies that show multivariate analysis results with a large number of patients diagnosed with major and minor SGCs[10-15]. Studies on SGCs were mostly performed by evaluating major salivary glands[9,16,17], minor salivary glands[18-21] or more often, parotid gland tumors[10,11,22-26].

In this study, various factors affecting LRFS, DFS, OS, and DMFS were evaluated in patients diagnosed with major and minor SGCs, who only received surgery followed by PORT.

Distant metastasis was detected in 13 (24.5%) patients. This rate is between 15% and 37% in some studies[14,16,27-29]. In the analyzed group of patients, distant metastasis development rate was found to be compatible with that in articles. As in the study of Renehan et al.[14], similarly, most of metastases occurred in the lung with 9 (69%) patients, 3 (23%) in the bone, and 1 (8%) in the brain.

In recent study, 10-year DFS rate was 38.8%. Similarly, this rate ranges between 37% and 69% in various studies[5,14,16,29,30]. Thus, the DFS rate, in our study, was found to be compatible with the literature.

In the current study, 9 (16.9%) patients developed local recurrence. This rate was found similar to the studies of Fitzpatrick and Theriault[29] and Borthne et al.[30]. However, higher rates have also been reported in some studies. In the study of Fu et al.[28], which included 100 patients with SGCs, the 5-year recurrence rate was 28%. In a historically important study published by Spiro[27] in 1986, the rate of locoregional recurrence was found to be as high as 39% in the parotid gland, and 60% in the submandibular gland and minor salivary gland tumors. However, in this study, most patients received only surgical treatment. Nevertheless, in another study published by Spiro et al.[16], lower recurrence rates were reported (local recurrence rate was 21%, and regional recurrence rate was 10%). The difference of this study from the previous study was that more patients received RT postoperatively. Similar results were also found in the study of Armstrong et al.[12] which showed that while the locoregional control rate was 69% in the arm of PORT, it was lower (40%) in the arm of only surgery. However, in our study, the 5-year locoregional control rate was 79.2%, which was slightly higher than in many studies. May be, the reason of this is that all selected patients in the recent study received both treatment modalities, surgery and PORT.

In the current study, the location of most tumors was in the parotid gland at a rate of 75.5% (40 patients), as mentioned in the literature, and 7 (13.2%) patients' diseases were in the minor salivary glands and 6 (11.3%) in the submandibular glands, respectively. Also, in a Swedish epidemiological study[31], which included 3305 patients, similar rates were detected (58% in parotid, 11% in submandibular, 23% in minor salivary gland location). In the analyzed group of patients, 3 of 7 patients diagnosed with minor SGCs were located in the maxilla, 1 in the nasal cavity, 1 in the tonsil, 1 in the hard palate, 1 in the mouth floor. These findings were incompatible with some studies in the literature. Conversely, in some studies[18,27], the most common location of minor SGCs appeared in the palate.

In our cohort, lymph node involvement was detected in 14 (26.4%) patients. Thirteen of these 14 patients' tumor sites were in the parotid gland and 1 in minor salivary gland. None of the patients with lymph node involvement was detected in submandibular gland. However, in various studies, lymph node involvement rate in the submandibular gland tumor was reported to be between 19% and 25%[32,33]. In the present study, the rate of developing metastasis to the lymph node in patients with tumors located in the parotid gland was 32.5%, which was similar or close to many studies' results in some published articles[10-12,22,26].

In the current study, the location of tumor was not found as a prognostic factor affecting LRFS, OS, DFS, and DMFS in either univariate analysis or multivariate analysis. While these results are similar to those in some studies in the literature[29], opposite results have also been reported in some other studies[15,34]. In the study of Vander Poorten et al.[22], it was found that histological grade in parotid gland tumors had no effect on DFS. However, in this study, grade was divided into two categories as high grade and low grade; moreover, MEC and acinic cell carcinoma were classified as low grade. These results differed from the results of the studies of Kane et al.[25] and Frankenthaler et al.[11]. In these two studies, ACC was included in the low-grade group and high-grade tumors associated with decreased survival. In the analyzed group of patients,

histological grade was related to LRFS in univariate analysis (p<0.005) and close to significance (p<0.06) on DFS, and in multivariate analysis, it was related to LRFS (p<0.038) and DFS (p<0.015), and close to significance on OS (p<0.057).

In the present study, most common histological subtype was ACC in 13 (24.5%) patients. In these 13 patients, the tumor location was in the submandibular gland in 5, in the minor salivary gland in 5 and in the parotid gland in 3. These rates were similar to the rates of other studies[31,32,34]. In our cohort, the lymph node involvement rate of the patients with ACC was 7.6%, which is similar to some other publications[10,33]. In the current study, the rate of perineural invasion was found to be 33.9%, similar to a very large patient-numbered study by Westergaard-Nielsen et al.[5]. While ACC histology may remarkably show local recurrence by neural invasion, in our study, ACC histology was not found to be a factor reducing local control as in the study of Terhaard et al.[34] This may be due to the combined treatment modality being used. As in a large patient-numbered study[35], acinic cell carcinomas were more frequently observed in the parotid gland (in our study, all 5 acinic cell carcinomas were noticed in the parotid gland). In the study of Hoffman et al.[35] including 1353 cases, lymph node involvement was found around 10% in tumors with acinic cell histology and distant metastasis rates ranged from 3% to 17% in various studies[34,35]. In the analyzed group of patients, no lymph node or distant metastases were detected in any of the 5 patients with acinic cell histology.

In the current study, 10 (18.9%) patients had MEC histology. All of them originated from the parotid gland and it constituted 25% of parotid gland tumors. This rate had a wide range of 10% to 38% in various studies[10,11,16,22,23,25,30,31]. In our study, lymph node involvement was present in 50% of patients diagnosed with MEC. This result was slightly higher than the rates (14-45%) in the literature[12,33,36,37]. Additionally, distant metastasis developed in 30% of patients in the recent study. This rate was lower (16%) in the study of Terhaard et al.[34]. Over and above this, in the current study, MEC histology was found to be associated with distant metastasis development and DFS in multivariate analysis.

All 9 (17%) cases with SCC histology were observed in the parotid gland. In general, these tumors were observed in older men. In the patients with SCC histology, lymph node involvement was around 22% in the present study. In the study of Terhaard et al.[34], a higher rate of 30% was found. Because of the high lymph node metastasis rate of SCC histology, the authors generally recommend elective neck treatment. In addition, distant metastasis rate was found as low as 11%. In the study of Terhaard et al.[37], the 10-year distant metastasis rate was found to be 35%.

In the analyzed group of patients, histological subtype was found to be one of the factors affecting LRFS and DFS in univariate analysis. In multivariate analysis, it was found that MEC histology was associated with distant metastasis development and DFS. Apart from the study of Therkildsen et al.[15], in many publications, histological subtype was not found as an independent factor affecting either locoregional control[13,26] or distant metastasis development[11]. While some studies[9,10,14,16,19,20] have found histological subtype prognostically important, on the contrary, in some studies[11,13,22,25,26], it is prognostically not important.

In the present study, while age was the only factor affecting OS in univariate analysis, it was not a prognostic factor in multivariate analysis. Although the male and female ratios were almost close to each other, women generally seemed to get diagnosed with SGC at younger ages and more likely to have histologies of ACC, adenocarcinoma, and acinic cell carcinoma. While gender was found as a prognostic factor affecting DFS in univariate analysis, no significance was found in multivariate analysis. In the study of Terhaard et al.[34], gender was found to be an independent factor for distant metastasis development. In some studies[25], male gender was associated with low OS rate.

According to the results of some studies, facial nerve dysfunction in parotid tumors is a prognostic factor affecting LRFS[26] and DFS[11,22,26]. However, in our study, it was not found as a factor affecting prognosis.

In the current study, when creating patient groups, we evaluated the tumor diameter by 4 cm and above and below 4 cm, and evaluated the lymph node status as positive or negative. In univariate analysis, we could not find significance in both parameters. In multivariate analysis, we found lymph node status as a statistically significant factor on OS and DFS. According to most studies in the literature, nodal stage is an independent factor associated with DFS[10,11,19,22,25,26]. In the study of Terhaard et al.[34] T and N stages were found to be independent prognostic factors for the development of distant metastasis. At the same time, they found a relationship between local control and T stage, and regional control and N stage.

Although surgical margin is found to be as an independent factor affecting prognosis in some studies[15,23,34], in this limited-numbered cohort, it was not found to be as a factor affecting prognosis neither in univariate analysis nor in multivariate analysis.

Extraglandular extension was found to be a prognostic factor affecting LRFS and DFS in univariate analysis, and OS in multivariate analysis, as one of the remarkable results. In some studies, extraglandular extension was found to be an independent prognostic factor affecting local recurrence

development[34] and OS[23,34]. While no prognostic effect of perineural invasion was demonstrated in recent study, in the study of Terhaard et al.[34], perineural invasion was found to be an independent prognostic factor that increased the risk of distant metastases by 2.2 times.

All patients included in our study were selected only from patients who received PORT. Since all patients underwent only PORT, they were not compared to any other treatment modality. RT generally increased both local control and OS in retrospective series with large numbers of patients, comparing only surgical and PORT treatment modalities[8,11,12].

In head and neck cancers, the time interval between surgery and RT is important for locoregional control. This does not seem to be very valid for salivary gland tumors[36]. In the study group, the period from surgery to RT onset was median 57 days (range 25-155), and it was not detected as a prognostic factor.

In most studies, no significant correlation was found between received dose of RT and prognosis. However, in our analyzed group, dose of RT was found to be an independent prognostic factor affecting LRFS ($p<0.019$) and OS ($p<0.040$). Particularly, this effect became evident at doses over 60 Gy. Many factors are taken into account when choosing dose of RT to be delivered. Particularly, resection margin status is a very critical issue that is taken into account when deciding on delivered dose of RT[37]. In the study of Garden et al.[26], a trend towards higher local control was observed at doses of 60 Gy and above. Some authors recommend applying a radiation dose of 65 Gy and over for high-risk (incomplete resection) patients and 70 Gy RT for gross residual disease[37].

Study Limitations

Due to the rarity of SGCs in the population, a small number of samples were included in the study. The study was conducted in a single center, and it was a retrospective study. These situations are the main limitations of the study.

CONCLUSION

PORT increases both local control and OS with higher doses of delivered RT (60 Gy≤). Additionally, histological subtype, histological grade, lymph node involvement and extraglandular extension were determined as independent factors affecting prognosis. Institutions dealing with health sciences should collaborate to design prospective randomized studies on diseases with low frequency in the population. Multi-institutional randomized clinical trials are needed in this regard.

Ethics

Ethics Committee Approval: The study protocol was reviewed and approved by İstanbul University Cerrahpaşa-Cerrahpaşa Faculty of Medicine Clinical Research Ethics Committee, with decision date no of 06/11/2014-235913.

Informed Consent: Written informed consent forms were read by each patient and signed approvals were obtained before their treatment.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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ORIGINAL ARTICLE

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Comparing the Use of Subgaleal and Subdural Drain in Non-acute Subdural Hematomas: Does the Hematoma Age Affect the Results?

Akut Olmayan Subdural Hematomlarda Subgaleal ve Subdural Diren Kullanımının Karşılaştırılması: Kanama Yaşı Sonuçları Etkiler mi?

Kutsal Devrim SEÇİNTİ

Kahramanmaraş Sütçü İmam University Faculty of Medicine, Department of Neurosurgery, Kahramanmaraş, Turkey

ABSTRACT

Aim: Surgical treatment of non-acute subdural hematomas is to place a closed drainage system in the subdural area following burr-hole trepanation, but it has tendency to drain related complications. Subgaleal drain is also suggested as an alternative, but there is no consensus yet. The aim of this study is to examine the effect of hematoma age and drain insertion style on surgical outcomes.

Materials and Methods: The data of 79 patients were retrospectively analyzed. The patients were divided into two groups as "subdural drain" and "subgaleal drain". Each group was further subgrouped as "chronic hematoma" and "subacute hematoma". They were compared in terms of the capacity of draining the hematoma and complications such as pneumocephalus, recurrence and others. The effect of hematoma age on results was examined.

Results: It was determined that hematoma could be evacuated more effectively in the subgaleal drain group (p=0.045). It was found that in subacute hematomas, subgaleal drain resulted in more recurrence, but prevented drain-related complications. Subgaleal drain was found to be acceptable for chronic subdural hematomas.

Conclusion: The use of subgaleal drain may be an option to avoid drain-related complications. However, if the hematoma is subacute, the rate of recurrence increases.

Keywords: Subdural, hematoma, drain, subgaleal, recurrence

ÖZ

Amaç: Kronik ve subakut subdural hematomların kabul görmüş cerrahi tedavisi burr-hole trepanasyonu takiben subdural alana kapalı diren sistemi yerleştirmektir. Direne bağlı komplikasyonlara açık olan bu yönteme alternatif olarak direnin subgaleal alana yerleştirilebileceği de önerilmektedir, ancak henüz bu konuda görüş birliği sağlanamamıştır. Bu çalışmada amaç, kronik veya subakut subdural hematomun cerrahi tedavisinde kanamanın yaşının ve direnin subgaleal alana veya subdural alana yerleştirilmesinin cerrahi başarı üzerindeki etkisini incelemektir.

Gereç ve Yöntem: Kriterlere uyan ve takiplerine eksiksiz ulaşılabilen 79 hastanın verileri geriye dönük incelendi. Hastalar, kullanılan cerrahi yönteme göre "subdural diren grubu" ve subgaleal diren grubu" olarak iki gruba ayrıldı. Her bir grup ayrıca kendi içinde "kronik kanaması olanlar" ve "subakut kanaması olanlar" olarak iki alt gruba daha ayrıldı. Gruplar, uygulanan yöntemin kanamayı boşaltabilme kapasitesi, pnömosefali, rekürrens ve diğer komplikasyonlar açısından kıyaslandı. Kanamanın yaşının sonuçlara etkisi incelendi.

Bulgular: Subgaleal diren grubunda kanamanın daha etkili şekilde boşaltılabildiği belirlendi (p=0,045). Subakut kanamada subgaleal direnin daha fazla rekürrens ile sonuçlandığı ancak direne bağlı komplikasyonları önlediği fark edildi. Kronik kanaması olan hastalarda ise subgaleal diren yerleştirilmesinin kabul edilebilir olduğu saptandı.

Address for Correspondence: Kutsal Devrim SEÇİNTİ MD, Sütçü İmam University Faculty of Medicine, Department of Neurosurgery, Kahramanmaraş, Turkey
Phone: +90 505 605 76 92 **E-mail:** devrimsecinti@yahoo.com **ORCID ID:** orcid.org/0000-0003-4345-0805
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Sonuç: Direne bağlı komplikasyonlardan kaçınabilmek için subgaleal diren kullanılması seçenek olabilir. Ancak subakut hematom varlığında rekürrens oranı artmaktadır.

Anahtar Kelimeler: Subdural, hematom, diren, subgaleal, rekürrens

INTRODUCTION

Symptomatic subdural hematomas are very common in neurosurgery practice and are predominantly detected in elderly patients. Both the increase in life expectancy in developed countries and the more accessible and more frequent use of antiplatelet and antiaggregant drugs cause an increase in chronic subdural hematoma cases[1,2]. Surgery is generally recommended when subdural hematoma becomes symptomatic. The recommended treatment in chronic or subacute subdural hematoma surgery is evacuation of the hematoma with a burr-hole craniostomy and closed drainage system[3]. Surgical complications are generally stated as infection, acute hematoma, seizure, symptomatic hygroma, and symptomatic pneumocephalus[4]. It is also known that this method, which has proven to be quite effective, can cause epileptic seizure due to cortex irritation or motor deficit and parenchymal hematoma due to cortex injury[4,5].

In recent years, the method in which the drain is placed in the subgaleal (subperiosteal) area instead of the subdural area following burr-hole opening has attracted attention[5-7]. Because placing the drain in the subgaleal area seems to protect the physician and the patient from these complications[6,7]. It is claimed that it is equal to or superior to subdural drain placement in terms of both complications and capacity to drain hematoma[5-7]. Although there are studies focused on this subject in the literature, the effect of the hematoma age on the results has not been examined before.

In our clinic, mainly subgaleal drain application has been performed since 2018 in order to avoid complications related to the drain. Burr-hole diameters are kept the same as those in the classical method and blunt-ended soft silicone drains are used as standard. Regardless of the number of burr-holes, a single drain is always used for the same hemisphere. In patients undergoing two burr-hole craniostomies on the same side, a single drain is placed under the skin from back to front, crossing both burr-hole openings. Our clinical observations are that our surgical success has not changed, but our complication rates have decreased. The presented study was carried out with the aim of confirming these observations.

MATERIALS AND METHODS

Patient Selection and Determination of Groups

Following the approval document obtained from the Local Ethics Committee of Kahramanmaraş Sütçü İmam University (decision no: 2020/17-04), 138 patients who underwent surgical drainage with the diagnosis of subdural hematoma in our clinic between October 2017 and June 2020 in accordance with the Principles of the Declaration of Helsinki were retrospectively reviewed. Patients with acute subdural hematoma, patients who underwent craniotomy despite having subacute or chronic subdural hematoma, and pediatric patients with subdural hygroma or subdural hematoma due to shunt ovarian drainage were not included in the study. In order not to cause confusion in the discussion and interpretation of the data, patients who underwent bilateral surgery were also excluded from the study. Seventy-nine patients who met the criteria and whose data were fully accessible were included in the study. It was determined that 33 patients were treated with subdural drain (Figure 1) and 46 patients were treated with subgaleal drain (Figure 2). The patients were further divided into two more subgroups according to the stage of their hematoma as "those with subacute hematoma" and "those with chronic hematoma". Thus, it was aimed to determine whether the hematoma age affects the surgical success or not. Demographic data of the patients are summarized in Table 1.

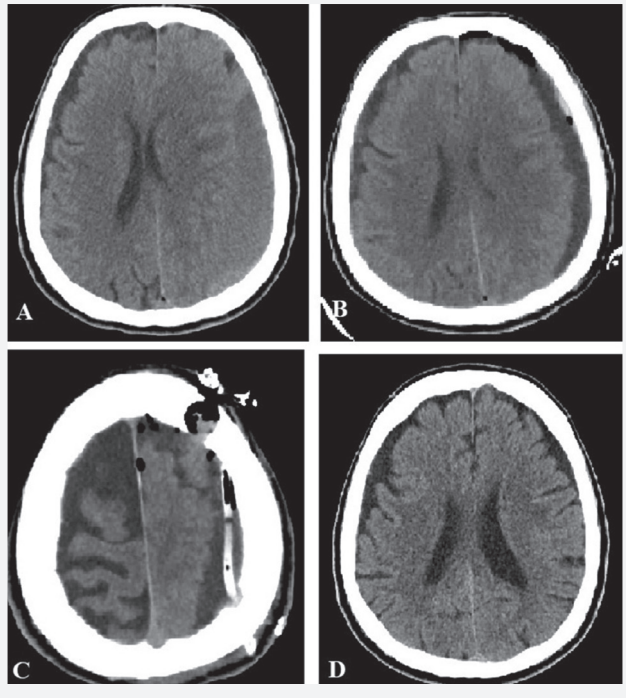

Figure 1. Cranial tomography images of a patient treated with a subdural drain. A) Preoperative hematoma status, B and C) Images obtained on the 3rd postoperative day [C) appearance of the drain in the subdural area], and D) Cranial tomography image at the 1st month postoperatively

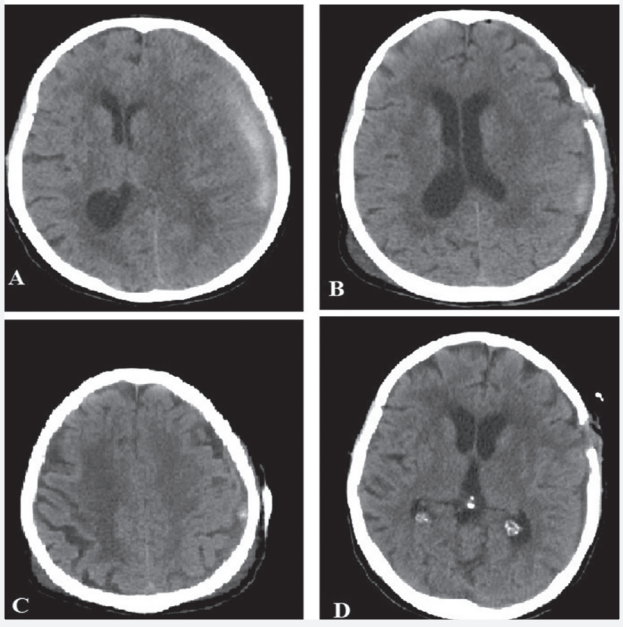

Figure 2. Cranial tomography images of a patient treated with subgaleal drain. A) Preoperative hematoma status, B and C) Images obtained on the 3rd postoperative day (position of the drain in the subgaleal area), and D) Cranial tomography image at the 1st month postoperatively

Measurements

The blood volumes at the time of admission, the blood volumes on the third postoperative day and the first month postoperative blood volumes of all patients were calculated from tomography images. The surgical results of the patients were evaluated in terms of insufficient drainage, recurrence, pneumocephalus, and the capacity of the techniques to drain blood. In addition, the effects of patient age and gender on surgical outcomes were also examined. X.Y.Z/2 formula, which is one of the accepted classical methods, was used in the calculation of blood volume and pneumocephalus volume[8].

Statistical Analysis

All data were analyzed with the Statistical Package for the Social Sciences v21 Software program. It was determined that in the normality test (Shapiro-Wilk test), the quantitative data [age, preoperative blood volume (V1), postoperative third day (V2) and postoperative first month (V3) blood volumes and pneumocephaly volumes and their percentage values] were not normally distributed and descriptive statistics were given as "mean, median, standard deviation, lowest and highest". Mann-Whitney U test, which is one of the nonparametric tests, was used in the analysis of quantitative data, and Fisher's Exact test was used in the analysis of qualitative data. Statistical significance value was accepted as $p<0.05$.

Table 1. Demographic data of patients and surgical results

	Subdural drain	Subgaleal drain
Number of patients	33	46
Age [mean (min-max)]	71.6 (35-92)	68.95 (37-90)
Female (n)	7	8
Male (n)	26	38
Chronic hematoma (n)	18	25
Subacute hematoma (n)	15	21
Preoperative blood volume (cm^3) (V1) (mean)	205.7	197.8
Postoperative third day blood volume (cm^3) (V2) (mean)	83.7	75.9
First month postoperative blood volume (cm^3) (V3) (mean)	44.8	28,9
Number and percentage of primary surgical complications	6[(I)] (18%)	1[(II)] (2%)
Number and percentage of secondary surgical complications	2[(III)] (6%)	5[(IV)] (10.9%)
Recurrence	0 (0%)	4 (8.7%)
Pneumocephalus volume (cm^3) (mean)	17.1	14.8

The table summarizes the combined results of the two surgical methods and the demographic data of the patients. The data of the table was created without considering the hematoma age (subacute/chronic) of the patients.

V1: patient's preoperative blood volume. V2: patient's blood volume on the third postoperative day. V3: patient's blood volume in the first postoperative month). [(I): drain-related seizure in one patient, cortical injury in three patients, insufficient drainage in two patients. (II): insufficient drainage in one patient. (III): death from pneumonia in two patients. (IV): death from pneumonia in three patients, hypertensive parenchymal hematoma in two patients (no surgery)]. min: Minimum, max: Maximum

RESULTS

79% of the patients were male patients. All patients with insufficient drainage and recurrence were male. This complication was never seen in women. However, statistical analysis was not carried out, as this situation may also be due to the size of the numerical difference between the genders.

It was observed that the blood volumes of female patients at the time of admission were statistically significantly lower compared to male patients. While the median value of blood volumes was 155 cm^3 in women at the time of admission, this rate was 195 cm^3 in men ($p=0.018$). However, there was no relationship between the volume of blood that could be drained and gender. Between men and women, early postoperative blood volume (V2) was found as $p=0.125$ and postoperative first month blood volume (V3) was found as $p=0.604$.

It was observed that the mean value of blood volumes at the time of admission of patients with subacute hematoma was higher than those presenting with chronic hematoma. However, it was not statistically significant ($p=0.779$). It was determined that the blood volume (V2) was slightly higher in the early postoperative period in subacute hemorrhage, but it was not statistically significant ($p=0.533$) (Table 2).

Table 2. The effect of hematoma age and drain location on the ability of hematoma to be drained

	Chronic (n=43)		Subacute (n=36)	
	Subdural drain (n=18)	Subgaleal drain (n=25)	Subdural drain (n=15)	Subgaleal drain (n=21)
Preoperative blood volume [cm^3 (%)]	196.33 (100%)	198.28 (100%)	216.35 (100%)	197.33 (100%)
Blood volume 3 days after the surgery [cm^3 (%)]	84.83 (43%)	73.32 (37%)	88.69 (41%)	79.09 (40%)
First month postoperative blood volume [cm^3 (%)]	51.11 (26%)	26.08 (13%)	41.76 (19%)	35.33 (18%)
Recurrence (%)	0 (0%)	0 (0%)	0 (0%)	4 (19%)
Insufficient drainage (%)	2 (11.1%)	0 (0%)	0 (0%)	1 (4.8%)

The data after the patients were divided into two subgroups as "chronic" and "subacute" according to the hematoma age are summarized in the table (% values represent the remaining blood volumes).

When both techniques were compared in terms of primary surgical complications, it was determined that inadequate drainage was observed in two (6.1%) of 33 patients in the subdural drain group and in one (2.2%) of 46 patients in the subgaleal drain group. There was no statistical difference between the groups (p=0.568). No other primary surgical complications such as acute subdural hematoma, acute epidural hematoma, or surgical site infection were encountered in either group (Table 1). This review was performed without including recurrence rates. When recurrences were added to the primary surgical complication list, it was determined that there was no statistical difference between the groups (p=0.512). When recurrence was evaluated under a separate heading, the recurrence rate was 0% (0/33) in the subdural drain group and 8.7% (4/46) in the subgaleal drain group. However, there was no statistical difference (Fisher's Exact test, p=0.136).

In terms of pneumocephalus rates, the mean air volume was found to be 17.1 cm^3 in the subdural drain group and 14.8 cm^3 in the subgaleal drain group (Table 1). Although it was seen that the subgaleal drain group was somewhat more advantageous in this regard, no statistical difference was found between the groups (p=828).

On the other hand, it was determined that all recurrences in the subgaleal drain group developed in patients with subacute hematoma (4/21) and none in the same group with chronic hematoma (0/25) developed recurrences. When analyzed in terms of recurrence rates, it was observed that both techniques were not superior to each other in patients with chronic hematoma, but the recurrence rate was higher in subgaleal drains placed in patients with subacute hematoma (Table 2). In the subdural drain group, the complication rate due to placement of the drain in the subdural space was 12% (4/33). There was no statistically significant difference between the genders in terms of primary surgical complications (Fisher's Exact test p=0.429). The drainage capacity of the drain site, regardless of the type of hematoma, is summarized in Table 3 for all groups.

When both techniques were compared in terms of patients only with subacute hematoma, no difference was found between the blood drainage capacities of both techniques (p=0.196). Again, when both techniques were examined only in terms of patients with chronic hematoma, it was determined that subgaleal drain was statistically more successful at the end of the first month (p=0.042). In addition, when chronic and subacute hematomas were evaluated together, it was determined that the rate of evacuation of the subgaleal drain at the end of the first month was statistically better (p=0.045). The combined effects of the type of hematoma (hematoma age) and the drain site on the rate of blood discharge are summarized in Table 4.

DISCUSSION

There is no consensus on the use of drains and drain localization in surgeries where symptomatic chronic and subacute subdural hematomas are drained by burr-hole. In a comprehensive study on this subject, it is stated that the choice of surgeons varies among geographical regions. For example, it is stated that 80% of surgeons in Africa, 83.7% in Europe and 100% in America use drains, while 50% of Asian surgeons do not use drains[9]. There are differences not only between regions but also between countries. According to studies, only 11% of surgeons in England state that they always use drains[10], while this rate rises to 80.6% in Canada[11]. The rate of drain use is 29.5% in India and 42.9% in Nigeria[12,13].

It seems that it is not absolutely necessary to use a drain in the surgical treatment of chronic or subacute subdural hematomas. However, the presence of a drain may suggest to the physician that hematoma will be drained more effectively. Twist drill craniostomy technique probably arose from the need to avoid complications related to the drain, on the one hand, and the sense of confidence given by the presence of the drain, on the other hand. Because, as is known, a drain is used in this technique, but unlike the classical method, this drain is not advanced in the subdural area, but is fixed just below the dura. In this way, it is aimed to avoid complications related to the

Table 3. Drainage capacity of the drain site independent of hematoma age

GROUP		Age	V1	V2	V3	Remaining V2%	Remaining V3%
Subdural drain (chronic+subacute)	N	33	33	32	31	32	31
	Mean	71,64	205,6667	83,7500	45,5484	42,0395	21,6665
	Median	**74,00**	**190,0000**	**75,0000**	**41,0000**	**39,0420**	**14,3860**
	SD	13,788	76,56438	56,90513	56,04929	25,02327	24,84841
	Minimum	35	91,00	0,00	0,00	0,00	0,00
	Maximum	92	369,00	267,00	260,00	106,80	104,00
Subgaleal drain (chronic+subacute)	N	46	46	46	43	46	43
	Mean	68,96	197,8478	75,9565	29,9535	37,1462	14,0767
	Median	**71,00**	**188,5000**	**67,0000**	**11,0000**	**37,9130**	**4,2683**
	SD	14,525	82,38904	56,51979	51,04478	21,04019	23,19783
	Minimum	37	70,00	0,00	0,00	0,00	0,00
	Maximum	90	442,00	237,00	225,00	84,75	94,74
Total	N	79	79	78	74	78	74
	Mean	70,08	201,1139	79,1538	36,4865	39,1537	17,2562
	Median	**72,00**	**190,0000**	**73,5000**	**16,0000**	**38,5890**	**9,4381**
	SD	14,194	79,59996	56,44008	53,38674	22,73049	24,03275
	Minimum	35	70,00	0,00	0,00	0,00	0,00
	Maximum	92	442,00	267,00	260,00	106,80	104,00

V1, V2, and V3: represent the preoperative blood volumes, postoperative third day, postoperative first month, respectively. The common descriptive data of patients in whom the drain was placed in the subdural space and in the subgaleal space are summarized in the table. Since the data are not normally distributed, it is recommended to consider the median values instead of the mean values when interpreting the table. As can be seen in the last column, the residual blood volume (remaining V3%) at the end of the first month was 14.38% of the preoperative blood volume in the subdural drain group, while the same rate was 4.26% in the subgaleal drain group. SD: Standard deviation

drain[14]. This technique did not reach its intended prevalence as it required unique equipment/kits, but it probably pioneered the application of subdural drains by showing that even a surface drain is effective.

The data of another study confirms all these concerns about the use of drain. In the related study, it is stated that the surgeon's experience and the preference to use a drain are inversely proportional, and this is simply explained as "to abstain from using a drain after a previous drain-related complication"[15]. The reason for starting subgaleal drain application in our clinic is that similar complications have been experienced.

When it is decided to use a drain, there is still no consensus in the literature on whether it is more appropriate to place it in the subdural or subperiosteal (subgaleal) area. According to a study by Soleman et al.[9], the use of subdural drains is still 50% among surgeons, while the rate of use of subgalel drains is 27%. They attribute the reason for this situation to the low evidence value of the publications on the use of subgaleal drains and the fact that it is a partially new approach. Again, according to the same study, it is stated that if a drain is to be used, experienced surgeons prefer subdural drain, while new generation (less experienced) surgeons prefer subgaleal drain. This situation is briefly explained as "experienced surgeons have difficulty in giving up their old habits, but new surgeons adapt more easily to new methods"[9]. In short, the experienced surgeon chooses not to use a drain if possible[15], but when he decides to use a drain, he cannot give up his old habit and uses a subdural drain[9].

In another study on the use of subgaleal drains, it was reported that the complication rates, including pneumocephalus, were higher in the subdural drain group compared to the subgaleal drain group[16]. In another study, pneumocephalus was not evaluated, but it was reported that the subgaleal drain technique was more advantageous in terms of all other complications, including recurrence[17]. In both studies, it was stated that there was no statistical difference between the techniques and the hematoma drainage capacities have been reported to be comparable to each other[16,17]. Soleman et al.[18], on the other hand, stated that the use of subgaleal drains reduced the rates of recurrence and infection, but the difference was again not statistically significan.

In the present study, it was determined that there was no difference between the groups in terms of insufficient drainage and other "drain unrelated" primary surgical complications. In terms of recurrence numbers, recurrence was observed in 4 patients in the subgaleal drain group, but not in any patient in the subdural drain group. The findings are also consistent with

Table 4. Joint effect of hematoma type and drain site on blood drainage rate

Group		V1	V2	V3	Remaining V2%	Remaining V3%
Subdural drain (chronic hematoma)	N	18	18	17	18	17
	Mean	196,3333	84,8333	51,1176	41,9276	23,9828
	Median	**192,5000**	**75,0000**	**41,0000**	**36,7160**	**17,1717**
	SD	62,89581	63,86313	68,53091	28,19363	30,28547
	Minimum	120,00	0,00	0,00	0,00	0,00
	Maximum	358,00	267,00	260,00	106,80	104,00
Subgaleal drain (chronic hematoma)	N	25	25	25	25	25
	Mean	198,2800	73,3200	26,0800	34,9974	9,9507
	Median	**185,0000**	**47,0000**	**,0000**	**33,0357**	**0,0000**
	SD	85,03750	59,70254	47,82339	20,26949	13,34893
	Minimum	91,00	0,00	0,00	0,00	0,00
	Maximum	442,00	237,00	225,00	76,76	50,90
Subdural drain (subacute hematoma)	N	15	14	14	14	14
	Mean	216,8667	82,3571	38,7857	42,1833	18,8540
	Median	**184,0000**	**75,0000**	**34,0000**	**43,3879**	**13,5042**
	SD	91,37354	48,83348	37,14251	21,29933	16,74700
	Minimum	91,00	0,00	0,00	0,00	0,00
	Maximum	369,00	181,00	140,00	82,42	47,18
Subgaleal drain (subacute hematoma)	N	21	21	18	21	18
	Mean	197,3333	79,0952	35,3333	39,7044	19,8073
	Median	**190,0000**	**79,0000**	**11,5000**	**38,9222**	**8,0337**
	SD	81,20796	53,77072	56,17619	22,14465	31,90841
	Minimum	70,00	0,00	0,00	0,00	0,00
	Maximum	379,00	190,00	180,00	84,75	94,74

The descriptive data of the patients after subgrouping as chronic and subacute according to hematoma age are summarized in the table. Since the data are not normally distributed, it is recommended to use "median" values in the evaluation of the table. As can be seen from the table, in the case of using a subgaleal drain in patients with chronic hematoma, the median value of the residual blood volume percentage (remaining V3%) at the end of the first month was 0.00%, while the same volume was measured as 17.17% in the patient group with chronic hematoma but whose drain was placed in the subdural area.

SD: Standard deviation

the literature[16-18]. What is interesting is that all recurrences in the subgaleal drain group were observed in patients with subacute hematoma, but not in patients with chronic hematoma, and as far as we can determine, this is a new finding for the literature. This may be related to the viscosity change that will develop depending on the hematoma age. Subgaleal drain seems to be less effective in draining subacute hematomas, which are expected to have higher viscosity.

When all patients were evaluated together, regardless of the hematoma age, it was observed that the subgaleal drain was able to drain the hematoma more successfully (p=0.045). This finding is inconsistent with the general literature. Because, although the findings in this direction are generally reached in the studies, it is stated that the results are not statistically significant[7,16-18]. However, it was seen that the results of both methods were not statistically different in terms of early postoperative blood volumes (V2). In other words, the change that made a statistical difference occurred in the last month when the patients had no drains. Therefore, we could not explain the reason why subgaleal drain placement is superior to the classical method. It was examined retrospectively whether the situation was related to the number of burr-holes, burr hole diameter and drain type, but it was determined that it was not related to these either. When the demographic data of the groups were compared, it was determined that both groups had similar characteristics for each parameter examined.

On the other hand, in the presented study, while there was no statistical difference between the two methods in patients with subacute hematoma, it was found that subgaleal drain was statistically more effective in patients with chronic hematoma. The reason why the findings of the study differ from the existing literature may be that hematomas were not examined separately as chronic and subacute in the mentioned studies.

Study Limitations

The limitation of the presented study is the low number of patients. If the number of patients is increased, it is also possible that the statistical difference between the two methods can be eliminated in terms of drainage capacity of the hematoma.

CONCLUSION

In conclusion, both methods are quite similar to each other in their capacity to drain the hematoma. Subdural drain placement has the potential to cause complications related to the drain, while subgaleal drain placement increases recurrence rates (especially in patients with subacute hematoma). If the hematoma is chronic, subgaleal drain seems to be more advantageous, but this decision is still controversial in subacute hematomas. The physician should decide according to his own experience whether he should take the risk of complications related to the drain or the risk of recurrence.

Ethics

Ethics Committee Approval: The study were approved by the Kahramanmaraş Sütçü İmam University of Local Ethics Committee (decision no: 2020/17-04).

Informed Consent: Retrospective study.

Peer-review: Externally and internally peer-reviewed.

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Distribution and Characterization of Aeroallergens in the Etiology of Allergic Rhinitis Patients in İstanbul Kartal Region

İstanbul Kartal Bölgesi Alerjik Rinit Hastalarının Etiyolojisindeki Aeroalerjenlerin Dağılımı ve Karakterizasyonu

Kadriye TERZİOĞLU[1], Meltem AĞCA ÇOBAN[2]

[1]*Kartal Dr. Lütfü Kırdar City Hospital, Clinic of Internal Disease, İstanbul, Turkey*
[2]*İstanbul Süreyyapaşa Chest Diseases and Thoracic Surgery Training and Research Hospital, Clinic of Intensive Care, İstanbul, Turkey*

ABSTRACT

Aim: Allergic rhinitis (AR) is a common disease and the first step in its treatment is to determine the allergen responsible for its etiology and to take preventive measures. The aim of this study was to determine the distribution of aeroallergens in AR patients in İstanbul Kartal region and to investigate other factors affecting the severity of AR.

Materials and Methods: The study was performed in a tertiary hospital on the Anatolian side of İstanbul among patients who were admitted to the allergy and clinical immunology outpatient clinic and diagnosed with AR. Data of patients were obtained from medical records retrospectively. Age, gender, concomitant atopic disease, family transition history, serum total IgE and serum eosinophil (Eo) levels were evaluated. Skin prick tests were performed with 25 allergens.

Results: One hundred and sixty patients were included in our study. Ninety-six (65.8%) of the patients were female and the median age of the study population was 31 (18-75) years. Distribution of aeroallergens according to skin test results was as follows; D. pteronyssinus (78.1%), D. farinae (75.2%), Tyrophagus putrescentlae (59.6%), Acurus siro (57.5%), Lepidoglyphus destructor (40.4%), cereals mix (37.7%), and cat hair (33.6%). It was found that there was a significant increase in total IgE levels ($p<0.001$) consistent with the increase in serum Eo level.

Conclusion: In our study, mite sensitivity has been found to be the most common allergen sensitivity in accordance with the geographic and climatic characteristics of the İstanbul region. Secondly, cat hair sensitivity and pollen (grasses mix) sensitivity were similarly high due to the increase in the number of domestic animals in the modern age. We think that our study contributes to the literature by examining the distribution of these allergens that cause AR and patients are guided to take preventive measures in this regard.

Keywords: Allergic rhinitis, aeroallergen, sensitization

ÖZ

Amaç: Alerjik rinit (AR) yaygın görülen bir hastalıktır ve tedavisinde ilk basamak, etiyolojisindeki sorumlu alerjeni saptayıp koruyucu önlemleri almaktır. Çalışmamızın amacı, İstanbul Kartal yerleşkesinde yaşayan AR hastalarının etiyolojisinde rol alan aeroalerjenlerin dağılımını tespit etmek ve AR şiddetini etkileyen faktörleri araştırmaktır.

Gereç ve Yöntem: Çalışma, İstanbul Anadolu yakasında bulunan üçüncü basamak bir hastanede gerçekleştirildi. Alerji ve klinik immünoloji polikliniğine rinit şikayeti ile başvurup AR tanısı konulan hastalar çalışmaya dahil edildi ve dosyaları geriye dönük olarak incelendi. Hastaların yaş, cinsiyet, eşlik eden diğer atopik hastalıkları, aile geçiş öyküsü, serum total IgE ve serum eozinofil (Eo) seviyeleri değerlendirildi. Deri prick testleri 25 adet alerjen ile yapıldı.

Bulgular: Çalışmamıza 146 hasta dahil edildi. Hastaların 96'sı (%65,8) kadındı. Medyan yaş 31 (18-75) yıl olarak saptandı. Deri prick test sonuçlarına göre aeroalerjenlerin dağılımı sırasıyla; D. pteronyssinus (%78,1), D. farinae (%75,2), Tyrophagus putrescentlae (%59,6), Acurus siro (%57,5),

Address for Correspondence: Kadriye TERZİOĞLU MD, Kartal Dr. Lütfü Kırdar City Hospital, Clinic of Internal Disease, İstanbul, Turkey
E-mail: dr.kadriyete@gmail.com **ORCID ID:** orcid.org/0000-0002-0674-417X
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Lepidoglyphus destructor (%40,4), cereals mix (%37,7), kedi tüyü (%33,6) şeklindeydi. Hastaların serum Eo seviyesindeki artışa paralel olarak total IgE düzeylerinde de anlamlı artış olduğu (p<0,001) görüldü.

Sonuç: Çalışmamızda, İstanbul bölgesinin coğrafik yapısı ve iklimi ile uyumlu olarak en fazla akar duyarlılığı saptandı. İkinci olarak modern çağda ev içi hayvan besleme sayısında artmaya bağlı olarak kedi tüyü duyarlılığı ve polen (grasses mix) duyarlılığı benzer şekilde yüksek oranlarda saptandı. Çalışmamızın AR'ye sebep olan aeroalerjenlerin dağılımını inceleyerek literatüre katkı sağladığını ve hastaların bu yönde koruyucu önlemlerini almaları için yol gösterici olacağını düşünmekteyiz.

Anahtar Kelimeler: Alerjik rinit, aeroalerjen, duyarlılık

INTRODUCTION

Allergic rhinitis (AR) is a type 1 IgE-mediated disease of the nasal mucosa and is characterized by recurrent sneezing, runny nose, and nasal congestion[1]. Although its prevalence varies according to countries and age, it affects 10-20% of the population[2]. Detection of aeroallergens that cause allergic sensitization in AR and minimizing exposure are important components of the treatment plan.

Distribution of aeroallergens varies depending on geographical structure, seasons, socioeconomic and cultural structures[3]. In recent years, with the increase in AR prevalence, differences in the distribution of aeroallergens have begun to be detected. Various factors have been identified that lead to this situation, some of which are the hygiene hypothesis, the increase in the time spent indoors in parallel with the technological development, the increase in the release of various allergens such as ragweed pollen and aspergillus fumigatus into the atmosphere due to global climate change and gases released in the air[4-6].

Our aim in the study is to investigate the distribution of aeroallergens that play a role in the etiology of AR patients living in İstanbul Kartal region and the factors affecting AR severity.

MATERIALS AND METHODS

The study was carried out in a tertiary healthcare institution located on the Anatolian side of İstanbul. Patients who applied to the allergy and clinical immunology outpatient clinic with rhinitis symptoms and were diagnosed with AR were included in the study. The study were approved by the Kartal Dr. Lütfi Kırdar City Hospital Clinical Research Ethics Committee (protocol number: 2020/514/180/17, date: 26.06.2020).

Patient Selection

The files of patients admitted with AR between January and March 2020 were retrospectively reviewed. AR diagnosis of all patients was confirmed according to Allergic Rhinitis and its Impacts on Asthma (ARIA) guidelines, and patients with positive skin prick test were included in the study. Patients' age, gender, severity of rhinitis, year of AR, blood eosinophil (Eo) and total IgE values, accompanying atopic diseases (asthma, urticaria, eczema), comorbidity, and family history were investigated. Values of 0.2 µgr (normal range 0-0.2 µgr) for Eo elevation and 100 UI/mL (normal range 0-100 UI mL) for IgE elevation were accepted. The AR classification and severity of the patients were made according to ARIA (mild intermittent, moderate-severe intermittent, mild persistent, moderate-severe persistent). Intermittent AR; symptoms less than four days a week and lasting less than four consecutive weeks. Persistent AR; symptoms more than four days a week, lasting for four consecutive weeks. Symptoms were classified as mild if they did not affect sleep, daily activities, work-school activities, and as moderate-severe if they affected[7] them.

Skin Prick Test

Skin prick test was conducted with 25 allergens (grasses mix, cereals mix, tree mix, Dermatophagoides (D.) pteronyssinus, Dermatophagoides farinae, Acarussiro, cockroach, cat hair, dog hair, Aspergillus fumigatus, Alternaria, Clodosporium, Lepidoglypus destructr, Tyrophagus putrescentlae, wormwood, stickygrass, plantain, grapegrass, hazel, alder, ash, olive, birch, oak, and poplar) and standard commercial allergens were used (Alk-Abello, Lincoln Diagnostics, Dallas, TX, USA). The skin prick test was performed on the anterior surface of both arms in accordance with international guidelines. Histamine (10 mg/mL) was used as the positive control and 0.09% sterile saline was used as the negative control. The test was considered positive if the edema diameter at the test site after 20 minutes was found to be more than 3 mm compared to the negative control[8].

Statistical Analysis

Data statistics were performed using Statistical Package for the Social Sciences (SPSS) 22.0 (SPSS for Windows, version 19.0). The normality of the data distribution was checked with the Kolmogorov-Smirnov test. Quantitative variables were expressed as mean±standard deviation or median (25^{th}-75^{th} percentile), categorical variables were expressed as percentages. Student's t-test or Mann-Whitney U test was used to compare the differences of continuous variables. The x^2 test was used to compare categorical variables. Relationships between continuous variables were calculated with the Spearman correlation coefficient. P value of <0.05 was considered statistically significant.

RESULTS

Of the 146 AR diagnosed patients included in the study, 96 (65.8%) were female. The median age in the study group was 31 (18-75) years. When the severity of rhinitis was classified, mild persistent 35.6%, moderate-severe persistent 22.6%, mild intermittent 21.2%, moderate-severe intermittent 20.5% were found in order of frequency. In 45.9% of the patients, the rhinitis duration was longer than 5 years. As comorbid diseases, asthma was the first with 26.7%, eczema was 18.5% and urticaria was 6.8%. Elevated blood Eo was detected in 60 patients (41.1%), and total IgE elevation was found in 78 patients (53.4%) (Table 1). In Spearman correlation analysis, a positive and significant correlation was found between blood Eo value and total IgE (r=0.335; p<0.001) (Figure 1). When the family history of the patients was questioned; most of the patients (79.5%, n=116) had a family history. While the majority of patients with a family history were female (69.8%, n=81), the difference was significant compared to male gender (p=0.041).

Table 1. General data of patients with allergic rhinitis

	n	%
Gender		
Female	96	65.8
Male	50	34.2
Allergic rhinitis severity classification		
Mild intermittent	31	21.2
Moderate to severe intermittent	30	20.5
Mild persistent	52	35.6
Moderate to severe persistent	33	22.6
Duration of allergic rhinitis		
0-1 year	25	17.1
1-5 years	54	37
Over 5 years	67	45.9
Serum eosinophilia		
<0.2 µgr	86	58.9
>0.2 µgr	60	41.1
Total Ig E		
0-100 UI/mL	68	46.6
100-500 UI/mL	55	37.7
>500 UI/mL	23	15.8
Asthma		
Yes	39	26.7
None	107	73.3
Urticaria		
Yes	10	6.8
None	136	93.2
Eczema		
Yes	27	18.5
None	119	81.5
Comorbid		
Yes	17	11.6
None	129	88.4

Distribution of Sensitization to Aeroallergens

The allergens that were found to be positive in the skin prick test results of the patients, in order of frequency were: D. pteronyssinus (78.1%), D. farinae (75.2%), Tyrophagus putrescentlae (59.6%), Acurus siro (57.5%), Lepidoglyypus destructor (40.4%), cereals mix (37.7%), cat hair (33.6%), grasses mix (31.5%), dog hair (29.5%), cocroaches (21.2%), plantain (14.4%) and wormwood (13.7%) (Figure 2).

In total, any house dust mite and/or warehouse mite positivity was 89% (n=130), sensitivity to any pollen group (grass, grain, tree, weeds) was 52.7% (n=77) and sensitivity to any mold fungus was 13.7% (n=20) and no significant difference was found between the female and male genders in terms of sensitivity distributions (p=0.569, p=0.789, p=0.939). Among the aeroallergens, while cat hair sensitivity was found to be significantly higher in those who were sensitive to mold fungus and pollen (p=0.007, p<0.000, respectively), a similar relationship was not found in mite sensitivity (p=0.742).

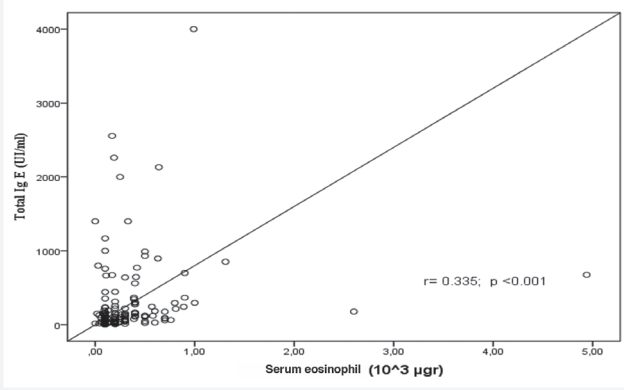

Figure 1. Correlation between serum eosinophil and total IgE

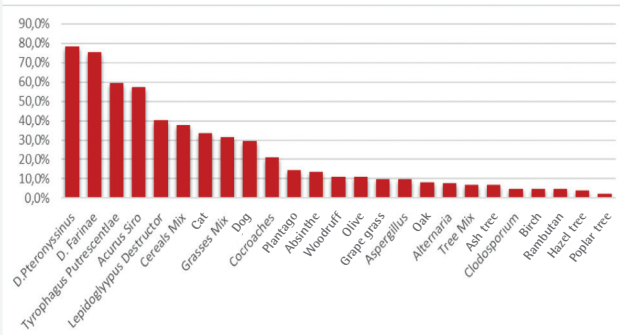

Figure 2. Distribution of sensitization to aeroallergens

When the characteristics and laboratory findings of the patients were evaluated according to the severity of AR, no significant difference was found (Table 2). AR severity and aeroallergen distributions are given in Figure 3 and Figure 4.

DISCUSSION

Allergens play an important role in the etiology of AR. The distribution of allergens and their effects on individuals may vary depending on geographical regions, socioeconomic level, living conditions and genetic structure. In our study, when the aeroallergen distribution of AR patients in the İstanbul Kartal region was examined, it was determined that D. pteronyssinus 78.1%, D. farinae 75.2%, Tyrophagus putrescentlae 59.6%, Acurus siro 57.5% and Lepidoglypus destructor 40.4% were in the top five with mite sensitivity and then pollen and cat hair sensitivity were determined to be effective. The ideal humidity for the reproduction and accumulation of house dust mites is 65-80% and they die at humidity levels below 50%[9]. In studies from various geographical regions around the world, it was shown that the level of dust mite was clinically insignificant at high altitudes such as New Mexico Alamos region (2,195 m), Italy Misurina region (1,756 m), and in cold climate regions such as Sweden Norbotten, where the average temperature is -10 to 15 °C and humidity is 0%[10-12]. However, İstanbul has an

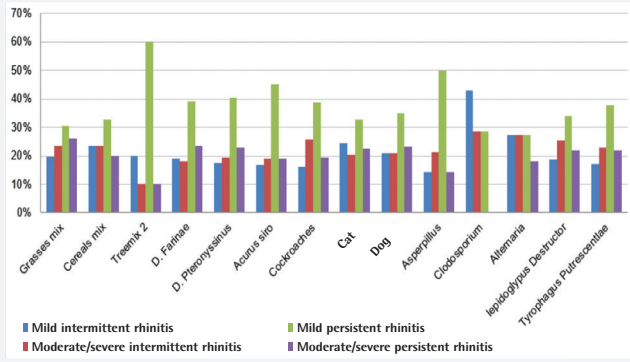

Figure 3. Distribution of aeroallergens by severity classification of allergic rhinitis

Table 2. Evaluation of factors affecting the severity of allergic rhinitis

	Mild intermittent n (%)	Moderate-severe intermittent n (%)	Mild persistent n (%)	Moderate-severe persistent n (%)	p*
Age (years)					
18-29	10 (6.8)	15 (10.3)	22 (15.1)	18 (12.3)	0.532
30-49	15 (10.3)	13 (8.9)	24 (16.4)	12 (8.2)	
>50	6 (4.1)	2 (1.4)	6 (4.1)	3 (2.1)	
Gender					
Female	25 (17.1)	20 (13.7)	35 (24)	16 (11)	0.058
Male	6 (4.1)	10 (6.8)	17 (11.6)	17 (11.6)	
Rhinitis duration (years)					
0-1	6 (4.1)	8 (5.5)	6 (4.1)	5 (3.4)	0.502
1-5	14 (9.6)	8 (5.5)	19 (13)	13 (8.9)	
>5	11 (7.5)	14 (9.6)	27 (18.5)	15 (10.3)	
Concomitant atopy					
Asthma+	8 (5.5)	7 (4.8)	17 (11.6)	7 (4.8)	0.649
Asthma-	23 (15.8)	23 (15.8)	35 (24)	26 (17.8)	
Urticaria+	2 (1,4)	2 (1.4)	5 (3.4)	1 (0.7)	0.709
Urticaria-	29 (19.9)	28 (19.2)	47 (32.2)	32 (21.9)	
Eczema+	4 (2.7)	6 (4.1)	13 (8.9)	4 (2.7)	0.386
Eczema-	27 (18.5)	24 (16.4)	39 (26.7)	29 (19.9)	
Serum eosinophilia (10^3 µgr)					
≤0.2	18 (12.3)	19 (13.0)	33 (22.6)	16 (11)	0.536
>0.2	13 (8.9)	11 (7.5)	19 (13)	17 (11.6)	
Serum total IgE (UI/mL)					
0-100 U	17 (11.6)	14 (9.6)	19 (13)	18 (12.3)	0.675
100-500	10 (6.8)	11 (7.5)	24 (16.4)	10 (6.8)	
>500	4 (2.7)	5 (3.4)	9 (6.2)	5 (3.4)	

+ / yes, - / none, *p<0.005

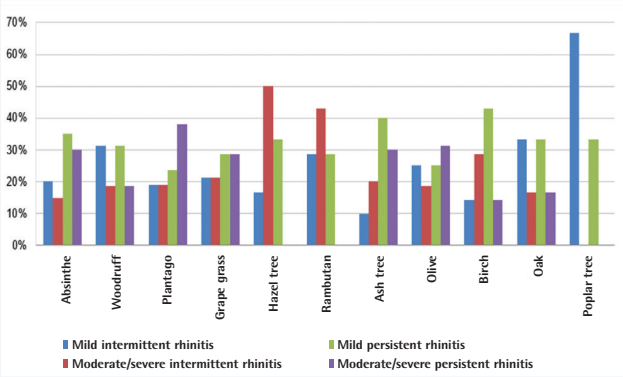

Figure 4. Distribution of aeroallergens by severity classification of allergic rhinitis

altitude at sea level, the lowest temperature is -11, the highest temperature is +40 degrees, the average relative humidity is 75%, and this rate goes up to 80-85% in certain months.

As a result, İstanbul has a climate with high humidity most of the year due to its geographical structure. For this reason, a high mite sensitivity rate is an expected result. In studies from different regions of Turkey, sensitivity rates for house mites D. pteronyssinus and D. farinae were 72.5% and 63.7% in Düzce, 62.2% and 51.3% in Eskişehir, 50% and 44% in Bursa, and 84% and 78.2% in the Eastern Black Sea region, which is similar to the data obtained in our study[13-16]. The high sensitivity to house dust mites in our study is thought to be due to the high humidity and mild climate of the İstanbul Kartal region, which is located at sea level. In a recent study by Ediger et al.[15], the most frequently observed aeroallergens in Bursa region after D. pteronyssinus and D. farine were grasses mix (38.6%), olive (33.2%), cereals pollen (32.3%), Acarus siro (26.3%) and cat hair (12%)[14].

In our study, the sensitivity rate of Acarus siro was found to be approximately 2 times higher with 57.5%, and the sensitivity rate of cereals mix (37.7%) and grasses mix (31.5%) was similar. It is an expected result that olive pollen causes high sensitivity similar to grasses mix due to olive cultivation in Bursa region. In our study, sensitivity to olive tree pollen was found to be lower (11%). This result shows that geographical vegetation closely affects the allergen etiology in AR patients. According to the results, it is important for allergy specialists to create an allergen panel according to the characteristics of the geography they are in.

Another remarkable point obtained in our study is that the rate of cat sensitivity (33.6%) in AR patients is higher than the grasses mix (31.5%), which is one of the main allergens, and it ranks third after the mite and cereals (grain) mix. The cat sensitivity rate in Europe is estimated at around 27%[17]. It is thought that this high sensitivity causes an increase in the amount of cat allergens in places where there are no cats (school, work, nursery) as a result of the increase in domestic cat feeding rates in industrialized countries, and this contributes to sensitization[18,19]. This result was supported by the study of Gulbahar et al.[20] in 387 patients. Although the cat sensitivity rate was found to be 44.7% in this study, it was reported that only 1.6% of the patients kept cats at home.

Another interesting point obtained in our study was that cat hair sensitivity was found to be significantly higher in individuals with mold and pollen sensitivity. Albumin-dependent cross-reactivity is known to be among cat hair and dog hair allergens, but no known cross-reactivity has been detected between molds and pollen groups. Further studies with larger series are needed for the significance of this data.

It is known that genetic transmission is an important factor in the formation of AR. If there is no history of allergy in the parents, the probability of AR is 0-10%, if one of the parents has a history of allergy, this rate is 30-40%, if both parents have a history of allergy, this rate can reach 60-70%[21]. In our study, the presence of allergy history in the mother and/or maternal relatives was 58.2%, and the family history positivity was found to be significantly higher in females than males. This result indicates that the female gender carries the diseased gene at a higher rate.

Recently, AR and asthma have been defined as the single airway diseases, and there are many studies showing elevated serum Eo and total IgE in parallel with airway inflammation in these diseases[22,23]. Similarly, in our study, the total IgE level of patients with AR was found to be significantly higher than the normal population. In the correlation analysis, it was observed that there was a positive correlation between serum total IgE and serum Eo count. These results show the importance of evaluating the total IgE and Eo levels together in patients with AR. However, no significant correlation was found between total IgE elevation and AR severity in our cohort. Similarly, no significant relationship was found between the severity of AR and age, gender, type of aeroallergen, and other concomitant atopic diseases.

Study Limitations

Compared to the population of İstanbul, the small number of patients in our study and the inability to measure serum specific IgE in correlation with the skin prick test are the limitations of our study.

CONCLUSION

AR is a disease whose prevalence increases every year and multifactorial factors play a role in its etiology. Along with genetic predisposition, allergens play an important role in the etiology. In this study, mites, pollen and cat hair were in the top three ranks in the distribution of aeroallergens. Considering the differences in the distribution of allergens according to climatic conditions, geographical structure and socioeconomic

levels, knowing the distribution of allergens in the population admitted to the hospital with AR will guide the patients to take preventive measures in this direction.

Ethics

Ethics Committee Approval: The study were approved by the Kartal Dr. Lütfi Kırdar City Hospital Clinical Research Ethics Committee (protocol number: 2020/514/180/17, date: 26.06.2020).

Informed Consent: Retrospective study.

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Authorship Contributions

Concept: K.T., Design: K.T., M.A.Ç., Data Collection or Processing: K.T., M.A.Ç., Analysis or Interpretation: M.A.Ç., Literature Search: K.T., Writing: K.T.

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ORIGINAL ARTICLE

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Relationship of Lymphocyte to Monocyte Ratio at Diagnosis with Prognosis in Patients with Diffuse Large B-cell Lymphoma: A Retrospective Study

Yaygın Büyük B Hücreli Lenfomalı Hastalarda Lenfosit Monosit Oranının Prognozla İlişkisi: Retrospektif Çalışma

Ali ESER

Bezmialem Vakıf University Faculty of Medicine, Department of Internal Disease, Division of Hematology, İstanbul, Turkey

ABSTRACT

Aim: Although there is a complete response and increase in survival rates with rituximab in diffuse large-B-cell lymphoma (DLBCL), approximately 30% of the patients face with relapse or refractory disease. International prognostic index (IPI) is the most widely used method used for identifying relapse and refractory disease. Recently, the lymphocyte monocyte ratio (LMR) that can be used in place of or in combination with IPI has been proposed as an effective prognostic factor to predict clinical survival in DLBCL patients.

Materials and Methods: Two hundred twenty three patients diagnosed with DLBCL at our center between 2012 and 2020 were included in the study. The age, gender, absolute lymphocyte count (ALC), absolute monocyte count (AMC), and follow-up time were recorded from the files of the patients. Patients were divided into two groups as: exitus group and alive group. LMR was calculated.

Results: The median age at diagnosis was 58 years. The median ALC was $1.5x10^3$/uL, the median AMC was $0.6x10^3$/uL, and the median LMR was 2.6. The median follow-up time was 53 months. Five-year overall survival and progression-free survival were 78% and 69%, respectively. The age was significantly higher in the exitus group than in the alive group ($p<0.05$). ALC, before and after treatment, was significantly higher in the exitus group than in the alive group ($p<0.05$). Pre-treatment AMC did not show a significant difference ($p>0.05$). Pre-treatment LMR level did not show a significance difference in the exitus and alive groups ($p>0.05$).

Conclusion: LMR alone has low prognostic determinacy. Therefore, it should be evaluated with other prognostic determinants.

Keywords: Diffuse large B-cell lymphoma, absolute lymphocyte count, absolute monocyte count, lymphocyte-to-monocyte ratio

ÖZ

Amaç: Yaygın büyük B hücreli lenfomada (YBBHL) rituksimab ile birlikte tam yanıt ve sağkalım oranlarının artmasına rağmen hastaların yaklaşık %30'u nüks veya dirençli hastalık ile karşı karşıyadır. Nüks ve dirençli hastalığın belirlenebilmesi için uluslararası prognostik indeks (UPİ) en yaygın kullanılan metottur. Son zamanlarda UPİ yerine veya birlikte kullanılabilecek lenfosit monosit oranı (LMO) YBBHL hastalarında klinik sağkalımı tahmin etmek için etkili bir prognostik faktör olarak önerilmiştir.

Gereç ve Yöntem: 2012-2020 yılları arasında merkezimizde YBBHL tanısı konulan 223 hasta çalışmaya dahil edildi. Hastaların dosyalarından yaşı, cinsiyeti, mutlak lenfosit sayısı (MLS), mutlak monosit sayısı (MMS), takip süresi kaydedildi. Hastalar ölüm ve yaşam grubu olarak ikiye ayrıldı. MLS MMS'ye bölünerek LMO hesaplandı.

Bulgular: Tanı anındaki medyan yaş 58 idi. MLS medyan $1,5x10^3$/uL, MMS medyan $0,6x10^3$/uL, LMO medyan 2,6 olarak saptandı. Medyan takip süresi 53 ay oldu. Beş yıllık genel sağkalım ve ilerlemesiz sağkalım sırasıyla %78 ve %69 olarak gerçekleşti. Ölüm grubunda hastaların yaşı yaşam grubundan anlamlı olarak daha yüksekti ($p<0,05$). Ölüm grubunda tedavi öncesi ve tedavi sonrası MLS yaşam grubundan anlamlı olarak daha yüksekti ($p<0,05$). Ölüm ve yaşam grubunda tedavi öncesi MMS açısından anlamlı farklılık görülmedi ($p>0,05$). Ölüm ve yaşam grubunda tedavi öncesi LMO değerinde anlamlı ($p>0,05$) farklılık görülmedi.

Address for Correspondence: Ali ESER MD, Bezmialem Vakıf University Faculty of Medicine, Department of Internal Disease, Division of Hematology, İstanbul, Turkey
Phone: +90 532 246 41 05 **E-mail:** dralieser@gmail.com **ORCID ID:** orcid.org/0000-0001-9423-928X
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Sonuç: LMO'nun tek başına prognostik belirleyiciliği düşüktür. Bu nedenle diğer prognostik belirteçlerle birlikte değerlendirilmelidir.
Anahtar Kelimeler: Yaygın büyük B-hücreli lenfoma, mutlak lenfosit sayısı, mutlak monosit sayısı, lenfosit monosit oranı

Introduction

Diffuse large B cell lymphoma (DLBCL) is the most common type of aggressive non-Hodgkin lymphoma[1]. The median survival is less than one year in untreated patients[2]. Rituximab plus cyclophosphamide, vincristine, and prednisolone (R-CHOP) are the standard treatment for DLBCL and have a complete response rate of 70-90%[3,4]. However, approximately 30% of the patients face with relapse and refractory disease[5]. Prognostic evaluation is required to estimate patients with a relapse potential and high risk of refractory disease in DLBCL. For this purpose, prognostic factors such as international prognostic index (IPI), post-treatment positron emission tomography/computed tomography (PET/CT) and early interim evaluation, gene expression profile, Ki-67 proliferation index are used[6-9]. On the other hand, most of these methods are expensive and difficult to interpret. Therefore, there is a need for new methods that are inexpensive and easily accessible and interpreted by everyone. Recently, several studies have shown that the ratio of absolute lymphocyte/monocyte count (LMR) at diagnosis, which is obtained from a complete blood count, can predict clinical outcome in DLBCL[10,11].

In this study, we aimed to demonstrate the efficiency of LMR in determining relapse and refractory cases and its usability with or without other prognostic determinants.

MATERIALS AND METHODS

The data from 223 adult patients with DLBCL, who were initially treated with R-CHOP at our hospital between 2012 and 2020, were retrospectively analyzed. A total of 27 patients were diagnosed with recurrent or primary refractory DLBCL. As a salvage therapy rituximab-ifosfamide, carboplatin, etoposide (R-ICE), rituximab-cisplatin, cytarabine, dexamethasone (R-DHAP), rituximab-bendamustine (R-Benda) were used, and afterwards autologous stem cell transplantation was performed. Laboratory levels were evaluated before starting R-CHOP chemotherapy. Diagnostic examinations of the patients were performed in our center. Laboratory results from the external center were not taken into consideration. Absolute lymphocyte count (ALC) and absolute monocyte count (AMC) were obtained from the complete blood count. LMR was calculated by using the ratio of ALC to AMC.

Bezmialem Vakıf University Ethics Committee approval was obtained from the Non-interventional Clinical Research Ethics Committee, with the protocol number of 20/380 and date of 01.12.2020.

Statistical Analysis

Mean, standard deviation, median, minimum, maximum value, frequency and percentage were used for descriptive statistics. The distribution of variables was checked with the Kolmogorov-Smirnov test. The Mann-Whitney U test was used for the comparison of quantitative data. The Wilcoxon test was used for the repeated measurement analysis. The chi-square test was used for the comparison of the qualitative data. Statistical Package for the Social Sciences 27.0 was used for statistical analyses. Values of $p<0.05$ were considered significant.

RESULTS

Two hundred twenty three patients diagnosed with DLBCL and treated with R-CHOP protocol were analyzed retrospectively in our center. The demographic characteristics of the patients are summarized in Table 1. The median age at diagnosis was 58 years (range: 17-89). The median ALC was 1.5 (range: 0.2-61.3) x 10^3/µL, the median AMC was 0.6 (range: 0.1-8.9) x 10^3/µL, and the median LMR was 2.6 (range: 0.3-64.1). The median follow-up duration was 53 (range: 5-105) months.

Five-year overall survival (OS) and progression-free survival (PFS) were 78% and 69%, respectively. The median age of the patients in the exitus group was significantly higher than in the alive group ($p<0.05$) (Figure 1).

The gender distribution in neither exitus nor alive group did not differ significantly ($p>0.05$) (Table 2). Pre and post-treatment

Table 1. Baseline patient characteristics

		Min-Max	Median	Mean±SD	n (%)
Age		17.0-89.0	58.0	57.0±16.2	
Gender	Female				114 (51.1)
	Male				109 (48.9)
ALC (µL)		0.2-61.3	1.5	2.02±4.30	
AMC (µL)		0.1-8.9	0.6	0.72±0.69	
LMR		0.3-64.1	2.6	3.23±4.58	
Mortality	(-)				147 (65.9)
	(+)				76 (34.1)
Follow-up time (month)		5-105	53.0	41.7±15.4	

ALC: Absolute lymphocyte count, AMC: Absolute monocyte count, LMR: Lymphocte/monocyte ratio, SD: Standard deviation, Min-Max: Minimum-maximum

ALC values were significantly higher in the exitus group than in the alive group (p<0.05) (Figure 2).

In the alive group, ALC decreased significantly (p<0.05) after the treatment compared to pre-treatment results. In the exitus group, ALC decreased significantly (p<0.05) after the treatment compared to pre-treatment. The decrease in ALC after the treatment in the exitus group was significantly higher than in the alive group (p<0.05) (Table 2). Pre-treatment AMC did not show a significant difference in both groups (p>0.05). Post-treatment AMC in the exitus group was significantly higher than in the alive group (p<0.05). Post-treatment AMC in both groups did not show a significant change compared to pre-treatment status (p>0.05). The change in AMC after the treatment was not significantly (p>0.05) different between the exitus and alive groups (Table 2) (Figure 3).

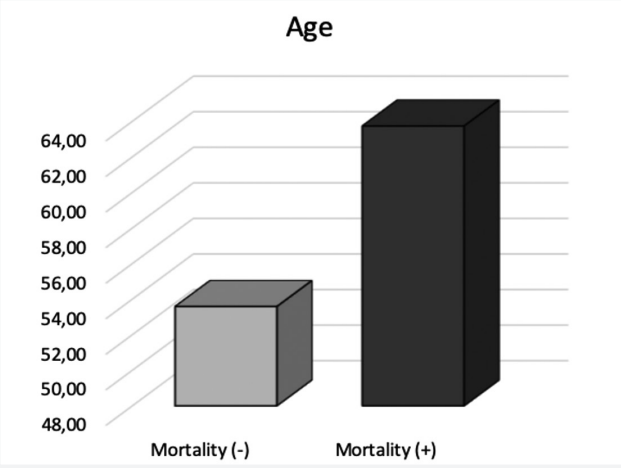

Figure 1. Age and mortality relationship in diffuse large-B-cell lymphoma patients

Pre-treatment LMR level did not show a significant difference in the exitus and alive groups (p>0.05). Post-treatment LMR in the exitus group was significantly higher than in the alive group (p<0.05). Post-treatment LMR in the alive group showed a significant decrease compared to pre-treatment levels (p<0.05). Post-treatment LMR in the exitus group did not show a significant difference compared to pre-treatment levels (p>0.05). Post-treatment LMR change was not significantly (p>0.05) different between both groups (Table 2) (Figure 4).

DISCUSSION

The most critical point in the management of DLBCL is to identify patients with high risk of relapse and refractory disease with standard therapy. For that purpose, IPI is the most commonly used prognostic index to predict the results in naive and relapsed/refractory DLBCL. Many studies have shown the prognostic importance of IPI during relapsed/

Table 2. Comparison of ALC, AMC and LMR of mortality and non-mortality groups before and after treatment

		Mortality (-)			Mortality (+)			p	
		Mean±SD	n (%)	Median	Mean±SD	n (%)	Median		
Age		53.59±15.84		56.0	63.74±14.92		66.0	**0.000**	m
Gender	Female		76 (51.7)			38 (21.6)		0.810	x^2
	Male		71 (48.3)			38 (21.6)			
ALC (µL)									
Before treatment		1.87±1.86		1.6	2.30±6.93		1.3	**0.043**	m
After treatment		1.46±0.78		1.3	1.60±4.35		0.8	**0.000**	m
Before/after difference		-0.41±1.85		-0.3	-0.70±3.34		-0.5	**0.043**	m
Intra group difference		***0.000***		w	***0.000***		w		
AMC (µL)									
Before treatment		0.74±0.79		0.6	0.68±0.44		0.5	0.587	m
After treatment		0.69±0.27		0.7	0.64±0.58		0.5	**0.002**	m
Before/after difference		-0.05±0.79		0.1	-0.04±0.65		-0.1	0.106	m
Intra group difference		0.077		w	0.345		w		
LMR									
Before treatment		3.48±5.47		2.7	2.75±1.87		2.3	0.285	m
After treatment		2.22±1.20		1.9	2,66±3.11		1.6	0.049	m
Before/after difference		-1.26±5.52		-0.6	-0.09±3.34		-0.4	0.398	m
Intra group difference		***0.000***		w	0.133		w		

m: Mann-Whitney U test, x^2: Chi-square test, w: Wilcoxon test.

ALC: Absolute lymphocyte count, AMC: Absolute monocyte count, LMR: Lymphocte/monocyte ratio, SD: Standard deviation

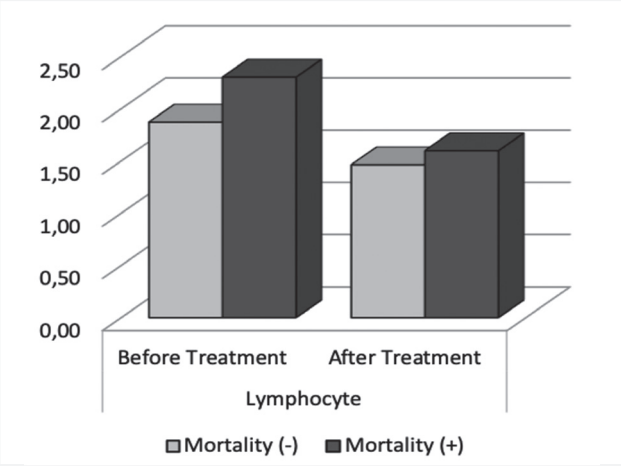

Figure 2. Absolute lymphocyte count and mortality relationship in diffuse large-B-cell lymphoma patients

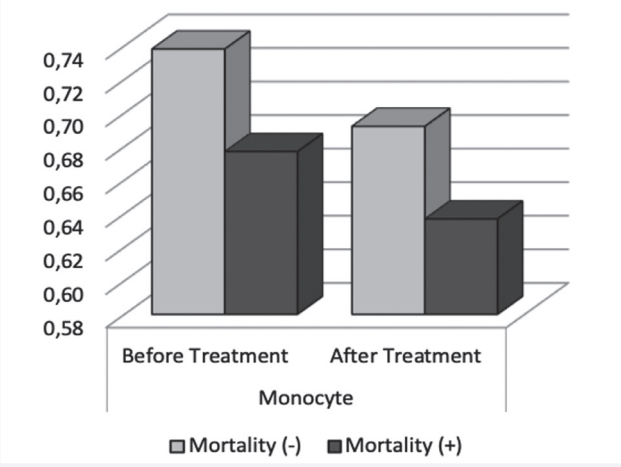

Figure 3. Absolute monocyte count and mortality relationship in diffuse large-B-cell lymphoma patients

refractory DLBCL[12,13]. In addition to this, it has been reported that IPI is less predictive and less beneficial during rituximab[14]. Therefore, some previous studies have shown that LMR could be a useful prognostic determinant for survival in DLBCL in order to demonstrate whether LMR improves predictive value in calculating the risk of patients with DLBCL[15-18]. ALC is a representative determinant of host immunity and AMC tumor microenvironment. Thus, LMR is a predictive biomarker for clinical outcomes in DLBCL[19]. However, one limitation point for LMR at initial diagnosis is that it cannot evaluate the host/tumor interaction during treatment[19].

Bento et al.[20] established the cut-off levels as 1.1 μL for ALC, 0.79 μL for AMC, and 2.25 for LMR in their study and emphasized that low LMR predicted both shorter PFS and also OS. In another study, Katoh et al.[21] found it statistically significantly longer for 2-year OS and PFS among patients with low (<2.6) and high

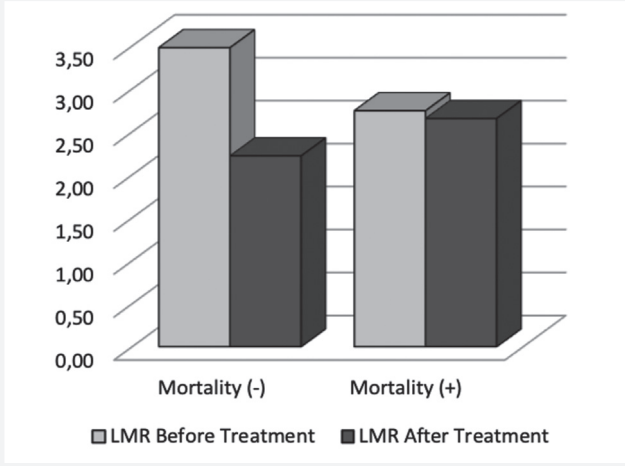

Figure 4. Lymphocte/monocyte ratio and mortality relationship in diffuse large-B-cell lymphoma patients

LMR: Lymphocte/monocyte ratio

LMR (>2.6) for patients with relapse/refractory DLBCL (79.4% vs. 22.4%; p<0.001 and 68.9% vs 0%; p<0.001). In addition, a low LMR was found in patients with primary resistance for recurrent diseases poor 2-year OS and PFS was predicted[21]. In our study, the median levels of ALC, AMC, and LMR were 1.6 μL, 0.6 μL, and 2.7, respectively. Contrary to previous studies[15-17], pre-treatment and post-treatment ALC in the exitus group was significantly higher than in the alive group (p<0.05). The decrease in ALC after the treatment in the exitus group was significantly higher than in the alive group (p<0.05) (Table 2). Pre-treatment AMC did not show a significant difference neither in both groups (p>0.05). Post-treatment AMC in the exitus group was significantly higher than in the alive group (p<0.05) (Table 2). Pre-treatment LMR did not show a significant difference in both groups (p>0.05). Post-treatment LMR level in the exitus group was significantly higher than in the alive group (p<0.05). Post-treatment LMR change did not differ significantly in both groups (p>0.05) (Table 2).

However, there are a few limitations of using only LMR to determine patient prognosis. The survival outcomes of patients are not affected only by the immune system, but also by the specific characteristics of some tumors such as precise genetic mutations, pathology of tumors, and tumor size[22,23]. Chemotherapy or radiotherapy could affect the function of the immune cells[24-26]. But the most importantly, count and functions of lymphocytes or monocytes could be regulated by tumor cells[27]. Therefore, LMR alone is probably not a perfect predictor of patients' clinical survival.

Similar to LMR, neutrophil-lymphocyte ratio (NLR) obtained from complete blood count can also be used to determine prognosis. Demircioglu et al.[28] showed that NLR was associated with IPI and disease stage.

Study Limitations

Our study has some limitations. The predictive value for OS and PFS, and the dynamic change of ratio during therapy, progression or relapse should also be confirmed in a larger population and at longer follow-up duration. Regular complete blood count should be performed to determine whether effective treatment will improve ALC and AMC.

CONCLUSION

In our study, contrary to previous studies, no significant effect of LMR on survival was detected. Therefore, LMR should be evaluated together with factors such as IPI, LDH, Ki-67 proliferation index, and PET/CT. A larger patient group is needed to obtain more effective data.

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Ethics

Ethics Committee Approval: Bezmialem Vakıf University Ethics Committee approval was obtained from the Non-interventional Clinical Research Ethics Committee, with the protocol number of 20/380 and date of 01.12.2020.

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

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ORIGINAL ARTICLE

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The Effect of Rosmarinic Acid on Cell Viability, Steatosis, Paraoxonase-1, and Paraoxonase-3 Protein Levels in Palmitate-induced Non-alcoholic Fatty Liver Disease Model in HepG2 Cells

Palmitat ile Non-alkolik Yağlı Karaciğer Hastalığı Modeli Oluşturulan HepG2 Hücrelerinde Rosmarinik Asitin Hücre Canlılığına, Yağlanmaya, Paraoksonaz-1 ve Paraoksonaz-3 Protein Düzeylerine Etkisi

Esra YAKŞİ[1], Eray ÖZGÜN[2]

[1]*Lüleburgaz City Hospital, Kırklareli, Turkey*
[2]*Trakya University Faculty of Medicine, Department of Medical Biochemistry, Edirne, Turkey*

ABSTRACT

Aim: We aimed to investigate the effect of rosmarinic acid (RA) on cell viability, steatosis, paraoxonase (PON)1, and PON3 protein levels in palmitate-induced non-alcoholic fatty liver disease (NAFLD) model in HepG2 cells.

Materials and Methods: To induce an experimental steatosis model, HepG2 cells were incubated with 1 mM palmitate for 24 hours. For the treatment, non-toxic RA concentrations were added to the cell culture medium simultaneously with the palmitate. Cell viability was evaluated by 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide assay. To evaluate steatosis, intracellular triglyceride levels were measured and the cells were examined microscopically with Oil-Red O staining. PON1 and PON3 protein levels were measured by Western blotting.

Results: 1 mM palmitate caused a significant decrease in cell viability and a significant increase in triglyceride levels, but it did not significantly change PON1 and PON3 protein levels. RA caused a significant increase in cell viability and a significant decrease in triglyceride levels in the palmitate-treated cells. Similar findings with the triglyceride levels of cells were shown in microscopic examination of cells that were stained with Oil-Red O. RA did not significantly change PON1 and PON3 protein levels in neither non-treated cells nor treated cells with palmitate.

Conclusion: Our study showed that RA increases cell viability and decreases steatosis, but it does not change PON1 and PON3 protein levels in palmitate-induced NAFLD model in HepG2 cells.

Keywords: Non-alcoholic fatty liver disease, rosmarinic acid, palmitate, paraoxonase-1, paraoxonase-3

ÖZ

Amaç: Palmitat ile non-alkolik yağlı karaciğer hastalığı modeli oluşturulan HepG2 hücrelerinde rosmarinik asitin (RA) hücre canlılığına, yağlanmaya, paraoksonaz (PON) 1 ve PON3 protein düzeylerine etkisini araştırmayı amaçladık.

Gereç ve Yöntem: Deneysel yağlanma modeli oluşturmak için, HepG2 hücreleri 1 mM palmitat ile 24 saat inkübe edildi. Tedavi olarak palmitat ile aynı anda hücre kültürü medyumuna, HepG2 hücrelerine toksik olmayan RA konsantrasyonları eklendi. Hücre canlılığı 3-(4,5-dimetil-2-tiazolil)-2,5-difenil-2H-tetrazolium bromür testi ile değerlendirildi. Yağlanmanın değerlendirilmesi için hücre içi trigliserid düzeyleri ölçüldü ve hücreler Oil-Red O ile boyanarak mikroskopik olarak incelendi. PON1 ve PON3 protein düzeyleri Western blot yöntemiyle ölçüldü.

Bulgular: 1 mM palmitat hücre canlılığında anlamlı bir azalmaya ve trigliserid düzeylerinde anlamlı bir artışa yol açtı, PON1 ve PON3 protein düzeylerini ise anlamlı olarak değiştirmedi. Palmitat ile oluşturulan deneysel non-alkolik yağlı karaciğer hastalığı (NAFLD) modelinde RA, hücre canlılığını anlamlı olarak artırdı ve trigliserid düzeylerini anlamlı olarak azalttı. Oil-Red O ile boyanan hücrelerin mikroskopik incelenmelerinde

Address for Correspondence: Dr. Eray ÖZGÜN, Trakya University Faculty of Medicine, Department of Medical Biochemistry, Edirne, Turkey
E-mail: drozgune@hotmail.com **ORCID ID:** orcid.org/0000-0002-6744-1519
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hücrelerin trigliserid düzeylerindeki değişimler ile benzer bulgular görüldü. RA hem palmitat uygulanmayan hem de palmitat uygulanan HepG2 hücrelerinde PON1 ve PON3 protein düzeylerini anlamlı olarak değiştirmedi.

Sonuç: Çalışmamız, RA'nın palmitat ile NAFLD modeli oluşturulan HepG2 hücrelerinde hücre canlılığını artırdığını, yağlanmayı azalttığını, ancak PON1 ve PON3 protein düzeylerini değiştirmediğini gösterdi.

Anahtar Kelimeler: Non-alkolik yağlı karaciğer hastalığı, rosmarinik asit, palmitat, paraoksanaz-1, paraoksonaz-3

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a disease characterized by the presence of different degrees of fatty liver in individuals who do not have a habit of use of alcohol. NAFLD is one of the important health problems with its prevalence estimated to be around 25% worldwide. Triglyceride accumulation in hepatocytes is increased in NAFLD patients[1,2]. Palmitate, a 16-carbon saturated fatty acid, is found in the structure of triglycerides in the liver and is one of the most abundant fatty acids in the livers of both healthy individuals and NAFLD patients[3].

NAFLD is not just a liver disease, but it is a multisystem disease. NAFLD has also been shown to be associated with metabolic syndrome and atherosclerosis[4,5]. Paraoxonase (PON) 1 and PON3 enzymes are anti-atherogenic enzymes mainly synthesized in the liver. PON1 and PON3 are secreted from the liver into the circulation and are transported in the circulation bound to high-density lipoprotein (HDL)[6].

HepG2 cells are commercially available human-derived hepatoma cells and are frequently used to induce an experimental NAFLD model with free fatty acids because of their similarity to hepatocytes[7-10]. It has been reported that the steatosis induced by 1 mM palmitate in HepG2 cells is a cellular model of NAFLD that can be used to investigate acute and toxic effects in liver cells due to fat accumulation[7].

Rosmarinic acid (RA) is phenolic acid, the ester of caffeic acid and 3,4 dihydroxyphenyllactic acid synthesized from L-phenylalanine and L-tyrosine amino acids[11]. RA is a polyphenol found naturally in plants such as rosemary, sage, and perilla steak grass, with shown antioxidant and antiinflammatory effects[12]. In the literature, we did not come across any study investigating the effect of RA on PON1 and PON3 enzymes.

In this study, it was aimed to investigate the effect of RA on cell viability, steatosis, PON1 and PON3 protein levels in palmitate-induced NAFLD model in human-derived hepatoma cells. With this study, the effect of RA on PON1 and PON3 levels both when administered directly and in NAFLD was shown for the first time.

MATERIALS AND METHODS

Chemical and Consumables

RA, sodium palmitate, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) and Oil Red O were purchased from Sigma-Aldrich (St. Louis, MO, USA). Eagle's minimal essential mediums were purchased from Wisent (St-Bruno, QC, Canada). Fetal bovine serum (FBS), antibiotic-antimycotic and chemiluminescent substrate were purchased from Thermo Fisher (Waltham, MA, USA). Primary antibodies to PON1, PON3, and alpha tubulin and secondary antibodies were purchased from Abcam (Cambridge, England). Fatty acid-free bovine serum albumin was purchased from Gold Biotechnology (MO, USA). Radioimmunoprecipitation lysis buffer (RIPA) was purchased from Santa Cruz (Heidelberg, Germany). Polyvinylidene difluoride (PVDF) membrane was purchased from Bio-Rad (Hercules, CA, USA). All chemicals were of analytical purity.

Cell Culture Applications

This study was approved by Trakya University Deanship of Faculty of Medicine Scientific Research Ethics Committee on 29.03.2017 with the protocol code of TUTF-BAEK 2017/95 and decision number 06/05. Cells were kept in an incubator at 37 °C in 5% CO_2 with Eagle's minimum essential medium containing 10% FBS and 1% antibiotic-antimycotic. For the experiments, cells between passages of 10 and 20 were used.

HepG2 cells were incubated with 1 mM palmitate for 24 hours to induce steatosis[7-9]. Sodium palmitate was dissolved in sterile water at 70 °C[13,14] and conjugated for at least 3 hours 15 with 0.7 mM fatty acid-free bovine serum albumin, which was dissolved in medium and its concentration was similar to human serum albumin concentrations and cells were incubated with mixture for 24 hours. In the control group, only medium containing 0.7 mM albumin was applied.

MTT Test

MTT test was performed to assess cell viability[16]. 10^4 cells were seeded in 96-well plates. RA alone and together with 1 mM palmitate was applied to the cells at different concentrations for 24 hours. After 24 hours, the medium was removed and replaced with MTT (5 mg/mL) dissolved in 10 µL of PBS and 100 µL of Eagle's minimum essential medium without phenol red, and incubated for 4 hours. The medium containing MTT was taken and formazan, was dissolved with 200 µL dimethyl sulfoxide and 25 µL Sorenson buffer (0.1 M glycine and 0.1 M sodium chloride; adjusted to pH: 10.5 with 0.1 M sodium hydroxide) and the resulting color was measured spectrophotometrically at 570/630 nm in a microplate reader[17].

The measured absorbances were divided by the mean of the control group on the same plate, and the results were given as a percentage of the control group.

Triglyceride Measurement

Cells were seeded into 25 cm^2 flasks for triglyceride measurement. Different concentrations of RA were applied to the cells simultaneously with 1 mM palmitate for 24 hours. After 24 hours, cells were washed with phosphate buffered saline (PBS) and then scraped with PBS buffer containing 1% protease inhibitor and 0.1% Triton X-100. After homogenization using glass beads, the homogenate was centrifuged at 10,000 g for 10 minutes. The supernatant in the upper part was used in the experiments. Intracellular triglyceride levels were measured using the autoanalyzer's original kits. Protein levels were measured according to the method of Lowry et al.[18], and triglyceride levels were divided to protein results and results of groups were given as fold of control by dividing the results to the mean value of control group.

Oil Red O Staining

For staining with Oil Red O, cells were seeded in 6-well plates. Different concentrations of RA were applied to the cells simultaneously with 1 mM palmitate for 24 hours. After 24 hours, cells were washed with PBS and fixed with 10% paraformaldehyde. After fixation, it was washed with 60% isopropanol and incubated with 60% isopropanol containing Oil Red O [stock 0.35% (w/v) Oil Red O dissolved in 100% isopropanol] and washed with distilled water and photographed under a phase contrast invert microscope using a microscope camera[9].

Western Blot Method

Cells were seeded in 75 cm^2 flasks. Different concentrations of RA were applied to the cells simultaneously with 1 mM palmitate for 24 hours. After the experimental procedure, cells washed with PBS were scraped with RIPA containing 1% of protease inhibitor cocktail, phenyl methyl sulfonyl fluoride and sodium orthovanadate solutions. After homogenization using glass beads, the homogenate was centrifuged at 10,000 g for 10 minutes. The supernatant in the upper part was used in the experiments. Protein measurement was performed according to the method of Lowry et al.[18]. A 4-12% polyacrylamide gel was prepared according to the Laemmli[19] method, and 20 µg of protein was separated by vertical sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Gels were transferred to a polyvinylidene difluoride membrane using a semi-wet transfer system. Membranes were blocked with 5% milk powder for 1 hour and incubated with primary antibodies (1:1,000 dilution for PON1 and PON3, 1:10,000 dilution of tubulin) overnight at 4 °C. Then, membranes incubated for 1 hour with secondary antibody (1:10,000 dilution) were visualized using a chemiluminescent substrate. Band intensity for each protein was calculated using the Image J program[20]. PON1 and PON3 protein levels were divided to the level of alpha tubulin protein used as the loading control of the same sample, and the results were given as a fold of control by dividing value to the value of control group in the same membrane.

Statistical Analysis

Statistical analyzes were performed using the Statistical Package for the Social Sciences 20 program. One-way ANOVA was used to compare experimental parameters, and Tukey and Tamhane tests were used for comparison between groups. Results were expressed as mean±standard deviation, and $p<0.05$ was considered statistically significant.

RESULTS

Cell viabilities after RA treatment to HepG2 cells for 24 hours were found as: 100±3 in the control group, 96±6% in cells treated with 5 µM RA, 106±8% in cells treated with 10 µM RA, 107±6% in cells treated with 25 µM RA, 122±9% in cells treated with 50 µM RA, 131±8% in cells treated with 100 µM RA, 119±8% in cells treated with 200 µM RA, 77±7% in cells treated with 300 µM RA, 73±5% in cells treated with 400 µM RA, and 68±6% in cells treated with 500 µM RA. 5, 10 and 25 µM RA did not significantly change cell viability compared to the control group ($p>0.05$ for all). 50, 100 and 200 µM RA significantly increased cell viability compared to the control group, 5, 10 and 25 µM RA ($p<0.05$ for all). 200 µM RA significantly decreased cell viability compared to 100 µM RA ($p<0.05$). 300, 400 and 500 µM RA significantly decreased

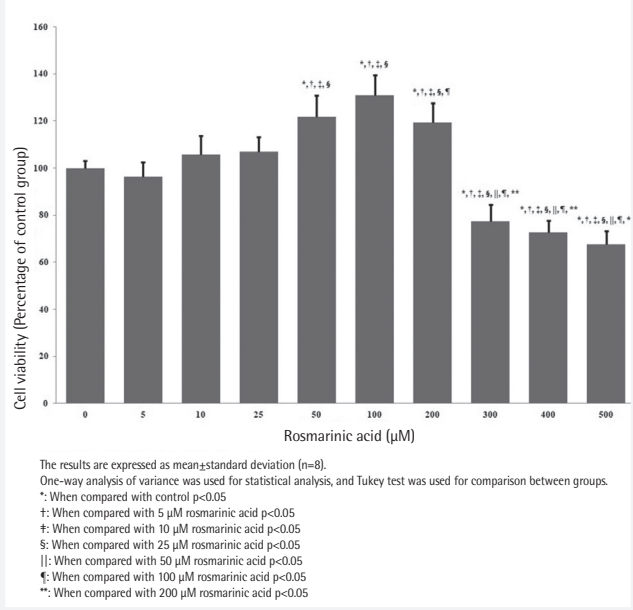

Figure 1. Effect of rosmarinic acid on cell viability in HepG2 cells

cell viability compared to control and 5, 10, 25, 50, 100 and 200 µM RA (p<0.05 for all) (Figure 1).

Cell viabilities after 24 hours treatment to HepG2 cells were found as: 100±6% in the control group, 54±4% in cells treated with only 1 mM palmitate; 54±6% in cells treated with 1 mM palmitate and 5 µM RA, 55±6% in cells treated with 10 µM RA, 59±6% in cells treated with 25 µM RA, 66±6% in cells treated with 50 µM RA, 75±7% in cells treated with 100 µM RA and 83±5% in cells treated with 200 µM RA. Cell viability was significantly reduced in all cells treated with 1 mM palmitate compared to the control group (p<0.05 for all). Treatment of 50 µM RA with 1 mM palmitate significantly increased cell viability compared to treatment of only 1 mM palmitate and 5 and 10 µM RA with 1 mM palmitate (p<0.05 for all). Treatment of 100 µM RA with 1 mM palmitate significantly increased cell viability compared to treatment of only 1 mM palmitate and 5, 10, and 25 µM RA with 1 mM palmitate (p<0.05 for all). Treatment of 200 µM RA with 1 mM palmitate significantly increased cell viability compared to treatment of only 1 mM palmitate and 5, 10, 25 and 50 µM RA with 1 mM palmitate (p<0.05 for all) (Figure 2).

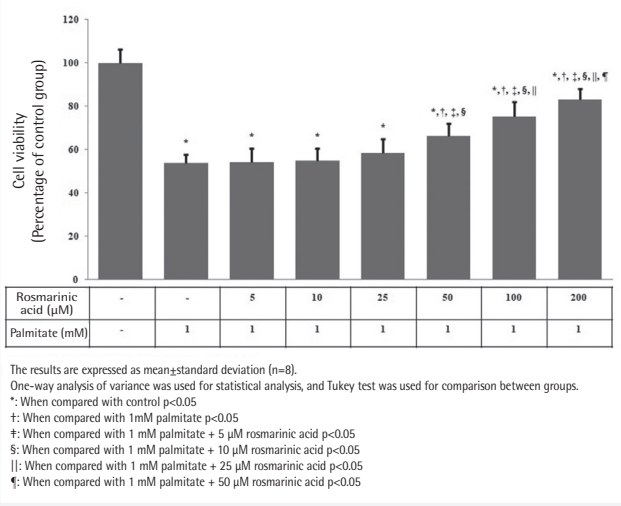

Figure 2. The effect of rosmarinic acid on cell viability in palmitate-induced steatosis in HepG2 cells

Triglyceride levels after 24 hours treatment to HepG2 cells were found as 1.00±0.07 in the control group, 2.54±0.06 in cells treated with only 1 mM palmitate; 2.55±0.04 in cells treated with 1 mM palmitate and 5 µM RA, 2.47±0.12 in cells treated with 1 mM palmitate and 10 µM RA 2.44±0.15 in cells treated with 1 mM palmitate and 25 µM RA 2.27±0.05 in cells treated with 1 mM palmitate and 50 µM RA 1.94±0.17 in cells treated with 1 mM palmitate and 100 µM RA and 1.77±0.27 in cells treated with 1 mM palmitate and 200 µM RA. Triglyceride levels increased significantly in all cells treated with 1 mM palmitate compared to the control group (p<0.05 for all). Treatment of 50 µM RA with 1 mM palmitate significantly reduced triglyceride levels compared to treatment of only 1 mM palmitate and treatment of 5 µM RA with 1 mM palmitate (p<0.05 for both). Treatment of 100 µM RA with 1 mM palmitate significantly reduced triglyceride levels compared to administration of only 1 mM palmitate and administration of 5, 10, and 25 µM RA with 1 mM palmitate (p<0.05 for all). Treatment of 200 µM RA with 1 mM palmitate significantly reduced triglyceride levels compared to treatment of only 1 mM palmitate and treatment of 5, 10, and 25 µM RA with 1 mM palmitate (p<0.05 for all) (Figure 3A).

The effect of RA on steatosis was examined microscopically by Oil Red O staining in HepG2 cells, in which palmitate steatosis was created. It was observed that the staining with oil red O was higher in the cells treated with 1 mM palmitate compared to the control group. It was observed that RA concentrations applied together with 1 mM palmitate reduced staining with Oil Red O, and this decrease was more pronounced especially at higher concentrations (Figure 3B).

PON1 protein levels after RA treatment to HepG2 cells for 24 hours were: 1.11±0.13 fold of control in cells treated with 100 µM RA, and 1.07±0.07 fold of control in cells treated with 200 µM RA. PON3 protein levels after 24 hours treatment to HepG2 cells were: 1.12±0.16 fold of control in cells treated with 100 µM RA and 1.25±0.28 fold of control in cells treated with 200 µM RA. There was no significant difference in PON1 and PON3 levels between 100 µM and 200 µM RA treated and control cells (p>0.05 for all) (Figure 4A).

After RA treatment to HepG2 cells with 1 mM palmitate for 24 hours, PON1 levels were 0.94±0.05 fold of control group in cells treated with only 1 mM palmitate; it was found to be 1.01±0.10 fold of control group in cells treated with 1 mM palmitate and 100 µM RA, and 0.92±0.05 fold of control group in cells treated with 200 µM RA. PON3 levels were 1.03±0.11 fold of control group in cells treated with only 1 mM palmitate; it was found to be 0.95±0.08 fold of control group in cells treated with 1 mM palmitate and 100 µM RA, and 1.04±0.17 fold of control group in cells treated with 200 µM RA. There was no significant difference in PON1 and PON3 protein levels between treatment of 1 mM palmitate, 100 µM and 200 µM RA with 1 mM palmitate and control cells (p>0.05 for all) (Figure 4B).

DISCUSSION

NAFLD is a multisystemic disease whose effects are not limited to the liver and is an important health problem with an increasing incidence all over the world[4,21]. NAFLD is an

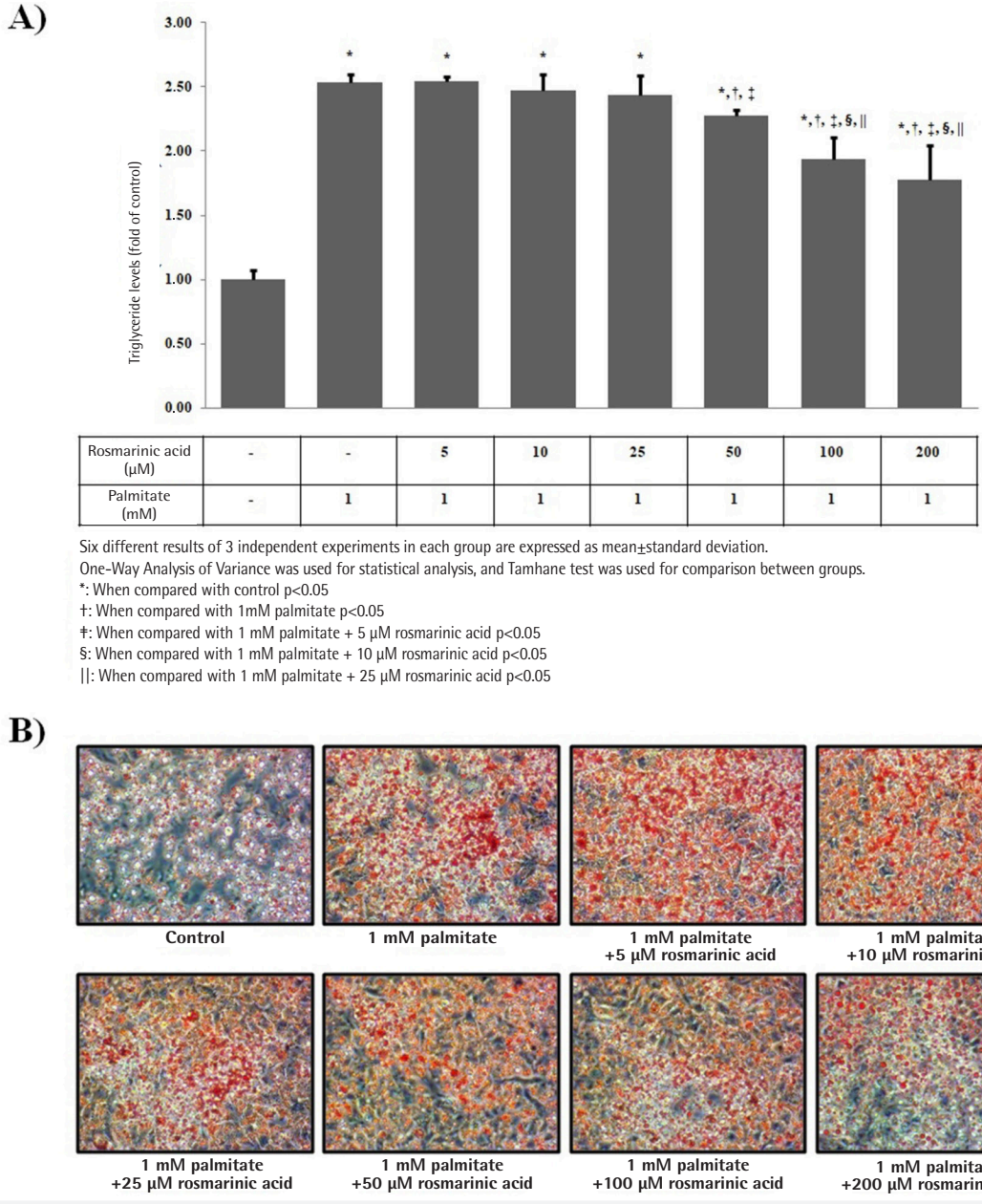

Figure 3. The effect of rosmarinic acid on A) triglyceride levels and B) steatosis (Oil Red O staining, 400x magnification) in palmitate-induced steatosis in HepG2 cells

independent risk factor for atherogenic dyslipidemia and has also been shown to be associated with atherosclerosis[2,5]. Although diet and exercise are recommended primarily in the treatment of NAFLD, it has been reported that antioxidant therapy, especially vitamin E, can be added to the treatment in these patients[1,22]. RA is a plant-derived polyphenol that protects against oxidative damage and scavenges free radicals[12,23]. The most common cause of death in NAFLD patients is cardiovascular diseases[24]. PON1 and PON3 enzymes are antioxidant enzymes mainly synthesized in the liver. These enzymes take part in the structure of HDL in the circulation and play a role in preventing the development of atherosclerosis[6].

The aim of our study was to investigate the effect of RA on cell viability, steatosis, PON1 and PON3 protein levels in palmitate-induced experimental NAFLD model in HepG2 cells.

There are conflicting results in the literature regarding the effect of RA on cell viability in HepG2 cells. Adomako-Bonsu et al.[25] reported that RA applied to HepG2 cells for 5 hours was not toxic up to 700 μM and caused 25% toxicity at 2800 μM

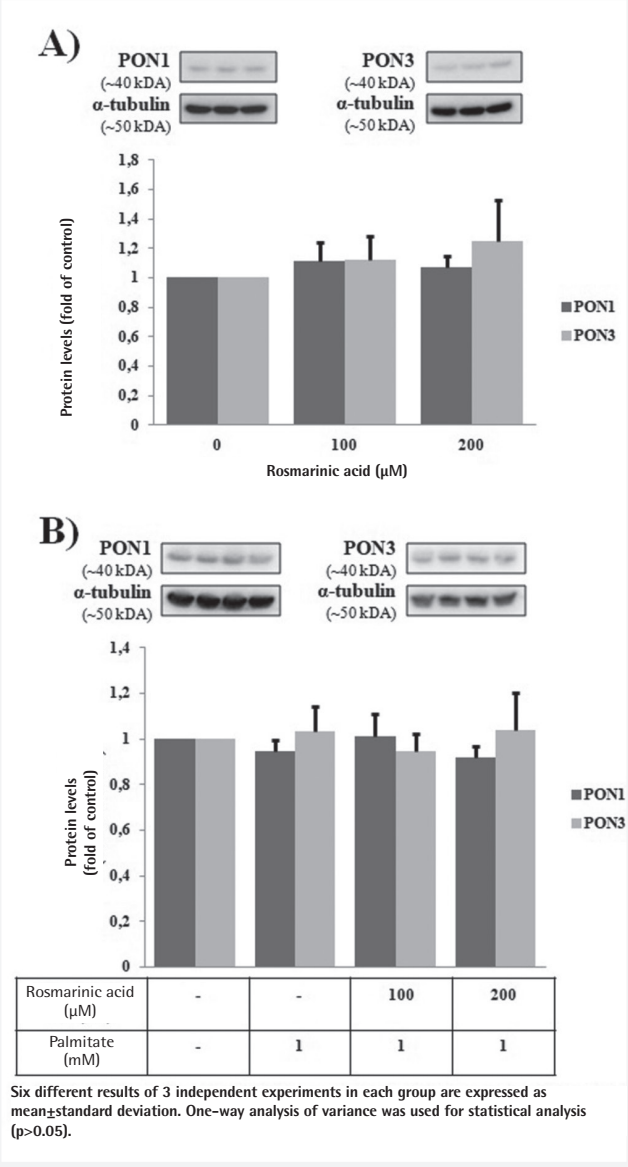

Figure 4. Effect of rosmarinic acid on levels of paraoxonase 1 and 3 when, A) administered alone and B) combined with 1 mM palmitate to HepG2 cells

concentration; Wu et al.[26] reported that 160 μM RA applied to HepG2 cells for 24 or 48 hours was not toxic. Ozgun and Ozgun[27] reported that 100 μM RA applied to HepG2 cells for 24 hours did not cause a decrease in cell viability, while 1000 μM RA reduced cell viability by more than 50%. On the other hand, Ma et al.[28] reported that approximately 35 μM of RA applied to HepG2 cells for 24 hours reduced cell viability. For this reason, in our study, the effect of RA on cell viability was investigated by applying RA concentrations up to 500 μM to HepG2 cells for 24 hours. While 50, 100 and 200 μM RA significantly increased cell viability in HepG2 cells, RA concentrations of 300 μM and above significantly decreased cell viability, and therefore RA concentrations up to 200 μM were applied against palmitate-induced steatosis.

It is known that in the palmitate-induced cellular NAFLD model in HepG2 cells, cell viability decreases and intracellular steatosis increases[7,8]. In our study, palmitate application significantly decreased cell viability and increased intracellular triglyceride level, which was consistent with the literature. It was also seen microscopically that palmitate application increased the intracellular lipid content. These findings prove that palmitate-induced cellular NAFLD model are formed in HepG2 cells in our study.

In our study, 50, 100 and 200 μM RA significantly reduced palmitate-induced cell death. In the literature, we could not find any study investigating the effect of RA on cell viability in the palmitate-induced cellular NAFLD model, but in a different experimental model that supports our findings, it has been reported that RA reduces tert-butyl hydroperoxide-induced cell death in HepG2 cells depending on the dose[29]. In our study, 50, 100 and 200 μM RA significantly reduced the increase in triglyceride levels caused by palmitate, and it was also seen microscopically with Oil Red O staining that RA reduced steatosis, especially at high doses. We found only one study investigating the effect of RA on palmitate-induced experimental steatosis, and in this study published in 2020, Kim et al.[10] reported that RA reduced the steatosis induced by 500 μM palmitate, in line with our study.

In our study, 1 mM palmitate administration in HepG2 cells did not significantly change PON1 and PON3 protein levels. In the literature, Özgün et al.[8] supportive of our study, reported that palmitate administration to HepG2 cells, in which an experimental NAFLD model was created with 1 mM palmitate, did not change PON1 and PON3 protein levels. At the same time, Kudchodkar et al.[30] reported that dietary tripalmitin did not change PON1 activity in rats, while Boshtam et al.[31] reported that there was no difference between palmitic acid content of HDL and PON1 activities.

There is no study in the literature investigating the effect of RA on PON1 or PON3 enzymes. In our study, 100 and 200 μM RA, the most effective doses on cell viability and intracellular steatosis, had no significant effect on PON1 and PON3 protein expressions both directly in HepG2 cells and in the palmitate-induced cellular NAFLD model in HepG2 cells. In the light of these findings, we can say that RA has no significant effect on PON1 and PON3 protein levels in HepG2 cells, neither when administered alone nor in combination with palmitate.

Study Limitations

Examination of the effects of RA only at the cellular level since it is an *in vitro* experimental study, experimental NAFLD model's being limited to 24 hours to avoid serum starvation in cells as stated in the literature, and the use of HepG2 cell line

instead of primary hepatocytes, although it is frequently used in the literature, are the limitations of this study.

CONCLUSION

Our study showed that RA increased cell viability and decreased steatosis, but did not change PON1 and PON3 protein levels in the palmitate-induced cellular NAFLD model in human hepatoma cells. In the light of our findings, we can say that RA may be effective in the prevention of NAFLD because it reduces steatosis, but it has no effect on the levels of antiatherogenic enzymes PON1 and PON3. However, our study is an *in vitro* experimental study and our findings should be supported by future animal and human studies.

Ethics

Ethics Committee Approval: This study was approved by Trakya University Deanship of Faculty of Medicine Scientific Research Ethics Committee on 29.03.2017 with the protocol code of TUTF-BAEK 2017/95 and decision number 06/05.

Informed Consent: Commercially available HepG2 cells were used in this study.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

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

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ORIGINAL ARTICLE

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Can Mean Platelet Volume Value Be Used as Inflammation Marker in Children with Familial Mediterranean Fever?

Ailesel Akdeniz Ateşi Tanılı Çocuklarda Ortalama Trombosit Hacmi Değeri Enflamasyon Belirteci Olarak Kullanılabilir mi?

Erhan AYGÜN[1], Fatih Mehmet KELEŞOĞLU[2], Rukiye ÖMEROĞLU EKER[2]

[1]*İstanbul University, İstanbul Faculty of Medicine, Department of Child Health and Diseases, İstanbul, Turkey*
[2]*İstanbul University, İstanbul Faculty of Medicine, Department of Child Health and Diseases, Division of Pediatric Rheumatology, İstanbul, Turkey*

ABSTRACT

Aim: Familial Mediterranean Fever (FMF) is disrupted response to inflammation by the organism as a result of mutations in the *MEFV* gene. It has been shown that the mean platelet volume could be used as a marker in many inflammatory diseases. In this study, it was aimed to determine inflammation by observing the changes in the mean platelet volume in the complete blood count.

Materials and Methods: The study included 570 children diagnosed with the FMF based on the Tel-Hashomer Criteria. We also included 73 healthy children as a control group. Demographic features, results of genetic analyses, complete blood count parameters, mean platelet volume levels, and C-reactive protein results were evaluated retrospectively. Data of the patients were recorded separately based on the acute attack and attack-free periods under treatment.

Results: The mean platelet volume was found to be 8.4±2.4 fL in the attack period and 9.5±1.9 fL in the attack-free period. The mean platelet volume was significantly lower in patients with an attack period compared to the patients at an attack-free period. Leukocyte count and C-reactive protein were statistically significantly higher in the attack period compared to the attack-free period.

Conclusion: The mean platelet volume decreases in patients with FMF with the effect of inflammation during the attack period. We think that it is important to consider mean platelet volume while evaluating the blood count.

Keywords: Familial Mediterranean Fever, inflammation, mean platelet volume

ÖZ

Amaç: Ailevi Akdeniz Ateşi (AAA), *MEFV* geninde meydana gelen mutasyonlar sonucu organizmanın enflamasyona verdiği yanıtın bozulmasıdır. Enflamatuvar hastalıkların pek çoğunda ortalama trombosit hacminin belirteç olarak kullanılabileceği gösterilmiştir. Bu çalışmada tam kan sayımındaki ortalama trombosit hacmindeki değişikliklere bakarak enflamasyonun belirlenmesi amaçlandı.

Gereç ve Yöntem: Çalışmaya Tel-Hashomer kriterlerine göre AAA tanısı almış 570 çocuk dahil edildi. Ayrıca 73 sağlıklı çocuk kontrol gurubu olarak dahil edildi. Hastaların demografik özellikleri, genetik tarama sonuçları, tam kan sayımı parametreleri, ortalama trombosit hacminin düzeyleri, C-reaktif protein değerleri retrospektif olarak değerlendirildi. Hastaların verileri tedavi altında ataklı ve ataksız dönemlerine göre ayrı ayrı kaydedildi.

Bulgular: Ortalama trombosit hacmi ataklı dönemde 8,4±2,4 fL iken ataksız dönemde 9,5±1,9 fL olarak saptandı. Hastaların ataklı dönem ortalama trombosit hacminin düzeyleri, ataksız döneme göre istatistiksel olarak anlamlı düşüktü. Beyaz küre sayıları ve C-reaktif protein seviyeleri atak döneminde ataksız döneme göre istatistiksel olarak anlamlı yüksekti.

Sonuç: AAA tanılı hastalarda ortalama trombosit hacmi, enflamasyonun etkisiyle atak döneminde azalmaktadır. Kan sayımı sonucu değerlendirilirken ortalama trombosit hacminin değerinin de göz önünde bulundurulmasının önemli olduğunu düşünmekteyiz.

Anahtar Kelimeler: Ailevi Akdeniz Ateşi, enflamasyon, ortalama trombosit hacmi

Address for Correspondence: Erhan AYGÜN MD, İstanbul University, İstanbul Faculty of Medicine, Department of Child Health and Diseases, İstanbul, Turkey
E-mail: dr.erhanaygun@gmail.com **ORCID ID:** orcid.org/0000-0002-4495-5824
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INTRODUCTION

Familial Mediterranean Fever (FMF) is an autosomal recessively inherited inflammatory disease characterized by short-term attacks of polyserositis accompanied by fever. It has been frequently described in communities of Mediterranean and Middle Eastern origin such as Turks, Armenians, Jews, and Arabs[1]. Finding the *MEFV* gene encoding the pyrin protein on the short arm of the sixteenth chromosome has revealed that the clinical course of the disease occurs due to the inability to suppress inflammation[2,3]. It has been found that acute inflammatory markers such as C-reactive protein (CRP), sedimentation, serum amyloid A, interleukin-2 (IL), IL-6, IL-8 and tumor necrosis factor-alpha (TNF-α) levels are high during FMF attack periods[4,5]. Moreover, it has also been reported that the inflammatory process continues in attack-free periods[6]. Mean platelet volume (MPV) is an inexpensive laboratory value that shows the MPV routinely measured in the complete blood count and shows platelet function and activation[7]. Large platelets are functionally, metabolically and enzymatically more active than small ones[8].

In the literature, it has been reported that MPV has an important role as an inflammation marker and can be used as a marker for the activity of the disease in chronic inflammatory diseases[9-13]. There are studies evaluating MPV in patients with FMF, but conflicting results have been reported.

In this study, it was aimed to determine inflammation by evaluating changes in MPV in complete blood count, which is a simple test in pediatric FMF patients.

MATERIALS AND METHODS

We retrospectively analyzed 570 patients who were diagnosed with FMF according to the Tel-Hashomer Criteria and followed up at regular intervals in the Pediatric Rheumatology Outpatient Clinic between 2014 and 2016. Mutation results were recorded. The patients were divided into two groups: The attack group (those with active clinical symptoms and CRP >10 mg/L) and the attack-free group. In addition, 73 cases who applied to the pediatric outpatient clinic and did not have inflammatory findings were included as the control group. Cases without mutation analysis results and clinical data or those who were treated with anakinra were not included in the study.

As laboratory parameters, white blood cell, platelet counts, MPV, and CRP levels were recorded. The examinations performed during the attack and non-attack periods were recorded separately. Complete blood count was studied in automatic cell counting devices (LH 780 Beckman Coulter) with blood samples taken into tubes containing dipotassium ethylenediamine tetraacetic acid (EDTA). CRP titers were analyzed on Beckman-Coulter DXC 800 brand devices in our laboratory using standard analyzers.

Our study was carried out in accordance with the principles of the Declaration of Helsinki. Research and publication ethics were complied with. Approval was obtained from the Ethics Committee of İstanbul University in March 2016 with the ethics number of 2016/363.

Statistical Analysis

As descriptive statistics, mean and standard deviation for numerical variables and number and percentage values for categorical variables were given. While the significance test of the difference between the two means was used in group comparison for numerical variables, the chi-square test was used for categorical variables. The Student's t-test was used to compare continuous data. To compare 3 groups in this study, one-way ANOVA followed by post-hoc Bonferroni test was performed in statistical analysis. Analyses were obtained using the Statistical Package for the Social Sciences v.21 software. The significance level was taken as $p<0.05$.

RESULTS

A total of 570 FMF patients and 73 healthy cases were included in the study. The attack group consisted of 33 boys (54%) and 28 girls (46%), the non-attack group included 239 boys (47%) and 270 girls (53%), and the control group consisted of 34 boys (47%) and 39 girls (53%). The mean age was 9.8±4.63 years in the FMF attack group, 11.2±4.46 years in the attack-free group, and 10.4±5.8 years in the control group. All patients included in the study were receiving colchicine treatment. One or more mutations were detected in a total of 527 (91.9%) patients. The frequency of mutations was 16.8% for M694V

Table 1. Mutation types

Mutation type	Number of patients
M694V heterozygous	89 (16.8%)
E148Q heterozygous	65 (12.3%)
M694V homozygous	59 (11.2%)
R202Q heterozygous	55 (10.4%)
M680I heterozygous	30 (5.7%)
M680I/M694V compound heterozygous	27 (5.1%)
R202Q/M694V compound heterozygous	24 (4.5%)
V726A heterozygous	22 (4.1%)
V726A/M694V compound heterozygous	16 (3%)
R202Q homozygous	13 (2.4%)
R202Q/M694V homozygous	12 (2.1%)
E148Q/M684V compound heterozygous	11 (2%)
E148Q/R202Q compound heterozygous	9 (1.7%)
M680I/R202Q homozygous	8 (1.5%)
Patients found to have other mutations	87 (16.5%)
Total	527 (91.9%)

heterozygosity as the most common mutation, followed by 12.3% for E148Q, 11.2% for M694V homozygosity, 10.4% for R202Q heterozygosity, and 5.7% for M6801 G/C heterozygosity. These groups were also categorized for specific mutations in Table 1. There was no significant difference between the groups in terms of age and gender. Groups were divided according to clinical attack status and mutation types. The mean follow-up time was 4.1±2.5 years, and the mean disease onset was 8.2±3.99 years. Sixty-one patients were in the attack period and 509 patients were in the attack-free period. All groups were evaluated in terms of CRP and leukocyte values. CRP and leukocyte levels were found to be significantly higher in the FMF attack group compared to the non-attack group and control group. The MPV value was found to be lower in the attack period compared to the attack-free and control period. There was no difference between the attack-free group and the control group in terms of MPV, CRP and leukocyte values. Each group is summarized in Table 2.

DISCUSSION

In our study, the mean MPV value was found to be lower in the attack period compared to the attack-free period.

Platelet activation is associated with increased atherosclerotic risk. MPV is an easily accessible and cost-effective test that can show platelet activation and function[14]. In a small number of studies comparing MPV values at the time of attack and in attack-free period in children with FMF, it was reported that there was no significant difference[15-18]. In the literature, there are studies in which MPV is significantly higher in FMF patients during attack-free periods[17,18]. On the contrary, there are also studies showing that platelet counts are significantly higher and MPV values are significantly lower during attacks in chronic diseases compared to control groups[6,19-21].

In our study, MPV values were significantly lower in patients in the attack period compared to the control group. Changes in the microtubular structure of platelets are thought to reduce the MPV value by changing the platelet shape during inflammation. In addition, a relative decrease in MPV in the circulation can be observed with the migration of large platelets to the area of inflammation[12]. Therefore, we concluded that the low MPV that we detected during an attack of FMF in our study might be useful to show episodes of attacks in FMF.

The use of colchicine may suppress platelet activation[22-24]. All of our patients were receiving colchicine treatment. This may partly explain the non-significant difference in MPV values between the attack-free group and healthy controls.

In the literature, studies comparing the CRP values of patients in the attack-free period and the control group have reported a significant increase in CRP value. However, it is seen that the sample size of these studies is not sufficient[22,25]. The strengths of our study are the size of sample in which each group included a sufficient number of subjects and the inclusion of an attack-free group with negative CRP values. Özer et al.[25] stated in their study that inflammation was higher in the attack-free period than the control group. However, in their study, FMF patients had higher CRP values in the attack-free period compared to the control group. Although it was stated that the patient group included in their study was in the attack-free period, the significant increase in CRP values in the FMF group should be considered as the weak point of the study. In our study, similar to the studies in the literature, elevated CRP and white blood cell were found at the time of attack. CRP values were normal in the attack-free period and in the control group. We thought that these results were related to the inflammation during the attack. It was observed that the incidence of amyloidosis was higher in the M694V genotype in the patient followed up with the diagnosis of Familial Mediterranean Fever. In addition, the incidence of arthritis and severe disease was found to be higher in M694V homozygous patients[26]. None of our patients had amyloidosis.

Study Limitations

The major limitations of our study are that it was performed in a single center, patients were on colchicine treatment, which can reduce inflammation, and it had a retrospective design. Other important limitations are that the personnel performing

Table 2. Comparison of the attack group, the attack-free group and the control group

	Attack group (Number of patients: 61)	Attack-free group (Number of patients: 509)	Control group (Number of patients: 73)	P1	P2	P3
Age (year)	9.8	11.2	10.4	0.695	0.262	0.035
Gender				0.997	0.597	0.580
Girl (n)	33	239	34			
Boy (n)	28	270	39			
CRP (mg/L)	50.25	1.46	1.12	<0.001	0.999	<0.001
MPV (fL)	8.4±2.4	9.5±1.9	9.6±1.1	<0.001	>0.05	<0.001
WBC (/mm³)	11460±4650	8460±2450	7860±1850	<0.001	>0.05	>0.05

CRP: C reactive protein, MPV: Mean platelet volume, WBC: White blood cell count, P1: Control and attack groups, P2: Control and attack-free groups, P3: Attack and non-attack groups

the blood collection were different, and the technique of blood collection and the time it took to be taken to the laboratory were not known. In prospective studies to be conducted in newly diagnosed patients who do not use colchicine, monitorization of MPV changes before, during and after an attack will yield safer results.

CONCLUSION

We showed that MPV decreased with the effect of inflammation during the attack periods in patients with FMF. We think that it is important to consider the MPV value while evaluating the blood count result, which is cheap, easy and can be checked in every clinic. Considering the conflicting results of the studies in the literature, more comprehensive studies are required to increase the reliability of MPV in clinical applications and to ensure its effective clinical use.

Ethics

Ethics Committee Approval: Approval was obtained from the Ethics Committee of İstanbul University in March 2016 (protocol no: 2016/363).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: E.A., F.M.K., R.Ö.E., Design: E.A., F.M.K., R.Ö.E., Data Collection or Processing: E.A., F.M.K., R.Ö.E., Analysis or Interpretation: E.A., F.M.K., R.Ö.E., Literature Search: E.A., F.M.K., Writing: E.A., F.M.K.

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Factors Causing Intraocular Lens Damage During Implantation in Phacoemulsification Surgery

Fakoemülsifikasyon Cerrahisinde İmplantasyon Sırasında Gelişen Göz İçi Lens Hasarına Neden Olan Faktörler

Ekrem ÇELİK[1], Tansu GÖNEN[2], Büşra ENGİN POTOĞLU[2], Lütfiye Ebru BABADAĞ[2]

[1]Tekirdağ Dr. İsmail Fehmi Cumalıoğlu City Hospital, Clinic of Ophthalmology, Tekirdağ, Turkey
[2]Tekirdağ Namık Kemal University Faculty of Medicine, Health Practice and Research Hospital, Clinic of Ophthalmology, Tekirdağ, Turkey

ABSTRACT

Aim: To evaluate the factors affecting intraocular lens (IOL) integrity during implantation in phacoemulsification surgery.

Materials and Methods: In this study, medical records of 3,856 patients (4,778 eyes) who underwent phacoemulsification and foldable IOL implantation by cartridge between August 2010 and July 2019 were retrospectively reviewed. The 11 eyes of 11 patients who had undergone IOL removal and reimplantation were noted for IOL damage during implantation. In other eyes, implanted IOL material, injector and cartridge type information were recorded.

Results: The properties of implanted 4,195 IOLs of 4,778 were hydrophobic acrylic (835 SA60AT, 2,438 AAB00, 922 ZCB00). The remaining 583 IOLs were hydrophobic surfaced high water content acrylic (UD 613). Hydrophobic acrylic IOLs with high water content were inserted with a disposable plastic syringe and foldable cartridge, and hydrophobic acrylic IOLs were inserted with a reusable metal syringe and disposable non-foldable cartridge. Of the 11 IOLs whose integrity was impaired during implantation, 8 were hydrophobic surfaced high water content acrylic and 3 were hydrophobic acrylic ($p<0.001$). There was no statistically significant difference between hydrophobic acrylic lenses ($p=0.103$).

Conclusion: The material structure of the one-piece hydrophobic surfaced high water content acrylic foldable IOLs, the implantation system and foldable cartridge used are the factors that can potentiate the development of damage in the IOL during implantation. For a problem-free implantation, it should be done carefully and slowly from inserting the IOL into the cartridge until it is placed in the capsule bag.

Keywords: Phacoemulsification, intraocular lens damage, intraocular lens exchange

ÖZ

Amaç: Fakoemülsifikasyon cerrahisinde implantasyon sırasında göz içi lens (GİL) bütünlüğünü etkileyen faktörleri değerlendirmek.

Gereç ve Yöntem: Bu çalışmada, Ağustos 2010-Temmuz 2019 tarihleri arasında fakoemülsifikasyon ve kartuş yardımlı katlanabilir GİL implantasyonu yapılmış 3.856 hastanın (4.778 göz) tıbbi kayıtları geriye dönük olarak incelendi. İmplantasyon sırasında GİL bütünlüğü bozulan 11 hastanın 11 gözü çalışmaya dahil edildi. Diğer gözlerin GİL ve implantasyon sistemi verileri kaydedildi.

Bulgular: İncelenen 4.778 GİL'nin 4.195'i hidrofobik akrilik (835'i SA60AT, 2.438'i AAB00 ve 922'si ZCB00) ve 583'ü hidrofobik yüzeyli yüksek su içerikli akrilik (UD 613) idi. Hidrofobik yüksek su içerikli akrilik GİL'ler tek kullanımlık plastik enjektör ve katlanan kartuş, hidrofobik akrilik GİL'ler yeniden kullanılabilir metal enjektör ve tek kullanımlık katlanmayan kartuş ile göz içine yerleştirilmişti. Bütünlüğü bozulmuş 11 GİL'in 8'i hidrofobik yüzeyli yüksek su içerikli akrilik iken 3'ü hidrofobik akrilikti ($p<0,001$). Hidrofobik akrilik GİL'ler kendi aralarında kıyaslandığında istatistiksel olarak anlamlı fark görülmedi ($p=0,103$).

Sonuç: Tek parça hidrofobik yüzeyli yüksek su içerikli akrilik katlanabilir GİL'lerin materyal yapısı, kullanılan implantasyon sistemi ve katlanabilir kartuş yapısı, implantasyon sırasında GİL'de hasar gelişimine neden olabilecek faktörlerdir. Sorunsuz bir implantasyon için, GİL'nin kartuşa yerleştirilmesinden kapsül içine yerleştirilene kadar geçen süreçte dikkatli ve yavaş uygulama yapılmalıdır.

Anahtar Kelimeler: Fakoemülsifikasyon, göz içi lens hasarı, göz içi lens değişimi

Address for Correspondence: Ekrem ÇELİK MD, Tekirdağ Dr. İsmail Fehmi Cumalıoğlu City Hospital, Clinic of Ophthalmology, Tekirdağ, Turkey
Phone: +90 505 516 98 35 **E-mail:** ekcelik@gmail.com **ORCID ID:** orcid.org/0000-0002-1455-4931
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INTRODUCTION

Intraocular lens (IOL) implantation during the modern phacoemulsification procedure is one of the basic principles of successful cataract surgery. With the introduction of foldable IOLs in modern surgeries, IOL implantation has become possible through a smaller corneal incision. In the historical development process of cataract surgery, many IOL and IOL implantation systems have been developed to increase the success of surgeries. While the IOL is placed in the eye with various injector systems and cartridges, complications requiring IOL replacement may occur. Some of these complications include damage to the lens capsule and zonule or damage to the IOL optic and haptic structure of the IOL during implantation. As a result of not being able to centralize the optic of the IOL, a decrease in visual acuity may occur after surgery. If the IOL optic cannot be centralized as a result of damage to the optic or haptic structure of the IOL, explantation and re-implantation should be performed[1,2].

In this study, we aimed to evaluate the effects of two different injector and cartridge systems used during intraocular implantation of one-piece hydrophobic and hydrophobic surfaced with high water content acrylic, two types of acrylic IOLs with different material properties, on possible IOL damage.

MATERIALS AND METHODS

4778 eyes of 3856 patients who underwent phacoemulsification and cartridge-assisted one-piece foldable IOL implantation between August 2010 and July 2019 in Tekirdağ Namık Kemal University Department of Ophthalmology and did not develop complications other than IOL damage during and after the procedure were analyzed retrospectively. The technical characteristics and IOL implantation systems of the IOLs used in all surgeries, the corneal incision length of the eyes containing the IOLs damaged during implantation, the location of the IOL damage, the IOL strength and the implantation order within their group were recorded. This study was approved by Tekirdağ Namık Kemal University Ethics Committee (numbered 2019.09.11.06) and complies with the 2008 Helsinki Declaration. All patients were informed about all stages of the surgical procedure and informed consent was obtained from the patients.

Phacoemulsification and IOL implantation were performed under topical, regional (subtenon and retrobulbar) or general anesthesia. For pupil dilation, phenylephrine 2.5%, cyclopentolate 1% and tropicamide 0.5% drops were applied 3 times with intervals of 5 minutes, 30 minutes before surgery. Topical anesthesia was provided with proparacaine hydrochloride 0.5% drops 4 times at 5 minute-intervals, 20 minutes before the procedure. Regional anesthesia was provided with a mixture of 2 mL 2% lidocaine hydrochloride and 2 mL 0.5% bupivacaine hydrochloride. Povidone iodine was used for surgical area cleaning. For endophthalmitis prophylaxis, 5% povidone iodine was dropped to the fornix; After 3 minutes, it was washed with Ringer's lactate solution. Two clear corneal side incisions were made with a 20 gauge (G) micro vitreoretinal knife. A cohesive viscoelastic agent (1.4% sodium hyaluronate) was injected into the anterior chamber. The main corneal incision was made with a slit blade (in various incisions between 2.2 mm and 3.2 mm). A flap was created in the anterior capsule with a 27 G cystotome. Continuous curvilinear capsulorhexis was performed with capsulorhexis forceps. After hydrodissection and hydrodelineation, the nucleus was aspirated by shredding with stop and chop and quick chop methods. Lens cortex and residuals were cleaned by bimanual irrigation and aspiration method. All procedures were carried out by experienced three surgeons. Two separate nurses experienced in phacoemulsification procedures inserted the IOL into the cartridge. The cartridge was first wetted with a balanced salt solution, then filled with a cohesive viscoelastic material, and the IOL was made ready for implantation. Hydrophobic acrylic IOLs SA60AT (AcrysoF, Alcon, Fortworth, TX, US), AAB00 (Sensar 1, Abbott Medical Optics, Santa Ana, CA, US) and ZCB00 (Tecnis 1, Abbott Medical Optics, Santa Ana, CA, US) were implanted by disposable non-folded cartridge and reusable metal syringe (Figure 1A); hydrophobic surfaced high water content acrylic IOL UD 613 (Acriva, VSY Biotechnology, İstanbul, Turkey) was implanted by folded disposable cartridges and disposable plastic syringe (Figure 1B)[3,4]. After filling the capsule with cohesive viscoelastic, the IOL was placed in the capsule. In order to remove the IOLs that were seen to be damaged during implantation and whose centralization could not be achieved, the IOL incision was made with micro scissors to the center of the IOL optic without expanding the corneal incision. The IOL was rotationally removed from the main corneal incision by holding one side of the incised IOL with colibri forceps. Another IOL of the same feature and diopter was implanted in the capsule. Drops containing topical steroid (prednisolone acetate 1%) and antibiotic (moxifloxacin 0.5%) were started in all patients after surgery. The drops were stopped by gradually reducing the

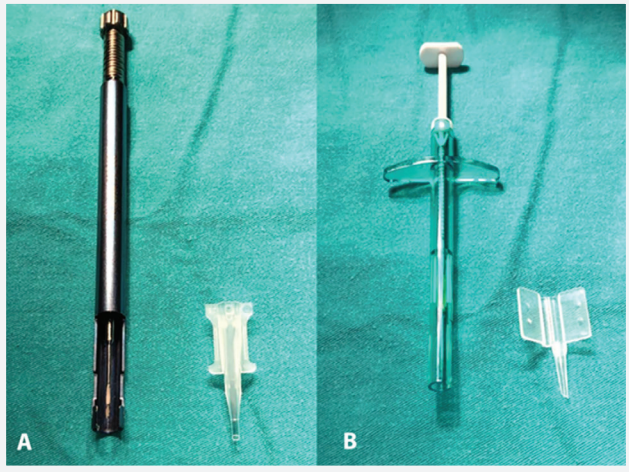

Figure 1. A) Reusable metal injector and disposable non-folded cartridge; B) disposable plastic injector and disposable folding cartridge

number of doses within two weeks. The IOLs used were divided into 2 groups as hydrophobic acrylic and hydrophobic surfaced high water content acrylic.

Statistical Analysis

The statistical analyses were performed using Statistical Package for the Social Sciences Statistics for Windows, version 24.0 (IBM, Statistical Product and Service Solutions, Inc., Chicago, IL, USA). The difference between groups was evaluated using the chi-square and Fisher's exact tests. P<0.05 was considered statistically significant.

RESULTS

Of the 4,778 IOLs implanted in the eye after phacoemulsification, 4,195 were hydrophobic one-piece acrylic (835 SA60AT, 922 ZCB00 and 2,438 AAB00) and 583 were hydrophobic surfaced high water content one-piece acrylic UD613 (Acriva) (Table 1). IOL integrity was impaired in 11 patients who were implanted (Table 2). Of the 11 IOLs whose integrity was impaired by damage, 8 were hydrophobic surfaced high water content and 3 were hydrophobic acrylic (p<0.001). When the hydrophobic acrylic IOLs with 3 different designs from two different manufacturers with 8 damaged lenses were compared, the difference was not statistically significant (p=0.103). No complications occurred during the explantation and re-implantation of the IOLs. Of the 8 IOLs with a hydrophobic surfaced high water content acrylic, 7 of them had haptic damage and 1 had optic damage. Haptic damage was observed in 3 of the hydrophobic acrylic IOLs. Two of the hydrophobic surfaced high water content acrylic IOLs had low diopter and were thin. The other 2 IOLs in the same group were damaged during the learning period of the cartridge insertion procedure. The main corneal incision length varied between 2.2 and 3.2 mm (Table 2).

Table 1. Intraocular lens included in the study

IOL, group	IOL, model	IOL, implanted, n	IOL, damaged, n	IOL, total, n	p value
Hydrophobic acrylic	SA60AT	835	2	4195	<0.001
	ZCB00	922	0		
	AAB00	2438	1		
Hydrophobic surfaced high water content acrylic	UD613	583	8	583	

IOL: Intraocular lens

DISCUSSION

The characteristics of the material which the IOL is produced, its optical and haptic design, the implantation system used, improper placement of the IOL in the cartridge, inappropriate placement of the cartridge in the implantation system, inappropriate and inadequate viscoelastic use, and inappropriate cartridges are factors that can adversely affect the IOL integrity during phacoemulsification procedures[5,6].

Foldable acrylic IOLs are the most commonly used materials in phacoemulsification and IOL implantation currently. Although there are one-piece, 3-piece, plate haptic or scleral fixation IOLs in use, we used only one-piece IOLs with haptics in our study. Acrylic IOLs can also be classified as hydrophilic acrylic, hydrophobic surfaced high water content acrylic or hydrophobic acrylic. Hydrophobic IOLs have a water content of less than 1%, while those with hydrophilic and hydrophobic surfaces contain water between 18% and 26%[5,7].

Hydrophilic IOLs consist of a hydroxyethyl methacrylate (polyHEMA) and hydrophilic acrylic monomer. They are soft and their hydrophilic nature provides excellent flexibility. It is possible

Table 2. Properties of damaged intraocular lens

IOL	IOL, model	IOL, design	IOL, implantation system	IOL, Damage localization	Corneal incision width (millimeter)	IOL, power (diopter)	IOL, implantation order
1	UD613	MC	T	Haptic	2.8	20.50	22/583
2	UD613	MC	T	Haptic	2.8	21.00	38/583
3	UD613	MC	T	Haptic	3.2	28.00	96/583
4	UD613	MC	T	Optic	3.2	24.00	244/583
5	UD613	MC	T	Haptic	2.8	19.00	547/583
6	UD613	MC	T	Haptic	2.8	18.00	575/583
7	UD613	MC	T	Haptic	2.4	-1.00	579/583
8	UD613	MC	T	Haptic	2.4	0.00	580/583
9	AAB00	C	Y	Haptic	2.4	21.50	1199/2438
10	SA60AT	C	Y	Haptic	2.4	22.00	681/835
11	SA60AT	C	Y	Haptic	2.2	21.50	782/835

IOL: Intraocular lens, MC: Modified C loop haptic, C: C loop haptic, T: Disposable plastic injector and folding cartridge, Y: Reusable metal injector and non-folding cartridge

to be implanted through corneal incisions below two millimeters. It is resistant to damage caused by folding. Because of the high rate of posterior capsule concentration development in hydrophilic IOL implantations, the use of hydrophobic surface and hydrophilic hybrid IOLs has become widespread. These are mostly coated with hydrophobic acrylic on the surface and contain ultrapure acrylate monomer that has a water content of 25%[5,8,9].

Hydrophobic IOLs consist of a series of acrylate and methacrylate copolymers. Rigid polymethylmethacrylate has been transformed into a foldable and durable material. Pull and push force can be applied to hydrophobic IOLs; IOLs can take their old shape in seconds. It can be inserted into the eye through a corneal incision of at least 2.2 mm[5,7,8].

SA60AT, which is a hydrophobic acrylic IOL, has an aspherical and biconvex front surface, while its back surface has a 360° sharp optical edge. Its haptics are "C" shaped and a continuation of optics[10]. ZCB00 and AAB00 are other hydrophobic acrylic IOLs; Biconvex, front surface aspherical and posterior surface has an optical square edge 360°[11]. The implantation of these IOLs is done with reusable metal injectors and disposable non-folding cartridges designed for these injectors. The IOL is placed in the cartridge groove and the plunger of the metal injector is pushed forward and inserted into the eye. The metal plunger is smaller than the cartridge groove and its rigid structure allows it to push the IOL without compressing the haptics and optics[4]. The resistant nature of the hydrophobic material to pulling, pushing and compression reduces the possibility of IOL damage[12].

UD613 is a hydrophilic acrylic IOL with a high water content (25%) with a hydrophobic surface and consists of one-piece optics and haptics. It is aspherical and has a modified "C" shaped haptics and is applied into the eye with a disposable plastic implantation system and a suitable disposable foldable cartridge[13]. In this system, the IOL is placed in the cartridge, then the cartridge flaps are closed and the IOL is folded. While the cartridge flaps are closed, the haptics or optical cartridge can remain between the flaps or the haptics may get stuck between the soft injector plunger and the cartridge while the IOL is injected into the eye. The high water content soft biomaterial structure of hydrophilic acrylic IOLs is also considered as another reason that increases the possibility of damage to the optic and haptics during folding and implantation[4,14]. In this study, optic damage was observed in 1 of the disintegrated lenses, while haptic damage developed in all of the others. According to our observations, the compression of the haptic between the plunger and the lumen of the cartridge caused damage during the closure of the cartridge after the IOL was placed in the cartridge or while it was pushed forward by the plunger in the cartridge lumen. If the IOL pushed by the plunger in the cartridge lumen is too thin or thick, that is, it has a very high or low power, it may cause difficulties in pushing the IOL forward. Thick IOLs can get stuck at the exit of the cartridge lumen; thin ones can remain between the piston and the lumen[4]. The very thin optical and haptic structures of the very low power (0.00 and -1.00 D) 2 IOLs in Table 2 may be the cause of their damage.

IOLs of different manufacturers are implanted into the eye with different cartridge and implantation systems designed for them. IOLs to be used for the first time may be damaged while being placed in the cartridge and eye during the learning process[5,6,14]. In our study, 2 of the hydrophobic surfaced high water content acrylic IOLs were damaged during the learning process. In the other group IOLs, the hydrophobic acrylic was not damaged during this process. Our observations suggest that features related to the cartridge and implantation system used, as well as experience, may also cause IOL damage.

Indication for IOL explantations are IOL damage, malposition, miscalculation of power and performance failure, opacification or staining. While damage rarely develops in one-piece acrylic IOLs, breakage can be seen mostly in the optic-haptic junction of three-piece IOLs[4,6,15]. Mamalis et al.[14] evaluated explantations and re-implantations, which they compiled from studies published until 2003, in terms of almost all types of IOLs currently in widespread use until that day. They also compared the damage-related change rate in one-piece hydrophobic acrylic IOLs with haptics changes due to other complications and found 7.5%. Other complications that cause IOL change are opacification, discoloration, incorrect lens power, dislocation, retinal surgery, decentration and glare. In one-piece hydrophilic acrylic IOLs with haptics, it was found that the change was mostly due to discoloration/opacification (70%). In the survey update study conducted by the same colleagues in 2007, no damaged lenses were reported in one-piece hydrophobic acrylic IOLs. They found that there was no notification of damaged lenses in one-piece hydrophilic acrylic IOLs, and the ratio of discoloration/opacification rate to other explantations this time was 60%. It was reported in the 2007 survey that the use rate of one-piece haptic hydrophobic acrylic IOLs increased compared to other material and design lenses, but no damaged lenses were seen. The effect of reducing the damage is the elimination of the imperfectness in implantation systems and cartridge technology, and the increase in the one-piece hydrophobic acrylic with haptics IOL using experience[14,15].

There are studies reporting that the choice of viscoelastic device used while placing the IOL in the cartridge causes IOL damage[6]. Insufficient viscoelastic substance into the cartridge or keeping the IOL in the cartridge for a long time may cause the IOL to stick to the cartridge[16]. Injecting a viscoelastic device with less pseudoplasticity into the cartridge may cause IOL damage[17]. In order to prevent the IOL from jamming during implantation, it is necessary to ensure sufficient wetting with a balanced salt solution and to ensure that the IOL is completely covered with viscoelastic. A slow and careful implantation while inserting into the cartridge and pushing with the injector plunger will help maintain IOL integrity[18].

Various techniques have been described for the removal of the damaged IOL[19-23]. The removal of the IOL can be performed by widening the main corneal incision or by dividing the IOL into many parts without expanding the incision site. After the viscoelastic device is injected into the anterior chamber and under the IOL, the IOL is divided into many parts with these techniques[2]. In addition, the IOL can be made suitable for removal by cutting haptics and optics using ND-YAG laser[24]. In the partial transection technique, the lens is cut in full thickness up to the center of the IOL optic, and it is held at one end with forceps and removed by rotation without expanding the corneal incision[21,25]. In our study, damaged IOLs were removed using the partial transection technique.

Study Limitations

The limitations of our study were the small number of cases, retrospective examination and the difference in the number of IOLs used between the groups. The fact that more than one surgeon perform the procedures has revealed the risk of low standardization between applications. The absence of such case series in the literature made it difficult to compare the study with other possibilities. We think that a study in which we will implant the same IOL type into the eye using different implantation systems will better reveal the factor that causes damage.

CONCLUSION

Today, there are many IOLs and their implantation systems made by different manufacturers with different materials and designs. The structural features of the hydrophobic surfaced high water content IOLs' cartridge and implantation system, which may have difficulties in the learning process, may cause damage to the IOL during implantation. For a problem-free implantation, we should be slow and careful during the period from inserting the IOL into the cartridge to pushing it forward into the capsule using the plunger.

Ethics

Ethics Committee Approval: This study was approved by Tekirdağ Namık Kemal University Ethics Committee (numbered 2019.09.11.06) and complies with the 2008 Helsinki Declaration.

Informed Consent: All patients were informed about all stages of the surgical procedure and informed consent was obtained from the patients.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: E.Ç., T.G., B.E.P., L.E.B., Concept: E.Ç., T.G., Design: E.Ç., T.G., L.E.B., Data Collection or Processing: E.Ç., T.G., B.E.P., L.E.B., Analysis or Interpretation: E.Ç., T.G., B.E.P., L.E.B., Literature Search: E.Ç., T.G., B.E.P., Writing: E.Ç., T.G., B.E.P., L.E.B.

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Cognitive, Affective and Behavioral Investigation of Turkish People's Attitudes Towards the COVID-19 Pandemic Quarantine Process

Türk Halkının COVID-19 Pandemisi Karantina Sürecine Yönelik Tutumlarının Bilişsel, Duyuşsal ve Davranışsal Boyutta İncelenmesi

Berkalp TUNCA[1], Ece ÖZGÖREN[2], İhsan BERK[3], Buket İPEK[4], Mustafa DEMİR[1], Nefise FERMANCI[1], Şevkiye BABACAN[1], Murat AKŞİT[5], Yemliha DURMAZ[5], Cengiz GAZELOĞLU[6], Sinan SARAÇLI[7]

[1]*Afyon Kocatepe University, Institute of Science, Department of Statistics, Afyonkarahisar, Turkey*
[2]*Eskişehir Technical University, Institute of Science, Department of Statistics, Eskişehir, Turkey*
[3]*Sanko University, Faculty of Medicine, Department of Biostatistics, Gaziantep, Turkey*
[4]*Karadeniz Technical University, Institute of Science and Technology, Department of Statistics, Trabzon, Turkey*
[5]*Private Sector, İstanbul, Turkey*
[6]*Süleyman Demirel University, Faculty of Arts and Sciences, Department of Statistics, Isparta, Turkey*
[7]*Afyon Kocatepe University, Faculty of Arts and Sciences, Department of Statistics, Afyonkarahisar, Turkey*

ABSTRACT

Aim: The aim of study is to determine the attitude of the Turkish people (in cognitive, affective and behavioral dimensions) and the factors affecting this attitude during the coronavirus epidemic that has affected the whole world since December 2019.

Materials and Methods: The data collected by a scale were modeled with explanatory factor analysis (EFA), confirmatory factor analysis (CFA) and structural equation modeling (SEM), and the effects of the dimensions and the importance of the effective items in each dimension were determined.

Results: 61.4% of the participants were male and 65.4% were under the age of 40 years. Individuals' "questioning their purpose of coming to life again" and "desire of being more sensitive to the events around them than in the past" were found to be significant, respectively. Other results obtained from the study are given in the relevant tables and figures.

Conclusion: It was determined that the affective dimension had the highest effect on the results of EFA, CFA and SEM analyses, which were effective in examining the attitudes of individuals towards an event with these sub-dimensions.

Keywords: COVID-19, epidemic processes, attitude and behavior, statistical modeling

ÖZ

Amaç: Bu çalışmanın amacı, Aralık 2019'dan beri tüm dünyayı etkisi altına alan koronavirüs salgını süresince Türk halkının sergilediği tutumu (bilişsel, duyuşsal ve davranışsal boyutlarda) ve bu tutum üzerinde etkili olan etmenleri belirlemektir.

Gereç ve Yöntem: Bir ölçek aracılığı ile derlenen veriler açıklayıcı faktör analizi (AFA), doğrulayıcı faktör analizi (DFA), yapısal eşitlik modellemesi (YEM) ile modellenerek, boyutlar arası ilişkiler ile her bir boyutta etkili olan maddelerin önemleri belirlenmiştir.

Bulgular: Katılımcıların %61,4'ü erkek ve %65,4'ü 40 yaş altıdır. Bireylerin tutumlarını açıklayan alt boyutlardan davranışsal boyut üzerinde bireylerin ekonomik tedbirlere vereceği önemin en etkili değişken olduğu, bilişsel ve duyuşsal boyutlarda ise sırası ile bireylerin hayata geliş amaçlarını tekrar

Address for Correspondence: Cengiz GAZELOĞLU MD, Süleyman Demirel University, Faculty of Arts and Sciences, Department of Statistics, Isparta, Turkey
Phone: +90 246 211 43 53 **E-mail:** cengizgazeloglu@sdu.edu.tr **ORCID ID:** orcid.org/0000-0002-8222-3384
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sorgulamaları ve çevrelerindeki olaylara karşı geçmiştekinden daha duyarlı olacaklarının önemi anlamlı bulunmuştur. Çalışmadan elde edilen diğer sonuçlar ilgili tablo ve şekillerde verilmiştir.

Sonuç: Bireylerin bir olaya karşı olan tutumlarının bu alt boyutlar ile incelenmesi noktasında etkili olan istatistiksel yöntemlerden AFA, DFA ve YEM analizi sonuçlarında duyuşsal boyutun etkisinin en yüksek olduğu belirlenmiştir.

Anahtar Kelimeler: COVID-19, salgın süreçleri, tutum ve davranış, istatistiksel modelleme

INTRODUCTION

In our country, the time spent at home has increased within the scope of the measures taken after the first coronavirus disease-2019 (COVID-19) case observed on March 11th. It has been observed that our citizens' awareness and sensitivities on some issues have increased. In addition, it is observed that the shares they make through social media have increased and they question their purpose of life and the values they give to their loved ones. While many individuals state that they have accomplished the things they wanted to do but did not have the opportunity to do before due to the hustle and bustle of life, it should not be overlooked that each of them has shared how important it is to be able to walk freely on the street, to eat at a restaurant and to drink tea in a cafe with their friends, without realizing it. Depending on these, it is seen that they express how important social solidarity is and that material things such as the house, the car, etc do not mean anything without humanity and it is remarkable that there has been an increase in shares about that they support aid campaigns for people in economic difficulties and that they are more sensitive towards street animals during this period. While some people are bored with staying at home, some people have to work without the luxury of staying at home because they provide for their families and the contribution of their work to this process is indispensable, the importance of which has been well-understood. In addition to these, it is another remarkable point that although people understand the importance of their health once again, they are psychologically tense with the fear of facing the deadly virus as a result of the slightest carelessness, and that intra-familial conflicts have increased both around the world and in our country.

All these indicators can be evaluated as a result of individuals' attitudes towards daily events, which differ in cognitive, affective and behavioral dimensions in this process. In this context, the aim of this study is to reveal the differences related to these dimensions both at the national and international levels and to contribute to humanity by emphasizing the importance of the positive and negative variables that cause it.

The research hypotheses of the study are given as items below.

1. What are the cognitive attitudes of individuals towards the COVID-19 pandemic quarantine process?

2. What are the affective attitudes of individuals towards the COVID-19 pandemic quarantine process?

3. What are the behavioral attitudes of individuals towards the COVID-19 pandemic quarantine process?

4. Is there a significant difference among individuals'cognitive, affective and behavioral attitudes towards the COVID-19 pandemic quarantine process?

5. Do individuals' attitudes towards the COVID-19 pandemic quarantine process differ significantly according to their demographic characteristics?

6. Do individuals' cognitive and affective attitudes towards the COVID-19 pandemic quarantine process have a significant effect on their behaviors?

Literature Review

Although the origin of the word pandemic is a combination of the Greek words "pan" (all) and "demos" (people), this word is often used for epidemic infectious diseases that are widespread in the whole country or in one or more continents at the same time[1].

However, the use of this word with its definition in many medical texts has been neglected in the last 20 years. This word did not find a place in the indexes of many authoritative studies and texts about the pandemic, even in comprehensive sources on the history of medicine, in classical epidemiological sources, and in the effective infections report of the institute of medicine in 1992[2]. Internationally accepted known and common definition of pandemic in the epidemiology dictionary is "an epidemic that occurs worldwide, or that originates in large areas and transcends international borders, affecting large masses of people"[3,4].

Among all known pandemic pathogens, influenza (flu infection) has been the main cause since the 16th century due to its potential seriousness and irregular occurrence[5,6].

In all other pandemics and the COVID-19 pandemic process, the most effective individual measures, apart from the measures taken by the administration, can be listed as giving maximum importance to hygiene, eating natural and healthy, increasing mobility and exercise, and paying attention to social isolation[7]. At this point, the importance of education

shows itself once again. The higher the number of conscious individuals in a society as a result of education, the higher the probability of success in the fight against epidemics. In this context, it should be emphasized that by creating a permanent awareness, people should act in line with ethical values and care about other people's lives.

It is known that it is easier to fight the pandemic in societies where healthy individuals are in the majority, and it has also been observed during the COVID-19 process and in the past that individuals with a healthy immune system are more likely to win the struggle for their lives, even if the virus is transmitted.

In the COVID-19 process, it is seen that the ethics in the behavior of individuals who make up all societies with their physicians, scientists, politicians affect the period we live in. The COVID-19 pandemic offers us an opportunity to reconsider our values, from individual ethics to social ethics, from professional ethics to political ethics. In this process, it has been realized that we need to balance the value of life and the profitability of the capitalist system in terms of bioethics and environmental ethics, and that the policies we create with human-centered thinking cannot be isolated from other life forms[8].

The Effects of Pandemic on Human Attitudes and Behaviors

When people are faced with a contagious disease epidemic, they can take some preventive actions against the negative effects of the epidemic in terms of health and economy to reduce the risk they face[9].

Studies in the literature on behavioral responses to the flu epidemic can be found in related sources[9-12]. There are many theories about risk perception, such as protection motivation theory (PMT)[13], health belief model[14], extended parallel process model[15] and precaution adoption process model[16]. The basic idea on which these theories are based is that people react to any threat. PMT distinguishes two phases called assessment of threat and assessment of coping. Assessment of threat is perceived personal susceptibility (or perceived vulnerability) combining states of fear for the threat (belief in the possibility of contracting the disease) and for perceived severity of the threat (having a serious feeling of contracting the disease). Assessment of coping is defined as variables related to the proposed protection response.

These are the perceived response effect (What protective behaviors will help?), the individual effect on the perceived response (Am I a confident person to exhibit protective behaviors?), and the consequences of the reactions (What are the disadvantages of protective behaviors?). According to PMT, assessment of the threat triggers the intention to act, while assessment of coping triggers the type of behavior exhibited[17]. Following the SARS epidemic that emerged in 2003, a population-based hypothetical study on people's protective measures in an influenza epidemic was conducted in 3 Asian and 5 European countries[9]. With some exceptions, the potential protective measures in each country were similar for individuals. Even in places where the risk was low, public transport was often cited as the most risky factor for disease transmission. Participants stated that in the event of a new epidemic, they would stay away from public transportation, entertainment places and shopping centers unless they are of vital importance. Participants also stated that although they cared about the high risk of contamination, they did not hesitate to go to health institutions. Moreover, participants working in one job stated that they took less protective measures than others. Interestingly, risk perception variables did not significantly affect their precautionary behaviors. They were only sensitive about public transportation[17].

Available knowledge, attitudes, and belief systems about the risk associated with a disease may change over time. Mathematical models are a powerful tool for estimating the potential contagiousness of disease and for investigating effective control measures. For models with a complex structure, it has also been shown that contagion can be prevented by preventing direct interaction of other individuals with this network if individual precautions are taken sufficiently considering infected individuals in the social network[18]. There are increasing efforts to motivate people to maintain social distance and to limit their interaction with other people and accordingly the risk of social diseases. Social distancing is not a new concept and has been used for centuries to quarantine infected individuals and avoid illness, but new approaches must be introduced to deal with modern social interactions[19]. Although it is imperative to provide a balance between informing the public and not creating panic[20], the community expects the latest up-to-date information and timely and satisfactory explanations of what and why to do from administrators[21-23].

During the COVID-19 epidemic, which is still ongoing in our country, compulsory measures were taken by the state on issues such as maintaining a distance of at least 1 meter and using masks in public transportation, public and common social areas, and announcements and statements were made about raising a general awareness among citizens.

It is an inevitable fact that all these effects experienced during the COVID-19 process cause people to re-judge their values in their lives. From this point of view, it is thought that the attitudes exhibited in this process are also effective for the future periods and it is aimed to determine the factors that have effects on the attitudes of individuals in this study.

Concept of Attitude

Attitude is not a directly observable feature, but a tendency attributed to that individual by inferring from the observable behaviors of the individual. In other words, an attitude is not a behavior that can be observed, but a tendency to prepare for behavior. The tendency of a person to show positive or negative behavior towards any event, object or person is called attitude. A mental assessment is the minimum requirement for a trend to be considered as an attitude, but most established attitudes that people develop over time include affective and behavioral elements. In other words, attitudes cannot be observed directly, but are revealed by the individual's other behaviors[24-26]. According to Fishbein and Ajzen[27], behavioral, affective and cognitive elements must be in a consistent relationship with each other in the formation of attitude[28]. Smith[29] has explained the concept of attitude as "the tendency that is attributed to the individual and that regularly forms the thoughts, feelings and behaviors of the individual about a psychological object".

Attitude is expressed as learned self-tendency, which appears in the form of being for or against a concrete object or an abstract concept, and directs the thoughts and feelings of the individual[30]. According to Petty and Cacciopo[31], attitudes are people's general evaluations of themselves, others, or other objects, events, or problems. These general assessments are based on many behaviors, affective and cognitive bases and affect their developments, changes and formations[32]. According to Thurstone[33], an attitude is a positive or negative intensity ranking and rating directed towards a psychological object. It is accepted by scientists that some variables play a role in the formation of attitude, and scientists examine and express these variables in different ways. Middlebrook, on the other hand, admits that attitude has three components[34]. These components are expressed as follows[35]:

1. Cognitive Component: It is the individual's thoughts and beliefs about the attitude.

2. Affective Component: It is the individual's liking or disliking of the subject of attitude.

3. Behavioral Component: It is the behavior of the individual regarding the subject of attitude.

The cognitive element is the rational component of attitudes consisting of ideas, knowledge and beliefs. The affective component includes positive (happiness, joy, appreciation and satisfaction) and negative feelings and emotions (regret, anger, boredom, fear, etc.) rather than neutral information. The behavioral element, on the other hand, expresses the tendency of an attitude to turn into a behavior[36,37]. Bloom[38] has revealed that affective features increase the cognitive achievement in the relevant area by about a quarter, that is, about a quarter of the variability in learning success is due to affective features[35]. The behavioral component expresses the tendency of an attitude to turn into a behavior[36]. The behavioral element reflects the tendency to act in accordance with the affective and cognitive elements and it is action-oriented[37,39].

Although Qiu et al.[4] examined the effects of the pandemic in their studies by considering them in health, economy, social and security dimensions, these dimensions will be evaluated as cognitive, affective and behavioral dimensions of the attitudes of individuals during their stay at home due to the COVID-19 pandemic in this study.

MATERIALS AND METHODS

In this study, it was primarily aimed to develop a new scale to determine the attitudes of Turkish people towards the COVID-19 pandemic quarantine process in cognitive, affective and behavioral dimensions. In this process, the evaluations of 10 scientists from different universities were taken to determine the expert opinions. These factors, namely cognitive, affective and behavioral dimensions, were examined with explanatory factor analysis (EFA), Confirmatory Factor Analysis (CFA) and structural equation model (SEM). The questionnaire used in the study was approved by the decision of Afyon Kocatepe University Scientific Research and Publication Ethics Committee dated 27.05.2020 and numbered 15.06.2020-E.17011.

Statistical Analysis

Statistical methods were used to determine the statistical validity and reliability of the item pool created for the attitude scale, and the internal consistency of the scale for the reliability analysis was determined by the Cronbach alpha coefficient. In order to determine the sub-dimensions and the items collected in these dimensions in this scale, whose validity and reliability were ensured, EFA was applied to the relevant data set and the obtained dimensions were tested with CFA again. Finally, the dimensions affecting the behavior of individuals during the COVID-19 process were modeled with SEM, and the interdimensional relations and the importance of the items that were effective in each dimension were determined. Statistical Package for the Social Sciences and LISREL programs were used in the analysis of the data used in the study.

Explanatory Factor Analysis

EFA can be defined as a multivariate statistics that aims to find and discover a small number of conceptually significant new variables (factors, dimensions) by bringing together a large number of interrelated variables. Factor analysis operates on the notion that measurable and observable variables can be reduced to fewer latent variables that share a common variance and are unobservable, which is known as reducing

dimensionality[40]. Rennie[41], on the other hand, defines EFA as an analytical technique with a computational logic based on the relationships between observed variables, aiming to reach a small number of explanatory factors (concepts) that explain the maximum variance[42].

Confirmatory Factor Analysis

When we review the literature in general, it is seen that CFA is a method that is mostly applied after classical factor analysis studies[43,44]. In such studies, the researchers subject the factor structures that they have determined with the EFA study to CFA. Thus, although it is a highly accepted practice, such practices actually contradict the nature of the SEM somewhat. Because here, in a sense, it is about testing the factor structures that the data set has directed us. However, it should be noted right away that although the exploratory factor analysis results of studies that do not have a strong theoretical basis are very good, frustration can be experienced in the phase of CFA.

Structural Equation Model

SEM is a statistical technique that is used to test the causal relationships between observed and unobserved (latent) variables, and analyzes especially latent variables with both dependent and independent variables in detail. It has also proven to be a useful technique in solving problems encountered in formulating theoretical structures. It is a systematic tool used especially in psychology, sociology, marketing and educational sciences to evaluate the relationships between variables and to test theoretical models. Technically, SEM is used to estimate the unknown parameters in the linear structure equation set. The variables in the equations are usually latent variables that are directly related to the observed variables.

SEM assumes that there is a causality structure between the set of latent variables and that latent variables can be measured through observed variables[45].

It gives better results than other multivariate statistical techniques such as multiple regression, path analysis and factor analysis. Other statistical techniques fail to take into account the interactions between dependent and independent variables. SEM can also present statistical efficiency and explanatory ability[46,47] in a model test with a single comprehensive method. SEM is a method that predicts and tests by revealing the linear relationships of the theoretical connection between the variables[48,49].

RESULTS

As a result of the data compiled from the questionnaire included in the study, descriptive statistics are given in Table 1, 2 and 3.

According to Table 2, 61.4% of the participants were male and 65.4% were under the age of 39 years. While 29.2% did not have a fixed income, 70.8% had minimum wage or higher income. 76.1% had undergraduate and graduate degrees, 23.9% were primary, secondary, high school and associate degree graduates. In addition, while the rate of individuals who had COVID-19 positive people around themselves was 26.7%, the rate of those who did not have was 73.3%.

According to Table 3, when the averages of affective, cognitive and behavioral attitudes of the participants were examined in terms of gender, it was seen that the averages of women (4.21, 3.33, 3.75) were higher than the averages of men (3.97, 3.10, 3.69).

When the averages of affective attitudes were examined in terms of age groups, the averages of individuals between the ages of 20 and 39 years (4.16 for those aged 20-29 years and 4.14 for those aged 30-39 years) were found to be higher than those of other age groups (4.05 for those aged 19 years and below, 4.07 for those aged 40-49 years, 4.05 for those aged 50 years and over). However, when the averages of cognitive attitudes were examined, it was seen that the averages of the participants aged 19 years and younger (3.47) were higher than the averages of the other age groups (3.22 for those aged 20-29 years, 3.24 for those aged 30-39 years, 3.20 for those aged 40-49 years, 3.25 aged 50 years and above). On the other hand, in the behavioral dimension, the averages of individuals aged 30 years and over (3.80 for those aged 30-39 years, 3.89 for those aged 40-49 years, 3.85 for those aged 50 years and above) were higher than those of participants in other age groups (3.71 for those aged 19 years and below, 3.56 for those aged 20-29 years).

When the averages of affective attitudes were examined in terms of income level, the averages of the participants who did not have a fixed income (4.14) and those with an income of 4001-7000 TL (4.15) were observed to be higher compared to the averages of participants with other income levels (4.00 for minimum wage, 4.08 for 2000-4000 TL, 4.08 for 7001-10000 TL, 4.00 for 10001 TL and above).

When the cognitive attitude averages were examined, it was seen that the averages of the participants who did not have a fixed income (3.28) and who had an income between 2000 TL and 7000 TL (3.25 for 2000-4000 TL, 3.28 for 4001-7000 TL) were higher than those of participants with other income levels (3.11 for minimum wage, 3.14 for 7001-10000 TL).

In addition, it is seen that the average of the cognitive attitudes of the participants whose income level is 10001 TL and above has a negative attitude with 2.81. In the behavioral dimension, the averages of the participants with an income level of 4001 TL and above (3.76 for 4001-7000 TL, 3.84 for 7001-10000

Table 1. Pandemics and their effects from the middle ages to the present.

Year of its beginning	Event	Geographical region	Estimated case/death rate	Estimated economic, social or political impact
1347	Plague	Eurasia	Death in 30-50% of the European population[50]	It accelerated the collapse of the feudal order in Europe[51]
Beginning of 1500s	Onset of smallpox	America	Death more than 50% in some countries[52]	It eliminated indigenous communities that facilitated the hegemony of European countries[53]
1881	5. cholera epidemic	Worldwide	Deaths more than 1.5 million[54]	Attacks on the Russian Tsarist government and health workers[55]
1918	Spanish flu	Worldwide	Deaths between 20 and 100 million[56]	3% loss in Australia, 15% loss in Canada, 17% loss in England and 11% loss in USA based on GNP[57]
1957	Asian flu	Worldwide	Deaths between 700000 and 1.5 million[58]	3% loss in GNP in Canada, Japan, England and USA[57]
1968	Hong Kong flu	Worldwide	1 million deaths[59]	Direct or indirect cost of 23-26 billion $ in USA[60]
1981[a]	HIV/AIDS	Worldwide	Cases more than 70 million, 36,7 million deaths[61]	Annual 2% loss of GNP in Africa[62]
2003	SARS	4 continents, 3 countries	8098 possible cases, 744 deaths[63]	In GNP: 4 billion $ loss in Hong Kong SAR and China, 3-6 billion $ loss in Canada and 5 billion $ loss in Singapore[64]
2009	Swine flu	Worldwide	Deaths between 151700 and 575500[65]	1 billion $ GNP loss in the Republic of Korea[66]
2012	MERS	22 countries	1879 symptoms, 659 deaths[67]	2 billion $ loss triggering 14 billion $ government promotion in the Republic of Korea[68,69]
2013[b]	West Africa Ebola epidemic	10 countries	28646 cases, 11323 deaths[70]	2 billion $ loss in Guinea, Liberia ve Sierra Leone[71]
2015	Zika virus	76 countries	2656 reported microcephaly central nervous system disorder[61]	7-18 billion $ loss in Latin America and Caribbeans[72]

[a]The effects of studies about HIV/AIDS on gross national product per capita were a little. [b]The West African Ebola Epidemic was seen between 2013 and 2016, but in 2014, it peaked and international effects were observed. GNP: Gross national product, HIV/AIDS: Human immuno deficiency virus/acquired immuno deficiency syndrome, MERS: Middle east respiratory syndrome, SARS: Severe acute respiratory syndrome, reference[6]

TL, 3.74 for 10001 and above) were found to be higher than those of participants with other income levels (3.68 for those without a fixed income, 3.61 for minimum wage, 3.69 for 2000-4000 TL).

When the averages of affective, cognitive and behavioral attitudes were examined in terms of education level, it was seen that the averages of individuals who were primary school graduates were higher than the averages of those with other education levels. In addition, considering the affective dimension, it was seen that the average was above 4 for every education level except secondary school graduates.

While it was found that the average of the participants with COVID-19 positive individuals in their close environment was 4.18 in the affective dimension, 3.24 in the cognitive dimension, and 3.71 in the behavioral dimension, the average of the participants who did not have COVID-19 positive individuals in their close environment was 4.09 in the affective dimension, 3.24 in the cognitive dimension and 3.73 in the behavioral dimension.

In addition, although the general average of the affective dimension was 4.12, the general average of the cognitive dimension was 3.24, and the general average of the behavioral dimension was 3.72, it was observed that the participants exhibited a positive attitude.

EFA results are given in Table 4.

According to Table 4, of the items that made up the affective dimension, for the item of affective (DUY1) "I have realized the meaning of life more", the factor load was 0.849 and the mean was 4.19; for the item of DUY2 "I have understood how valuable health is", the factor load was 0.814 and the mean was 4.40; for the item of DUY3 "My sensitivity to social issues has increased", the factor load was 0.788 and the mean was 4.03; for the item of DUY4 "I have realized that I should value the people I love more", the factor load was 0.703 and the mean was 3.97; for the item of DUY5 "I have understood the importance of the activities I did with my friends", the factor load was 0.659 and the mean was 4.18; for the item of DUY6 "I think that I will be a more sensitive individual in the future to events that I ignored in the past", the factor load was 0.619 and the mean was 3.98; and for the item of DUY7 "I was more worried about my close environment than myself", the factor load was found to be 0.568 and the mean was 4.07. In addition, the affective dimension factor explains 24.587% of the total variance. Of the items that made up the cognitive dimension, for the item of cognitive (BIL1) "My religious awareness has

Table 2. Frequency and percentage values for the participants

Demographic variables	Frequency	Percentage (%)
Gender		
Female	588	61.4
Male	370	38.6
Age		
19 years and below	55	5.7
20-29 years	373	38.9
30-39 years	199	20.8
40-49 years	150	15.7
50 years and above	181	18.9
Income level		
No fixed income	280	29.2
Minimum wage	39	4.1
2000-4000 TL	154	16.1
4001-7000 TL	368	38.4
7001-10000 TL	79	8.2
10001+	38	4.0
Education level		
Primary school	11	1.1
Secondary school	15	1.6
High school	124	12.9
Associate degree	79	8.2
Undergraduate degree	539	56.3
Graduate degree	190	19.8
The presence of any individual with positive COVID-19 in close environment		
No	702	73.3
Yes	256	26.7
Total	958	-

COVID-19: Coronavirus disease-2019

increased", the factor load was 0.894 and the mean was 2.96; for the item of BIL2 "My commitment to my religion has increased", the factor load was 0.828 and the mean was 3.07; for the item of BIL3 "The importance I attach to material has decreased", the factor load was 0.735 and the mean was 3.24; for the item of BIL4 "I have once again questioned my purpose of life", the factor load was 0.727 and the mean was 3.36; and for the item of BIL5 "I have realized that money is not everything", the factor load was 0.639 and the mean was 3.58. In addition, the cognitive dimension factor explains 20.147% of the total variance. From the items that made up the behavioral dimension, for the item of behavioral (DAV1) "I have limited my expenses as much as possible", the factor load was 0.846 and the mean was 3.62; for the item of DAV2 "I have increased my economic measures", the factor load was 0.806 and the mean was 3.71; for the item of DAV3 "I have tried to keep my expenses under control", the factor load was 0.752 and the mean was 3.79; for the item of DAV4 "I have avoided unnecessary shopping", the factor load was 0.600 and the mean was 3.49; for the item of DAV5 "I have provided my own transportation way instead of public transportation", the factor load was 0.551 and the mean was 4.02; for the item of DAV6 "I have paid attention to a healthy diet", the factor load was 0.545 and the mean was 3.72. The behavioral dimension factor explains 20.064% of the total variance. In addition, these 3 factors explain 64.798% of the total variance.

When Figure 1 is examined, it is seen that the most effective variable affecting the cognitive dimension factor is BIL4 "I have once again questioned my purpose of life" with a coefficient of 0.81. The cognitive dimension factor is affected by the item of BIL3 "The importance I attach to material has decreased" with a coefficient of 0.80, by the item of BIL1 "My religious awareness has increased" with a coefficient of 0.77, by the item of BIL5 "I have realized that money is not everything" with a coefficient of 0.68, and by the item of BIL2 "My commitment to my religion has increased" with a coefficient of 0.66. It is seen that it also fulfills the criterion of $X^2/df=6.44/3=2.14 <3$ which is among the criteria of goodness of fit.

According to Table 5, the composite reliabilit (CR) value of the cognitive dimension factor is 0.86 and the average variance extracted (AVE) value is 0.56. The CR value is expected to be greater than 0.70 and the AVE value to be greater than 0.50 and it is seen that the values calculated here are in accordance with these criteria.

When Figure 2 is examined, the most effective variable affecting the affective dimension factor is DUY1 "I have realized the meaning of life more" with a coefficient of 0.79. The affective dimension factor is affected by the item of DUY4 "I realized that I should value the people I love more" with a coefficient of 0.77, by the item of DUY2 "I have realized how valuable health is" with a coefficient of 0.75, by the item of DUY3 "My sensitivity to social issues has increased" with a coefficient of 0.74, by the item of DUY6 "I think that I will be a more sensitive individual in the future to events that I ignored in the past" with a coefficient of 0.72, by the item of DUY5 "I have understood the importance of the activities I did with my friends" with a coefficient of 0.69, and by the item of DUY7 "I was more worried about my close environment than myself" with a coefficient of 0.57. It is seen that it also fulfills the criterion of $X^2/df=13.26/8=1.65 <3$ which is among the criteria of goodness of fit.

According to Table 6, the CR value of the affective dimension factor is 0.88 and the AVE value is 0.52. The CR value is expected to be greater than 0.70 and the AVE value to be greater than 0.50 and it is seen that the values calculated here are in accordance with these criteria.

Table 3. Descriptive statistics on demographic variables (arithmetic mean, standard deviation)

Demographic variables	Affective		Cognitive		Behavioral	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
Gender						
Female	4.21	0.79	3.33	1.12	3.75	0.96
Male	3.97	0.90	3.10	1.11	3.69	0.94
Age						
19 years and below	4.05	0.91	3.47	1.08	3.71	0.95
20-29 years	4.16	0.77	3.22	1.09	3.56	0.96
30-39 years	4.14	0.83	3.24	1.11	3.80	0.84
40-49 years	4.07	0.92	3.20	1.24	3.89	0.95
50 years and above	4.05	0.92	3.25	1.12	3.85	0.99
Income level						
No fixed income	4.14	0.72	3.27	1.10	3.68	0.86
Minimum wage	4.00	1.03	3.11	1.10	3.61	1.01
2000-4000 TL	4.08	0.95	3.25	1.21	3.69	1.05
4001-7000 TL	4.15	0.85	3.28	1.10	3.76	0.98
7001-10000 TL	4.08	0.81	3.14	1.01	3.84	0.87
10001+	4.00	1.05	2.81	1.29	3.74	0.94
Education level						
Primary school	4.45	0.56	4.29	0.80	4.67	0.48
Secondary school	3.86	1.23	3.24	1.38	3.74	1.09
High school	4.04	0.87	3.29	1.06	3.77	0.98
Associate degree	4.04	0.94	3.50	1.07	3.83	0.98
Undergraduate degree	4.12	0.86	3.18	1.13	3.68	0.96
Graduate degree	4.20	0.72	3.20	1.12	3.72	0.87
The presence of any individual with positive COVID-19 in close environment						
No	4.09	0.87	3.23	1.13	3.73	0.95
Yes	4.18	0.76	3.24	1.08	3.71	0.95
General average	4.12	0.84	3.24	1.12	3.72	0.95

COVID-19: Coronavirus disease-2019

When Figure 3 is examined, the most effective variable affecting the behavioral dimension factor is seen to be DAV1 "I have limited my expenses as much as possible" with a coefficient of 0.93. The behavioral dimension factor is affected by the item of DAV2 "I have increased my economic measures" with a coefficient of 0.84, by the item of DAV3 "I have tried to keep my expenses under control" with a coefficient of 0.74, by the item of DAV6 "I have paid attention to a healthy diet" with a coefficient of 0.74, by the item of DAV4 "I have avoided unnecessary shopping" with a coefficient of 0.54, and by the item of DAV5 "I have provided my own transportation way instead of public transportation" with a coefficient of 0.51.

It is seen that it also fulfills the criterion of $X^2/df=12.75/5=2.55<3$, which is among the criteria of goodness of fit.

According to Table 7, the CR value of the behavioral dimension factor is 0.87 and the AVE value is 0.54. The CR value is expected to be greater than 0.70 and the AVE value to be greater than 0.50 and it is seen that the values calculated here are suitable for these criteria.

According to Table 8, the root mean square error of approximation (RMSEA) (0.035), normed fit index (NFI) (1.00), non-normed fit index (NNFI) (1.00), comparative fit index (CFI) (1.00), standardized root mean square residual (SRMR) (0.0064), goodness of fit index (GFI) (1.00) and adjusted goodness of fit index (AGFI) (0.99) values of the cognitive dimension factor are within the limits of excellent goodness of fit. The RMSEA (0.026), NFI (1.00), NNFI (1.00), CFI (1.00), SRMR (0.0087), GFI (1.00) and AGFI (0.99) values of the affective dimension factor are within the limits of excellent goodness of fit. For the behavioral dimension factor, the values of RMSEA (0.040), NFI (1.00), NNFI (0.99), CFI (1.00), SRMR (0.015), GFI (1.00) and AGFI (0.98) are within the limits of excellent goodness of fit.

Table 4. EFA, reliability analysis and descriptive statistics of the scale					
Factors/items		**Factor loads**	**Total correlation for corrected items**	**Cronbach alpha value if item is deleted**	**X̄±SD**
DUY eigenvalue: 8.357 Variance explanation rate: 24.587%					
DUY1	I have realized the meaning of life more	0.849	0.668	0.925	4.19±1.01
DUY2	I have understood how valuable health is	0.814	0.629	0.926	4.40±0.93
DUY3	My sensitivity to social issues has increased	0.788	0.611	0.926	4.03±1.06
DUY4	I have realized that I should value the people I love more	0.703	0.628	0.925	3.97±1.15
DUY5	I have understood the importance of the activities I did with my friends	0.659	0.682	0.924	4.18±1.05
DUY6	I think that I will be a more sensitive individual in the future to events that I ignored in the past	0.619	0.735	0.923	3.98±1.11
DUY7	I was more worried about my close environment than myself	0.568	0.544	0.927	4.07±1.19
BIL eigenvalue: 1.852 Variance explanation rate: 20.147%					
BIL1	My religious awareness has increased	0.864	0.598	0.926	2.96±1.39
BIL2	My commitment to my religion has increased	0.828	0.549	0.927	3.07±1.38
BIL3	The importance I attach to material has decreased	0.735	0.668	0.924	3.24±1.34
BIL4	I have once again questioned my purpose of life	0.727	0.657	0.925	3.36±1.37
BIL5	I have realized that money is not everything	0.639	0.665	0.924	3.58±1.33
DAV eigenvalue: 1.455 Variance explanation rate: 20.064%					
DAV1	I have limited my expenses as much as possible	0.846	0.642	0.925	3.62±1.22
DAV2	I have increased my economic measures	0.806	0.658	0.925	3.71±1.22
DAV3	I have tried to keep my expenses under control	0.752	0.698	0.924	3.79±1.20
DAV4	I have avoided unnecessary shopping	0.600	0.556	0.927	3.49±1.28
DAV5	I have provided my own transportation way instead of public transportation	0.551	0.531	0.928	4.02±1.27
DAV6	I have paid attention to a healthy diet	0.545	0.628	0.925	3.72±1.20
Total variance explanation rate: 64.798%					
SD: Standard deviation, DUY: Affective, BIL: Cognitive, DAV: Behavioral					

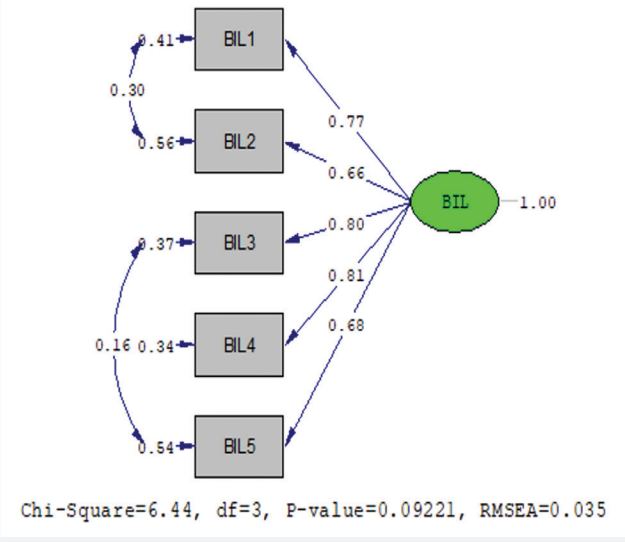

Figure 1. CFA model of cognitive dimension factor

CFA: Confirmatory factor analysis, BIL: Cognitive, RMSEA: Root mean square error of approximation

Table 5. For the items of cognitive dimension factor, findings on λ, t value and CR and AVE values					
Latent variable	**Observed variable**	**λ**	**t value**	**CR**	**AVE**
BIL	-	-	-	0.86	0.56
	BIL1	0.77	25.67		
	BIL2	0.66	20.94		
	BIL3	0.80	26.72		
	BIL4	0.81	27.55		
	BIL5	0.68	21.28		
BIL: Cognitive, CR: Composite reliabilit, AVE: Average variance extracted					

Level 2 CFA results of cognitive, affective and behavioral dimensions, which are sub-dimensions of attitude, are given in Figure 4.

The SEM model established with cognitive, affective and behavioral dimensions is given in Figure 5.

According to Figure 4, the most influential variable affecting the cognitive dimension factor was the item of BIL4 "I have

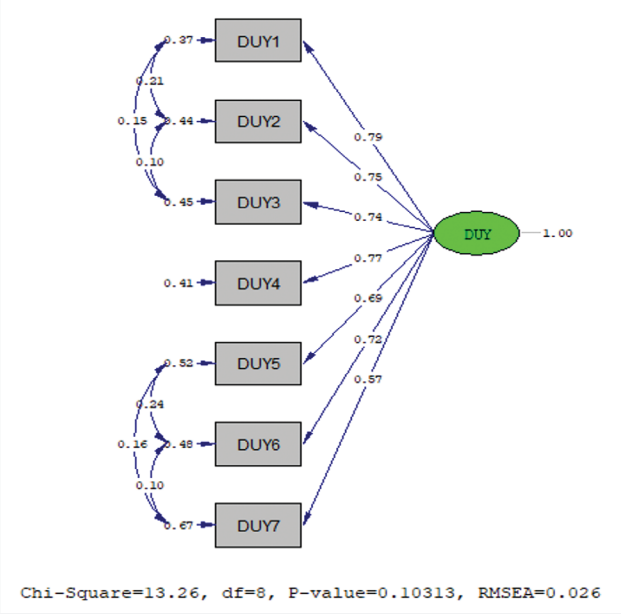

Figure 2. CFA model of affective dimension model

CFA: Confirmatory factor analysis, DUY: Affective, RMSEA: Root mean square error of approximation

Table 6. For the items of affective dimension factor, findings on λ, t value and CR and AVE values

Latent variable	Observed variable	λ	t value	CR	AVE
DUY				0.88	0.52
	DUY1	0.79	26.02		
	DUY2	0.75	23.80		
	DUY3	0.74	23.49		
	DUY4	0.77	25.55		
	DUY5	0.69	22.16		
	DUY6	0.72	23.26		
	DUY7	0.57	17.30		

DUY: Affective, CR: Composite reliabilit, AVE: Average variance extracted

once again questioned my purpose of life" with a coefficient of 0.88. The cognitive dimension factor is affected by the item of BIL5 "I have realized that money is not everything" with a coefficient of 0.84, by the item of BIL3 "The importance I attach to material has decreased" with a coefficient of 0.83, by the item of BIL1 "My religious awareness has increased" with a coefficient of 0.66, and by the item of BIL2 "My commitment to my religion has increased" with a coefficient of 0.58.

It is seen that the most effective variable affecting the affective dimension factor is the item of DUY6 "I think that I will be a more sensitive individual in the future to events that I ignored in the past" with a coefficient of 0.80. The affective dimension factor is affected by the item of DUY1 "I have realized the meaning of life more" with a coefficient of 0.74, by the item

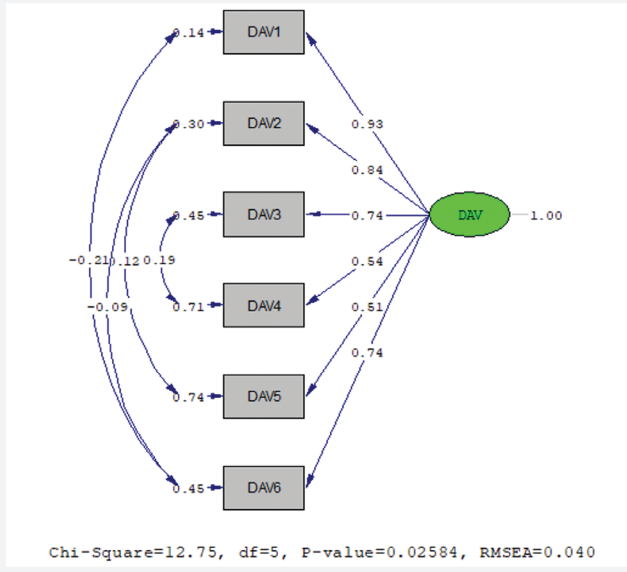

Figure 3. CFA model of behavioral dimension factor

CFA: Confirmatory factor analysis, DAV: Behavioral, RMSEA: Root mean square error of approximation

Table 7. For the items of behavioral dimension factor, findings on λ, t value and CR and AVE values

Latent variable	Observed variable	λ	t value	CR	AVE
DAV	-	-	-	0.87	0.54
	DAV1	0.93	35.00		
	DAV2	0.84	30.24		
	DAV3	0.74	26.04		
	DAV4	0.54	17.58		
	DAV5	0.51	16.61		
	DAV6	0.74	21.40		

DAV: Behavioral, CR: Composite reliabilit, AVE: Average variance extracted

Table 8. Findings on the goodness-of-fit criteria of CFA models established for cognitive, affective and behavioral factors

Goodness-of-fit criteria	Excellent fit	Acceptable fit	BIL	DUY	DAV
RMSEA	0< RMSEA <0.05	0.05≤ RMSEA ≤0.10	0.035	0.026	0.040
NFI	0.95≤ NFI ≤1	0.90< NFI ≤0.95	1.00	1.00	1.00
NNFI	0.97≤ NNFI ≤1	0.95≤ NNFI ≤0.97	1.00	1.00	0.99
CFI	0.97≤ CFI ≤1	0.95≤ CFI ≤0.97	1.00	1.00	1.00
SRMR	0≤ SRMR <0.05	0.05≤ SRMR ≤0.10	0.0064	0.0087	0.015
GFI	0.95≤ GFI ≤1	0.90≤ GFI ≤0.95	1.00	1.00	1.00
AGFI	0.90≤ AGFI ≤1	0.85≤ AGFI ≤0.90	0.99	0.99	0.98

CFA: Confirmatory factor analysis, BIL: Cognitive, DUY: Affective, DAV: Behavioral, RMSEA: Root mean square error of approximation, NFI: Normed fit index, NNFI: Non-normed fit index, CFI: Comparative fit index, SRMR: Standardized root mean square residual, GFI: Goodness of fit index, AGFI: Adjusted goodness of fit index

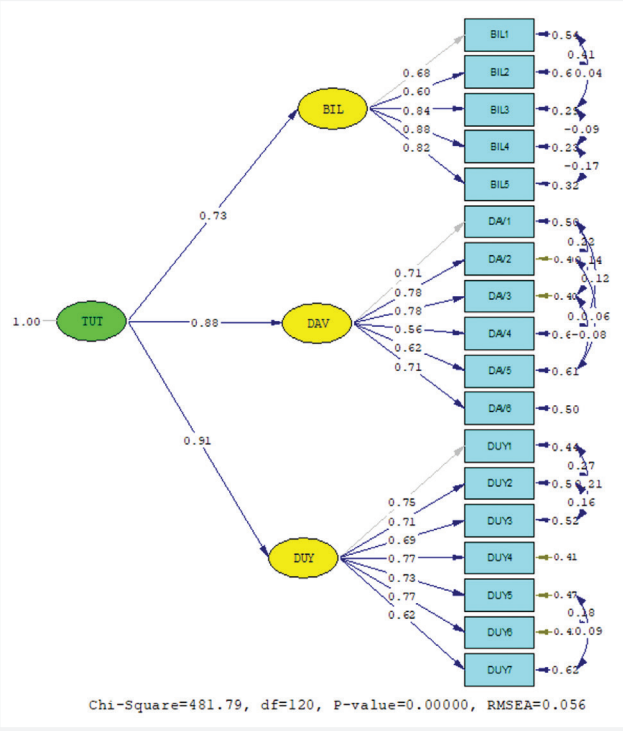

Figure 4. Second level confirmatory factor analysis results for cognitive, affective and behavioral dimensions

BIL: Cognitive, DUY: Affective, DAV: Behavioral, RMSEA: Root mean square error of approximation

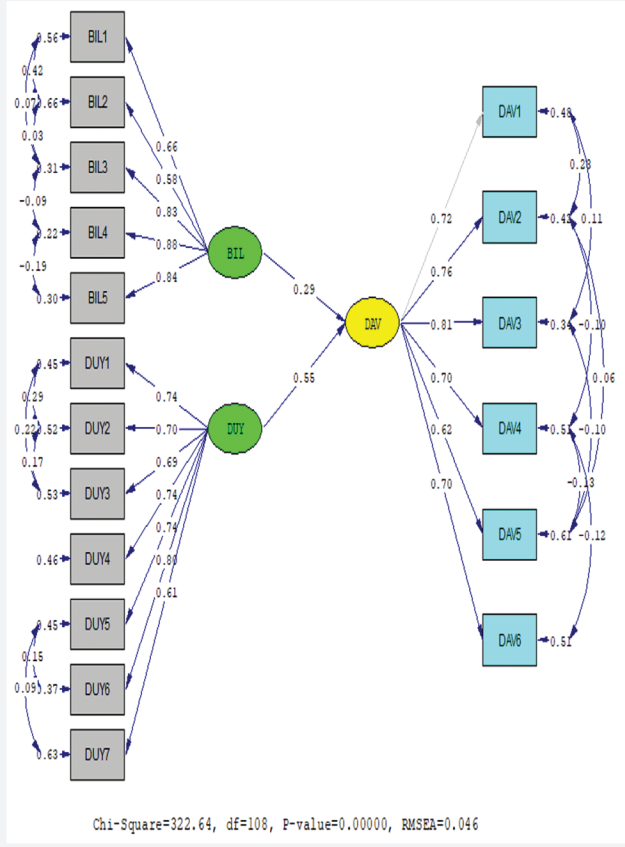

Figure 5. SEM model established with cognitive, affective and behavioral dimensions

BIL: Cognitive, DUY: Affective, DAV: Behavioral, RMSEA: Root mean square error of approximation

of DUY4 "I have realized that I should value the people I love more" with a coefficient of 0.74, by the item of DUY5 "I have understood the importance of the activities I did with my friends" with a coefficient of 0.74, by the item of DUY2 "I have understood how valuable health is" with a coefficient of 0.70, by the item of DUY3 "My sensitivity to social issues has increased" with a coefficient of 0.69, and by the item of "I was more worried about my close environment than myself" with a coefficient of 0.61.

With a coefficient of 0.81, the most influential variable affecting the behavioral dimension factor was DAV3 "I have tried to keep my expenses under control" The behavioral dimension factor is affected by the item of DAV2 "I have increased my economic measures" with a coefficient of 0.76, by the item of DAV1 "I have limited my expenses as much as possible" with a coefficient of 0.72, by the item of DAV6 "I have paid attention to a healthy diet" with a coefficient of 0.70, by the item of DAV4 "I have avoided unnecessary shopping" with a coefficient of 0.70, and by the item of DAV5 "I have provided my own transportation way instead of public transportation" with a coefficient of 0.62.

In addition, affective dimension (0.55) affects behavioral dimension more than cognitive dimension (0.29). It is seen that it meets the criterion of $X^2/df=322.64/108=2.98 <3$, which is among the criteria for goodness of fit.

According to Table 9, the CR and AVE values are 0.87 and 0.59 for the cognitive dimension factor, 0.88 and 0.52 for the affective dimension factor, and 0.87 and 0.52 for the behavioral dimension factor. The CR value is expected to be greater than 0.70 and the AVE value to be greater than 0.50 and it is seen that the values calculated here are suitable for these criteria.

According to Table 10, the RMSEA (0.046), NFI (0.99), NNFI (0.99), CFI (0.99), SRMR (0.035), GFI (0.96) and AGFI (0.94) values of the SEM model are within the limits of excellent goodness of fit. On the other hand, the RMSEA (0.056) value of the attitude model is within the limits of acceptable goodness of fit, while the NFI (0.98), NNFI (0.98), CFI (0.99), SRMR (0.042), GFI (0.95) and AGFI (0.92) values are within the limits of excellent goodness of fit.

Study Limitations

The data set used in the study was obtained through a 5-point Likert-type scale (1 strongly disagree and 5 strongly agree) in

Table 9. Findings on λ, t value, CR and AVE values for cognitive, affective and behavioral dimension factors

Latent variable	Observed variable	λ	t value	CR	AVE
BIL	-	-	-	0.87	0.59
	BIL1	0.66	22.00		
	BIL2	0.58	18.89		
	BIL3	0.83	28.30		
	BIL4	0.88	28.05		
	BIL5	0.84	28.49		
DUY	DUY1	0.74	25.27	0.88	0.52
	DUY2	0.70	23.09		
	DUY3	0.69	22.71		
	DUY4	0.74	25.10		
	DUY5	0.74	25.19		
	DUY6	0.80	27.99		
	DUY7	0.61	19.73		
DAV	DAV1	0.72	27.14	0.87	0.52
	DAV2	0.76	29.08		
	DAV3	0.81	26.77		
	DAV4	0.70	18.90		
	DAV5	0.62	16.69		
	DAV6	0.70	19.84		

CR: Composite reliabilit, AVE: Average variance extracted, BIL: Cognitive, DUY: Affective, DAV: Behavioral

Table 10. Findings related to goodness of fit criteria of SEM and attitude models

Goodness-of-fit criteria	Excellent fit	Acceptable fit	SEM	Attitude
RMSEA	0< RMSEA <0.05	0.05≤ RMSEA ≤0.10	0.046	0.056
NFI	0.95≤ NFI ≤1	0.90< NFI ≤0.95	0.99	0.98
NNFI	0.97≤ NNFI ≤1	0.95≤ NNFI ≤0.97	0.99	0.98
CFI	0.97≤ CFI ≤1	0.95≤ CFI ≤0.97	0.99	0.99
SRMR	0≤ SRMR <0.05	0.05≤ SRMR ≤0.10	0.035	0.042
GFI	0.95≤ GFI ≤1	0.90≤ GFI ≤0.95	0.96	0.95
AGFI	0.90≤ AGFI ≤1	0.85≤ AGFI ≤0.90	0.94	0.92

SEM: Structural equation modeling, RMSEA: Root mean square error of approximation, NFI: Normed fit index, NNFI: Non-normed fit index, CFI: Comparative fit index, SRMR: Standardized root mean square residual, GFI: Goodness of fit index, AGFI: Adjusted goodness of fit index, reference[73]

addition to demographic questions, which was applied to a total of 958 individuals that could be reached across Turkey between 1-30 June 2020.

DISCUSSION

The negative effects of the COVID-19 pandemic, which is the biggest problem of today, on human life are increasing day by day. In this study, which was carried out on the observation that the attitudes of individuals in their previous lives differed during the pandemic period, the cognitive, affective and behavioral attitudes of individuals during the pandemic process, the relationships among these attitudes and the variables that affected the attitudes were examined.

It was determined that the affective dimension had the highest effect in the results of EFA, CFA and SEM analysis, which are among the statistical methods that are effective in examining the attitude of individuals towards an event with these sub-dimensions.

When the other findings obtained from the study were examined in terms of demographic characteristics, it was observed that, in the affective dimension, the averages of individuals who were women, aged 20-29 years, had an income level of 4001-7000 TL, had a primary school education level and those with individuals who were found to be positive for COVID-19 in their close environment were high. In terms of demographic characteristics in the cognitive dimension, it has been observed that the averages of women, those aged 19 years and under, those with an income level of 4001-7000 TL, those with primary school education level and those with individuals with positive COVID-19 in their close environment were high.

Finally, in terms of demographic characteristics in behavioral dimension, it was observed that the averages of individuals who were female, aged between 40 and 49 years, had an income level of 7001-10000 TL, were primary school graduates, and had individuals without positive COVID-19 in their surroundings were higher.

As a result of SEM, it is seen that an increase of one unit in the affective dimensions of individuals causes an effect of 0.55 units in the behavioral dimensions, and an increase of one unit in the cognitive dimensions causes an increase of 0.29 units in the behavioral dimension.

While "individuals' questioning their purpose of life again" was the most effective item in the cognitive dimension, it was determined that individuals would be more sensitive individuals in the future than in the past and would attach importance to economic measures according to the items that were effective in the affective and behavioral dimensions.

CONCLUSION

It should not be forgotten that, like many pandemics in the past, the COVID-19 pandemic is a temporary process, even

if it has negative effects on the attitudes and behaviors of individuals.

With the hope that the ongoing vaccine studies will yield positive results as soon as possible, all individuals should take responsibility for this pandemic to be overcome with the least damage for our country and the whole world humanity. In order to inform people about responsibilities, taking into account the results of this research, which is an attitude determination study, it is necessary to assimilate the causes and consequences of the changes in the attitudes and behaviors of individuals in this process to learn a lesson from all these experiences and make positive contributions to the future.

Ethics

Ethics Committee Approval: Study was approved by the decision of Afyon Kocatepe University Scientific Research and Publication Ethics Committee dated 27.05.2020 and numbered 15.06.2020-E.17011.

Informed Consent: It is a survey study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: İ.B., M.A., Y.D., C.G., S.S., Design: B.İ., N.F., M.A., Y.D., C.G., S.S., Data Collection or Processing: B.T., M.D., Ş.B., C.G., S.S., Analysis or Interpretation: B.T., C.G., S.S., Literature Search: E.Ö., İ.B., B.İ., M.D., C.G., S.S., Writing: B.T., B.İ., M.D., N.F., Ş.B., C.G., S.S.

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The Effect of miR-34a-5p and miR-145-5p Ectopic Expression on Cell Proliferation and Target Gene Expression in the MDA-MB-231 Cell Line

miR-34a-5p ve miR-145-5p'nin MDA-MB-231 Hücre Hattındaki Ektopik Ekspresyonunun Hücre Proliferasyonu ve Hedef Gen Ekpresyonu Üzerindeki Etkisi

İlknur SUER[1], Murat KAYA[2], Emre ÖZGÜR[3]

[1]*İstanbul University-İstanbul Faculty of Medicine, Department of Medical Genetics, İstanbul, Turkey*
[2]*İstanbul University-İstanbul Faculty of Medicine, Department of Internal Diseases, Division of Medical Genetics, İstanbul, Turkey*
[3]*İstanbul University, Oncology Institute, Diagnostic Treatment and Care Services, Department of Basic Oncology, İstanbul, Turkey*

ABSTRACT

Aim: It was aimed to investigate the effect of miR-34a-5p and miR-145-5p on breast cancer cell line MDA-MB-231 and to determine the expression of target genes of these microRNAs (miRNAs).

Materials and Methods: Firstly, literature search and *in silico* analysis were performed to detect possible target genes of miR-34a-5p and miR-145-5p, which are known to be tumor suppressors. Mimic miR-34a-5p and miR-145-5p were transfected to the breast cancer cell line MDA-MB-231. Deregulated genes were investigated by the quantitative real-time polymerase chain reaction compared to control cells. Also, the effect of these miRNAs on proliferation was determined using the Water Soluble Tetrazolium Salt-8 method. Finally, the expressions of epithelial mesenchymal transition (EMT) markers, which are known to be important in the metastatic process, are examined.

Results: The proliferation of the miR-34a-5p or miR-145-5p transfected cells decreased compared to the control groups. The expression of E2F transcription factor 1 (*E2F1*) (p=0.009), mitogen activated protein kinase 1 (*MEK1*) (p=0.001) and cyclin dependent kinase 4 (*CDK4*) (p=0.005) genes, which were among the genes targeted by miR-34a-5p, were significantly reduced. EMT markers were significantly changed in miR-34a-5p transfected cells (*E-Cad* increase p=0.01; *Vimentin* decrease p=0.008). Kruppel-like factor 4 (*KLF4*) (p=0.007) targeted miR-145-5p were significantly reduced and EMT markers were significantly changed in miR-145-5p transfected cells (*E-Cad* increase p=0.0005; *Vimentin* decrease p=0.006).

Conclusion: miR-34a-5p and miR-145-5p may have an impact on the breast cancer cell line MDA-MB-231 proliferation and EMT mechanism. At the same time, according to our study results, it was revealed that *E2F1*, *MEK1* and *CDK4* genes, whose expression level decreased after transfection of mimic miR-34a-5p, could be targeted by miR-34a-5p in breast cancer, and that the expression level of *KLF4*, which decreased as a result of mimic miR-145-5p transfection, could be the target.

Keywords: MDA-MB-231, breast cancer, miRNA, miR-34a-5p, miR-145-5p

ÖZ

Amaç: Bu çalışmada miR-34a-5p ile miR-145-5p'nin meme kanseri hücre hattı MDA-MB-231 üzerindeki fonksiyonel etkilerinin araştırılması ve bu mikroRNA'ların (miRNA) olası hedef genlerinin miRNA mimik transfeksiyonu yapılan hücrelerde ifade seviyelerinin araştırılması hedeflenmiştir.

Gereç ve Yöntem: Tümör baskılayıcı özellikte oldukları bilinen miR-34a-5p ve miR-145-5p'nin meme kanserindeki olası hedef genlerinin tespiti için öncelikle literatür taraması ve *in silico* analiz çalışması yapılmıştır. Tespit edilen genlerin ifadelerindeki değişimler *in vitro* ortamda meme kanseri hücre hattı MDA-MB-231'de belirlenen miRNA'ların transfekte edilmesiyle kontrol hücrelerle karşılaştırmalı olarak kantitatif gerçek zamanlı polimeraz zincir reaksiyonu ile araştırılması hedeflenmiştir. Ayrıca bu miRNA'ların proliferasyona etkisi Water Soluble Tetrazolium Salt-8 yöntemi kullanılarak belirlenmiştir. Son olarak metastatik süreçte önemli olduğu bilinen epitelyal mezenkimal geçiş (EMT) belirteçlerinin ekspresyonuna bakılarak metastaza olan katkısının araştırılması hedeflenmiştir.

Address for Correspondence: İlknur SUER PhD, İstanbul University, İstanbul Faculty of Medicine, Department of Internal Diseases, Division of Medical Genetics, İstanbul, Turkey
Phone: +90 505 540 10 47 **E-mail:** ilknursuer@gmail.com, ilknursuer@istanbul.edu.tr **ORCID ID:** orcid.org/0000-0003-1954-4190
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Bulgular: miR-34a-5p transfeksiyonu yapılan gruptaki hücre proliferasyonunun kontrol grubu hücrelere göre azaldığı, EMT ile ilişkili genlerin ifadesinin anlamlı şekilde değiştiği (*E-Cad* artış p=0,01; *Vimentin* azalma p=0,008), miR-34a-5p'nin hedeflediği genlerden olan E2F transkripsiyon faktör 1 (*E2F1*) (p=0,009), mitojen aktive protein kinaz 1 (*MEK1*) (p=0,001) ve siklin bağımlı kinaz 4 *(CDK4)* (p=0,005) genlerinin ifadesinin belirgin şekilde düştüğü tespit edilmiştir. miR-145-5p transfekte edilen hücrelerde ise benzer şekilde hücre proliferasyonunda azalma ve EMT ile ilişkili genlerde anlamlı değişiklikler gözlenmiş (*E-Cad* artış p=0,0005; *Vimentin* azalma p=0,006), miR-145-5p'nin hedeflediği önemli proto onkogenlerden birisi olan Kruppel-benzeri faktör 4 (*KLF4*) (p=0,007) geninin ifadesinin önemli derecede azaldığı tespit edilmiştir.

Sonuç: Çalışma sonuçlarımız, miR-34a-5p ve miR-145-5p'nin meme kanseri hücre hattı MDA-MB-231 hücre proliferasyonu ve EMT mekanizması üzerinde etkili olabileceğini göstermiştir. Aynı zamanda çalışma sonuçlarımıza göre mimik miR-34a-5p transfeksiyon yapılması sonrasında ekspresyon seviyesi azalan *E2F1, MEK1* ve *CDK4* genlerinin meme kanserinde miR-34a-5p tarafından hedeflenebileceği, yine mimik miR-145-5p transfeksiyonu neticesinde ifade seviyesi azalan *KLF4* geninin miR-145-5p'nin hedefi olabileceği ortaya çıkmıştır.

Anahtar Kelimeler: MDA-MB-231, meme kanseri, miRNA, miR-34a-5p, miR-145-5p

INTRODUCTION

Breast cancer is the most common type of cancer in women, and it occupies a large place among cancer-related deaths in women all over the world, especially among women in underdeveloped countries[1]. MicroRNAs (miRNAs) are single-stranded, non-protein-coding RNAs containing approximately 19-24 nucleotides and repress their target mRNAs. Depending on the functions of target genes, miRNAs are biomolecules that play a vital role in various cellular processes such as differentiation, proliferation and apoptosis, and their deregulation is effective in the development of many types of cancer[2]. The miR-34 family, which was characterized in 2007, has three members, miR-34a-5p, miR-34b and miR-34c. The miR-34 family of tumor suppressor miRNAs is known to target many cancer-related genes, including p53. It has been reported that these miRNA family members are deregulated in a large number of cancer types[3]. One of the other most important tumor suppressor miRNA is miR-145-5p. The expression level of miR-145-5p has been shown to be frequently decreased in many cancers, including colorectal cancer, hematological cancers, prostate cancer, ovarian cancer, B-cell tumor, and breast cancer[4]. Undoubtedly, it is extremely important in terms of the molecular mechanism of cancer to learn that this decrease in the expression levels of miR-34a-5p and miR-145-5p, whose expression levels are found to be decreased in many cancers, contribute to the cancerization process by increasing their expression of target genes[4,5]. However, studies on miR-34a-5p and miR-145-5p miRNAs targeting which genes in breast cancer and through which pathways are effective in the cancer process are limited.

Within the scope of the study, it was aimed to investigate the effects of related miRNAs on cell proliferation by transfection of mimic miR-145-5p and miR-34a-5p to MDA-MB-231 cells, which are triple negative breast cancer cell line.

Afterwards, the expression levels of one of the target genes of miR-34a-5p, Mouse double minute 4 (*MDM4*), E2F1, Insulin-like growth factor 1 receptor (*IGF1R*), *MEK1, CDK4*, cluster of differentiation 44 (*CD44*), eukaryotic translation initiation factor 4E (*EIF4E*) targeted by neurogenic locus notch homolog protein 2 (*NOTCH2)* and target genes of miR-145-5p, *CDK4*, Cyclin-dependent kinase 6 (*CDK6*), Epidermal growth factor receptor (*EGFR*), SRY-Box Transcription Factor (*SOX2*), *KLF4*, jagged canonical notch ligand 1 (*JAG1*) genes were investigated and it was aimed to investigate whether the expression levels of related genes are correlated with miR-34a-5p and miR-145-5p. Finally, it was aimed to investigate the relationship of miR-34a-5p and miR-145-5p with metastasis by looking at the expression of epithelial mesenchymal transition (EMT) markers that are known to contribute to the metastatic process.

MATERIALS AND METHODS

Cell Culture and miRNA Transfection

For the functional study of miR-34a-5p and miR-145-5p non-coding RNAs *in vitro*, MDA-MB-231 breast cancer cell line 1% antibiotic (penicillin/streptomycin) and 10% fetal bovine serum (Gibco, Thermo Fisher Scientific Inc., Waltham, MA, USA) was cultured and replicated in RPMI-1640 (Roswell Park Memorial Institute-1640) (Gibco, Thermo Fisher Scientific, Waltham, MA, USA) medium at 37 °C in a 5% CO_2 incubator.

In our study, in which an *in vitro* functional experiment of miR-34a-5p and miR-145-5p synthetic mimic (Thermo Fisher Scientific Inc.) was planned separately in replicated cells, an oligonucleotide sequence control miRNA (non-targeting control miRNA, control nt miRNA) (Thermo Fisher Scientific Inc.) that did not target any gene was used (miR-145-5p 5'-GUCCAGUUUUCCCAGGAAUCCCU-3'), non-targeting control miRNA (5'-UCACAACCUCCUAGAAAGAGUAGA-3'), miR-34a-5p (5'-UGGCAGUGUCUUAGCUGGUUGU-3').

24 hours after seeding the appropriate number of cells (well/300,000 cells) on 6-well plates, transfection of miRNA mimic was performed in the cells. Mimics were transported into the cell with Lipofectamine 2000 reagent (Thermo Fisher Scientific Inc.) by applying the manufacturer's protocol in transfection. Then, the cells were incubated at 37 °C and 24

hours after transfection, cells transfected with miR-145-5p, miR-34a-5p and cells transfected with non-targeting control miRNA were taken separately in 1000 µL Trizol (MRC Inc.) in eppendorfs and RNA was then brought to -80 °C for isolation.

Isolation of RNA from Cells After Transfection

Total RNA was isolated from cells taken in trizol (MRC Inc.) in accordance with the manufacturer's protocol, and RNA concentration and purity were measured with the help of NanoDrop ND-2000c (Thermo Fisher Scientific Inc.) spectrophotometer.

Confirmation of miRNA cDNA Synthesis and Transfection by miRNA qRT-PCR

Using TaqMan probes, quantitative real-time polymerase chain reaction (qRT-PCR) was performed to determine the expression levels of miR-34a-5p and miR-145-5p in cell lines and to check whether the transfection processes were effective. MiRNA cDNA synthesis using total 30 ng RNA was performed using TaqMan MicroRNA reverse transcriptase kit (Applied Bio., Foster City, CA, United States) and miRNA RT primers according to the manufacturer's protocol. TaqMan assays were used for qRT-PCR, and qRT-PCR was performed on LightCycler® 480 (Roche) instrument using TaqMan Universal Master Mix (Thermo Fisher Scientific Inc.) kit with TaqMan miRNA probes (Thermo Fisher Scientific Inc.) and RNU43 (control miRNA) probes (Thermo Fisher Scientific Inc.) according to the manufacturer's protocol. Experiments were performed in duplicate and $2^{-\Delta\Delta Ct}$ method was used for relative quantitation analysis.

In Silico Prediction of Target Genes for Expression of miR-34a-5p and miR-145-5p After Transfection

Target prediction tools for prediction of related genes which are the tools such as miRBase (http://www.mirbase.org/), miRWalk (http://mirwalk.umm.uni-heidelberg.de/) and miRTargetLink (https://ccb-web.cs.uni-saarland.de/mirtargetlink/) and databases such as Genecards, NCBI, PubMed were used. That the relationship of the gene selected to be investigated selectively in the prediction phase of possible target genes and the related miRNA has not been shown in the MDA-MB-231 breast cancer cell line before, that the expression level of the selected gene in breast cancer has been shown to increase in breast cancer previously in the literature, and that the selected gene is targeted by the relevant miRNA at least in two different cancers were taken into account. The miRTargetLink database was also used to schematize genes with strong interactions of miR-34a-5p and miR-145-5p[6].

cDNA Synthesis and qRT-PCR Procedures for the Target Genes of miR-34a-5p and miR-145-5p

The cDNA extraction with SCRIPT cDNA synthesis kit (Jena Bioscience) and qPCR SybrMaster (Jena Bioscience) and qRT-PCR processes were performed in accordance with the manufacturer's protocol, using a total of 1000 ng of RNA obtained from the study group and control group cells.

While the expression levels of *MDM4*, *E2F1*, *IGF1R*, *MEK1*, *CDK4*, *CD44* and *NOTCH2* genes were determined in miR-34a-5p transfected cells, the expression levels of *EIF4E*, *EGFR*, *SOX2*, *CDK4*, *CDK6*, *JAG1* and *KLF4* genes were examined in miR-145-5p transfected cells. The primer sequences of the examined genes are shown in Table 1. qRT-PCR processes were performed on a LightCycler® 480 (Roche) device.

Table 1. Primer sequences

Primer	Primer Sequence	References
MDM4-F	5'-CTAAGTCCTTAAGTGATGATACCGATGT-3'	7
MDM4-R	5'-AACTTTGAACAATCTGAATACCAATCC-3'	
E2F1-F	5'-CAGAGCAGATGGTTATGG-3'	8
E2F1-R	5'-CTGAAAGTTCTCCGAAGA-3'	
IGF1R-F	5'-TTTCCCACAGCAGTCCACCTC-3'	9
IGF1R-R	5'-AGCATCCTAGCCTTCTCACCC-3'	
MEK1-F	5'ATGCATGGAAAGCATGCTTTGCTGCTACTGAA3'	10
MEK1-R	5'-TTCAGTAGCAGCAAAGCATGCTTTCCATGCA-3'	
CDK4-F	5'-TGCCAATTGCATCGTTCACCGAG-3'	11
CDK4-R	5'-TGCCCAACTGGTCGGCTTCA-3'	
CD44-F	5'-ACTGCAGCCAACTTCCGAGG -3'	12
CD44-R	5'-GGAATACACCTGCAAAGCGG-3'	
SOX2-F	5'-CTCCGGGACATGATCAGC-3'	
SOX2-R	5'-GGTAGTGCTGGGACATGTG-3'	
KLF4-F	5'-CCCAATTACCCATCCTTCCTG	
KLF4-R	5'-GTCTTCCCCTCTTTGGCTTG	
β-aktin-F	5'-GCCTCGCCTTTGCCGATC-3'	
β-aktin-R	5'-CCCACGATGGAGGGGAAG-3'	
NOTCH2-F	5'-GGGACCCTGTCATACCCTCT-3'	13
NOTCH2-R	5'-GAGCCATGCTTACGCTTTCG-3'	
EIF4E-F	5'-TACTAAGAGCGGCTCCACCAC-3'	14
EIF4E-R	5'-TCGATTGCTTGACGCAGTCTCC-3'	
EGFR-F	5'-ATGGTCAAGTGCTGGATG-3'	15
EGFR-R	5'-GAGGAAGGTGTCGTCTATG-3'	
CDK6-F	5'-TGGAGACCTTCGAGCACC -3'	16
CDK6-R	5'-CACTCCAGGCTCTGGAACTT-3'	
JAG1-F	5'-GCTTGGATCTGTTGCTTGGTGA -3'	17
JAG1-R	5'-ACTTTCCAAGTCTCTGTTGTCCTG-3'	
E-Cad-F	5'-TGCCCAGAAAATGAAAAAGG-3'	18
E-Cad-R	5'-GTGTATGTGGCAATGCGTTC-3'	
N-Cad-F	5'-ACAGTGGCCACCTACAAAGG-3'	
N-Cad-R	5'-CCGAGATGGGGTTGATAATG-3'	
Vimentin-F	5'-GAGAACTTTGCCGTTGAAGC-3'	
Vimentin-R	5'-GCTTCCTGTAGGTGGCAATC-3'	

Investigation of the Effect of miR-34a-5p and miR-145-5p on Cell Proliferation

After cells were transfected with miR-34a-5p and miR-145-5p synthetic mimic and control miRNA (non-targeting control miRNA), whether there was a change in the proliferation of cells were examined using the Cell Proliferation Reagent Water Soluble Tetrazolium Salt-8 (WST-8) (EcoTech Biotechnology) according to the manufacturer's protocol.

Cells seeded in triplicate at 5000 cells per well on 96-well plates were cultured at 37 °C in a 5% CO_2 incubator. Proliferation changes in cells transfected after 24 hours were measured at the following 24th, 48th and 72nd hours. For measurement, 10 µL of WST-8 (EcoTech Biotechnology) solution was added to each well and cells were incubated at 37 °C for three hours in the dark. Cell viability was evaluated using a plate reader (Thermo Varioskan flash) device capable of measuring absorbance at 450 nm.

Investigation of the Effect of miR-34a-5p and miR-145-5p on Cell Migration with EMT Marker

In order to examine whether the transfected miRNAs contribute to the migration and invasion potential of cells, the expression levels of *E-cadherin, N-cadherin, Vimentin* genes, which are important EMT markers in epithelial mesenchymal transition, were evaluated by qRT-PCR technique. qRT-PCR processes were performed using qPCR SybrMaster (Jena Bioscience) in accordance with the manufacturer's protocol.

Statistical Analysis

For normalization of gene expression, β-actin primers were used as internal control and experiments were performed in duplicate. The $2^{-\Delta\Delta Ct}$ method was used for the relative quantitation analysis. Statistical analyzes were performed using Student's t-test and data were presented as mean±standard deviation. Data with a p value less than 0.05 were considered statistically significant. GraphPad Prism 5 and Statistical Package for the Social Sciences 21 programs were used for graphic drawings.

RESULTS

Verification of Transfection Procedures

It was confirmed that the transfection occurred significantly for both miRNAs in miR-34a-5p and miR-145-5p transfected cells and control cells (Figure 1).

Effect of miR-34a-5p and miR-145-5p on Expression Level of Possible Target Genes in MDA-MB-231 Cells

As a result of the literature review and *in silico* studies, the expression of *E2F1*, *MEK1* and *CDK4* genes, which are among the genes that are predicted to be strongly associated with miR-34a-5p, were significantly suppressed in miR-34a-5p transfected cells compared to the control group (respectively p=0.009; p=0.001; p=0.005) and *KLF4* gene, which is among the genes predicted to be strongly associated with miR-145-5p, was significantly suppressed in miR-145-5p transfected cells compared to the control group (p=0.007) (Figure 2, 3, 4, 5).

Effect of miR-34a-5p and miR-145-5p on Proliferation and Migration of MDA-MB-231 Cells

It was determined that proliferation in MDA-MB-231 cells transfected with both miR-34a-5p and miR-145-5p decreased in correlation with the literature (Figures 6, 7).

As a result of expression evaluation of epithelial-mesenchymal cell markers with qRT-PCR to understand whether selected miRNAs have any regulatory role in cell migration, it was

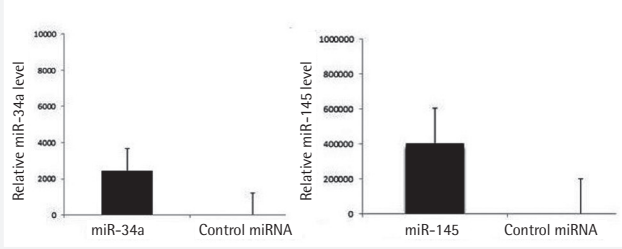

Figure 1. Verification of transfection
miRNAs: MicroRNAs

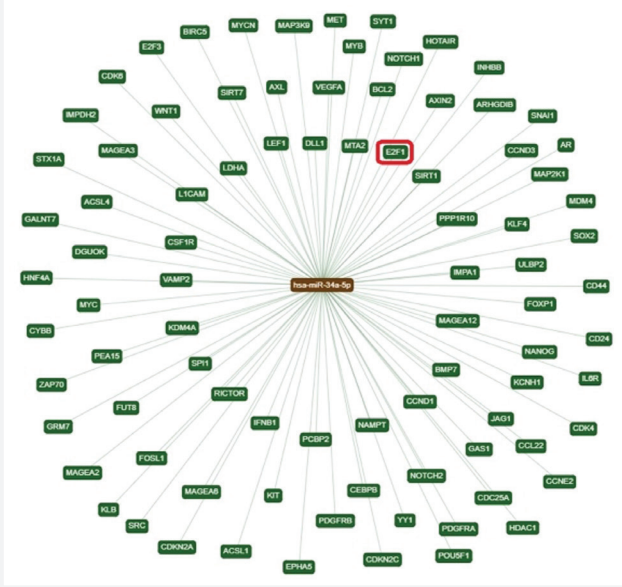

Figure 2. Genes predicted to be strongly associated with miR-34a-5p (https://ccb-web.cs.uni-saarland.de/mirtargetlink/network.php)

determined that E-Cad expression level was increased significantly (p=0.01; p=0.0005 respectively), Vimentin expression level was decreased significantly (p=0.008;p=0.006, respectively), and N-Cad expression level was not changed significantly (p>0.05) (Figure 8, 9) in cells transfected with miR-34a-5p and miR-145-5p compared to control cells.

As a result, it was determined that miR-34a-5p and miR-145-5p contributed to the suppression of cell migration in MDA-MB-231 breast cancer cells by regulating through EMT markers.

DISCUSSION

MiRNAs, which can act as oncogene (onco-mir) or tumor suppressor (TSmir) according to the genes they target, are among the most interesting research topics in recent years[19]. Abnormal expression changes in miRNAs may be involved in the cancer process by changing the expression of many genes targeted by the relevant miRNAs. The fact that miR-34 was the first miRNA to be tested in phase 1 in relation to the use of

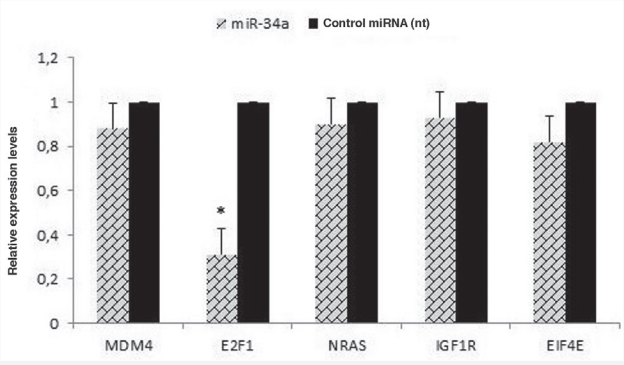

Figure 3. Effect of miR-34a-5p on expression levels of possible target genes in MDA-MB-231 cells

miRNAs: MicroRNAs

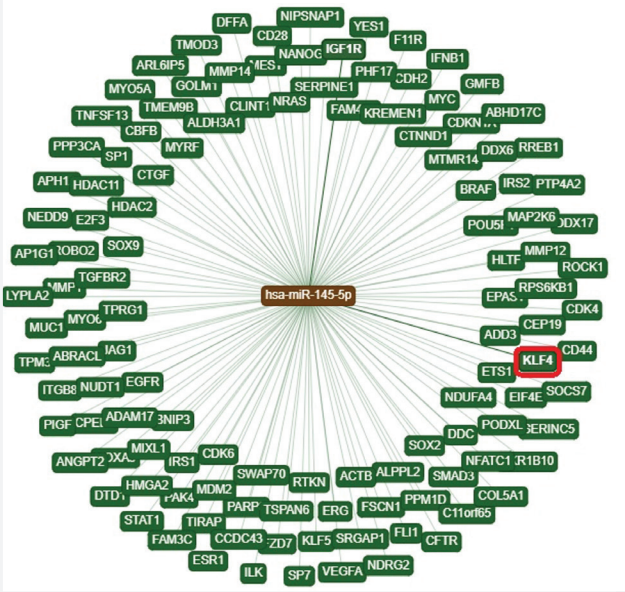

Figure 4. Genes predicted to be strongly associated with miR-145-5p (https://ccb-web.cs.uni-saarland.de/mirtargetlink/network.php)

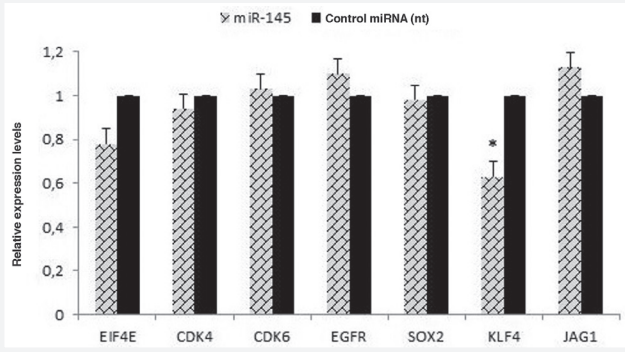

Figure 5. Effect of miR-145-5p on expression levels of possible target genes in MDA-MB-231 cells

miRNAs: MicroRNAs

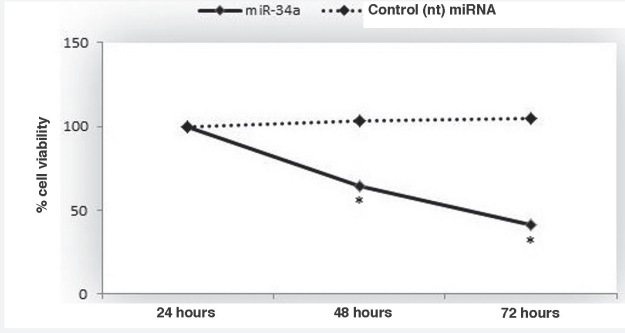

Figure 6. Effect of miR-34a-5p on proliferation in MDA-MB-231 cells

miRNAs: MicroRNAs

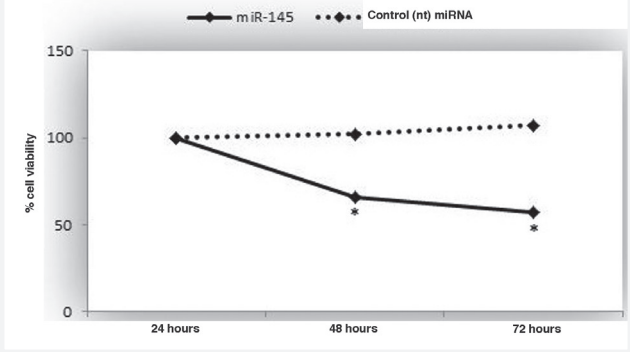

Figure 7. Effect of miR-145-5p on proliferation in MDA-MB-231 cells

miRNAs: MicroRNAs

miRNAs in therapy indicates that miR-34 may be one of the most promising miRNAs in the future[20].

Studies with miR-34a-5p localized at 1p36.22 have shown that ectopic expression of miR-34a-5p directly or indirectly causes a decrease in the expression of many proto-oncogenes, resulting in decreased cancer cell proliferation and elimination of metastasis[5]. The fact that *p53*, which is shown as the guardian of the genome, and its homologous *p63* and *p73* genes are controlled by miR-145-5p, and many important genes are targeted by this miRNA, indicates that miR-145-5p can have critical importance in early diagnosis and possible miRNA-based therapies[4]. Determining which genes control various cellular processes by regulating miRNAs is extremely important for understanding diseases at the molecular level[21]. miRNAs are involved in regulating the expression of most protein-coding genes at the post-transcriptional stage. Therefore, many bioinformatics tools have been created to predict the interaction between miRNA and target mRNA.

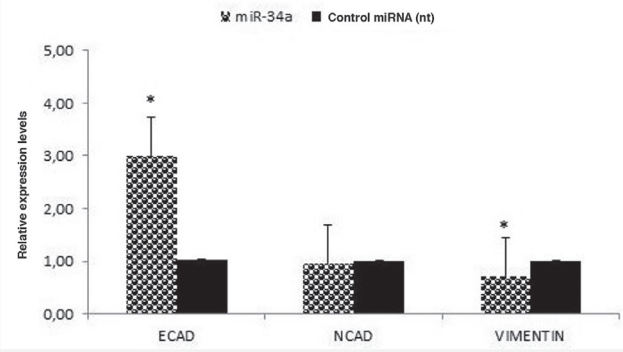

Figure 8. Effect of miR-34a-5p on expression levels of epithelial mesenchymal transition genes in MDA-MB-231 cells

miRNAs: MicroRNAs

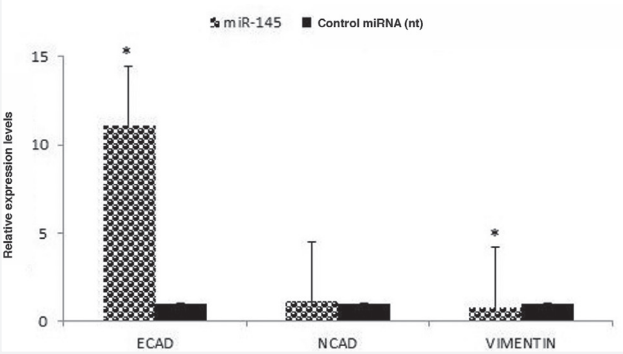

Figure 9. Effect of miR-145-5p on expression levels of epithelial mesenchymal transition genes in MDA-MB-231 cells

miRNAs: MicroRNAs

Supporting *in vitro* and *in vivo* predictions based on base pairing between miRNA and mRNA *in silico* may be important for the development and progression of many diseases, especially cancer, and the development of biomarkers for these diseases[2]. Figure 2 and Figure 4 show that miR-34a-5p and miR-145-5p can target hundreds of genes *in silico*. In the literature, it has been shown that the expression levels of miR-34a-5p and miR-145-5p are considerably reduced in breast cancer patient tissue samples and MDA-MB-231 cells, as in many other cancer types[22,23]. Therefore, according to the results of our transfection study to investigate the effects of miR-34a-5p and miR-145-5p on MDA-MB-231 cells, miR-34a-5p and miR-145-5p have been found to have a significant effect on MDA-MB-231 cell proliferation. The effects of miR-34a-5p and miR-145-5p on cell proliferation may be related to decreased expression of various genes targeted by these miRNAs. The *KLF4* gene is a gene that has been found to be highly expressed in more than 70% of breast cancers and has been reported to function as an oncogene[24]. The *E2F1* gene is also a gene with increased expression in breast cancer and associated with breast cancer metastasis[25]. *MEK1* and *CDK4* genes, whose expression levels have been found to be increased in many cancers, including breast cancer, are important oncogenes that play critical roles in apoptosis, migration, invasion and cell proliferation processes[26,27]. miR-34a-5p of the *E2F1*, *MEK1* and *CDK4* genes; despite the fact that *KLF4* has been shown to be effective in various cancer processes by being targeted by miR-145-5p, there are no studies in the literature on this subject in breast cancer[28-32]. In our study to fill this gap in the literature, it was determined that *E2F1*, *MEK1*, *CDK4* and *KLF4* gene expression levels were significantly decreased in MDA-MB-231 cells transfected with mimic miR-34a-5p and mimic miR-145-5p.

Study Limitations

Considering our study results and the information in the literature together, it can be predicted that miR-34a-5p may contribute to the cancer process by targeting *E2F1*, *MEK1* and *CDK4* genes, and miR-145-5p targeting *KLF4* in breast cancer. Among the limitations of the study; the fact that the research was carried out in cell line, it was not confirmed in the tissue, and the genes whose expression level was examined were examined only at the mRNA level. It is obvious that more meaningful results can be obtained if the findings obtained in our study at the level of RNA expression are supported by methods such as Western-Blot and luciferase activity determination in new studies on the subject.

It was seen that *CD44*[33], *NOTCH2*[34], *MDM4*[35], *IGF1R*[36] genes, which have been shown as targets of miR-34a-5p both *in silico* and *in vivo*, and similarly the expression levels of *EIF4E*[37], *EGFR*[38], *SOX2*[21], *CDK4*[37], *CDK6*[39], *JAG1*[40] genes which have been shown as targets of miR-145-5p did not have a statistically

significant change in MDA-MB-231 cells in which transfection of miRNA mimics was carried out in our study.

CONCLUSION

The data obtained from our study may be evidence that the miRNA-mRNA pairing created by theoretical prediction or the miRNA-mRNA relationship shown in vivo media in different cancer types may not be seen likewise in all cancer types due to the complex nature of cancer.

Acknowledgement

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Ethics

Ethics Committee Approval: Ethics committee approval form was not received because it was studied with cell lines.

Informed Consent: Patient samples were not studied.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept-Design: İ.S., M.K., E.Ö., Data Collection or Processing: İ.S., M.K., E.Ö., Analysis or Interpretation: İ.S., M.K., E.Ö., Literature Search: İ.S., M.K., E.Ö., Writing: İ.S., M.K., E.Ö.

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

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ORIGINAL ARTICLE

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Evaluation of the Hearing System in Chronic Obstructive Pulmonary Disease Patients

Kronik Obstrüktif Akciğer Hastalığı Hastalarındaki İşitme Sisteminin Değerlendirilmesi

Oral Burak DEMİREL, Tolga ERSÖZLÜ, Mahmut DENİZ

Tekirdağ Namık Kemal University Faculty of Medicine, Department of Otorhinolaryngology, Head and Neck Surgery, Tekirdağ, Turkey

ABSTRACT

Aim: This study aimed to examine the effect of chronic hypoxemia on auditory functions in chronic obstructive pulmonary disease (COPD) patients.

Materials and Methods: Sixty patients who had pulmonary function test (PFT) and diagnosed with COPD were included in the study. Four study groups and a control group were created in the study. Each study group was determined based on forced expiratory volume (FEV1) and FEV1/forced vital capacity (FVC) ratio. Groups consisted of mild, moderate, severe and very severe COPD patients. Each group included 15 patients. The control group consisted of 30 patients with an FEV1/FVC ratio of >70%. All patients underwent pure tone audiometry and otoacoustic emission (OAE) test.

Results: According to the audiological evaluation, a statistically significant difference was found between the severe and very severe group and the control group ($p<0.01$).

Conclusion: The results of the present study showed that auditory mechanisms may also be affected in patients with severe and very severe COPD. The authors of this study argue that necessary measures should be taken in the early stages of the disease for COPD patients to prevent the negative effects of chronic hypoxemia on the auditory system.

Keywords: Chronic obstructive pulmonary disease, hypoxia, auditory pathway, audiometry, otoacoustic emissions spontaneous

ÖZ

Amaç: Bu çalışmada, kronik hipokseminin kronik obstrüktif akciğer hastalığı (KOAH) hastalarında işitsel işlevler üzerindeki etkisini incelemeyi amaçladık.

Gereç ve Yöntem: Çalışmaya solunum fonksiyon testi (SFT) yapılan ve KOAH tanısı alan 60 hasta dahil edildi. Çalışmada dört çalışma grubu ve bir kontrol grubu oluşturulmuştur. Her çalışma grubu zorlu ekspiratuvar hacim (FEV1) ve FEV1/zorlu vital kapasite (FVC) oranına göre belirlendi. Gruplar hafif, orta, şiddetli ve çok şiddetli KOAH hastalarından oluşuyordu. Her grup 15 hastayı içeriyordu. Kontrol grubu FEV1/FVC oranı >%70 olan 30 hastadan oluşuyordu. Tüm hastalara saf ton odyometri ve otoakustik emisyon (OAE) testi uygulandı.

Bulgular: Odyolojik değerlendirmeye göre şiddetli ve çok şiddetli grup ile kontrol grubu arasında istatistiksel olarak anlamlı farklılık bulundu ($p<0,01$).

Sonuç: Bu çalışmanın sonuçları, şiddetli ve çok şiddetli KOAH'lı hastalarda işitme mekanizmalarının da etkilenebileceğini göstermiştir. Bu çalışmanın yazarları, kronik hipokseminin işitme sistemi üzerindeki olumsuz etkilerini önlemek için KOAH hastalarında hastalığın erken evrelerinde gerekli önlemlerin alınması gerektiğini savunmaktadır.

Anahtar Kelimeler: Kronik obstrüktif akciğer hastalığı, hipoksi, işitsel yolak, odyometri, otoakustik emisyonlar spontan

Address for Correspondence: Tolga ERSÖZLÜ MD, Tekirdağ Namık Kemal University Faculty of Medicine, Department of Otorhinolaryngology, Head and Neck Surgery, Tekirdağ, Turkey
Phone: +90 533 571 16 53 **E-mail:** tersozlu@nku.edu.tr **ORCID ID:** orcid.org/0000-0001-8629-6022
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INTRODUCTION

Hypoxemia is a decrease in the partial pressure of oxygen (pO2) in the blood. It is observed in almost all known pulmonary diseases. pO2 is used to demonstrate pulmonary functions. On the other hand, hypoxia is a decrease in tissue oxygenation and results from hypoxemia. This hypoxic state, which occurs in tissues, triggers various events over time, causing destruction in cells[1].

Hypoxemia is known to have a significant negative effect on the central and peripheral nervous system by disrupting the hemodynamic and biochemical regulatory mechanisms. As a result of chronically low oxygen saturation in the blood, a variety of pathologies, such as a decrease in adenosine levels and disruptions in the neurotransmitter cycle of neurons, are observed in the central nervous system[2-4]. In addition, it has been suggested that endothelial dysfunction occurs in the microcirculation due to recurrent oxidative stress secondary to chronic hypoxemia, and it disrupts the blood supply to peripheral nerves, causing inevitable damage to neuronal function[5,6].

The auditory system consists of two mechanisms, the peripheral and central hearing, and is extremely sensitive to the effects of hypoxemia. For a normal auditory function, the transmission from the cochlea to the auditory cortex must be complete, and sufficient and continuous oxygen support must be provided to this nervous system[7].

Chronic obstructive pulmonary disease (COPD) is a disease that causes a decrease in expiratory flow rates. Moreover, patients have irregular ventilation that causes arterial hypoxemia and hypercapnia. As a result of this chronic hypoxemia, oxygen deprivation occurs in tissues and various comorbidities may develop. These abnormalities can be detected by pulmonary function tests (PFT) and arterial blood gases[8].

Therefore, the present study was conducted considering that chronic hypoxemia observed in COPD patients might be harmful to the transduction and conduction mechanisms in the auditory pathways. This study aimed to examine the effect of chronic hypoxemia on auditory functions in COPD patients and to discuss the results within the context of the current literature.

MATERIALS AND METHODS

The study was conducted in a tertiary research hospital by obtaining the approval Tekirdağ Namık Kemal University of the Local Ethics Committee (2015/118/11/01) and all procedures were in accordance with the ethical standards of responsible committee on human experimentation (institutional and national) and with the 1975 Declaration of Helsinki, as revised in 2008. Additional informed consent was obtained from all patients whose data were included in this article. This study included patients with COPD who underwent a PFT with the pre-diagnosis of probable dyspnea between November 2015 and February 2016. PFT analyses were carried out in the PFT laboratory in the thoracic diseases department of the same faculty.

Comprehensive otorhinolaryngological examinations of all participants were performed (with 0 and 70-degree endoscopes). Those with conditions known to have a negative effect on the mechanism of hearing (patients with acute or chronic otorhinolaryngological disorders, otitis media, eustachian tube dysfunctions, Sino-nasal disorders, etc.), as well as chronic systemic diseases such as hypertension, diabetes mellitus, and chronic heart disease, which are known to be associated with microvascular circulation disorders, were not included in the study. Four study groups and a control group were created in the study.

Each study group was determined based on forced expiratory volume (FEV1) and FEV1/forced vital capacity (FVC) ratio. The first, second, third and fourth groups consisted of patients with Stage 1 (mild) FEV1/FVC <70% and FEV1 ≥80%, Stage 2 (moderate) FEV1/FVC 70% and 50%≤ FEV1 80%, Stage 3 (severe) FEV1/FVC 70% and 30%≤ FEV1 50%, and Stage 4 (very severe) FEV1/FVC 70% and FEV1 <30% COPD, respectively (Table 1). Each group included 15 patients. The control group consisted of 30 patients with an FEV1/FVC ratio of >70%.

All patients underwent pure tone audiometry and otoacoustic emission (OAE) test. Pure tone audiometry was performed by the same audiologist using a two-channel audiometry (Interaccoustics, A/S, Denmark) in accordance with the international standards. Patients with a hearing threshold higher than 25 dBHL were considered to have hearing loss. The severity of hearing loss was categorized as mild (26 to 40 dBHL), moderate (41 to 55 dBHL), moderate-severe (56 to 70 dBHL), severe (71 to 90 dBHL) and profound (91+ dBHL). The Madsen AccuScreen TE device (Otometrics, Denmark) was used for the OAE test.Normal outer hair cell function was determined

Table 1. GOLD Criteria for severity of airflow obstruction in COPD

GOLD stage	Severity	Spirometry
I	Mild	FEV1/FVC <0.7 and FEV1 ≥80% predicted
II	Moderate	FEV1/FVC <0.7 and FEV1 ≥50% but <80% predicted
III	Severe	FEV1/FVC <0.7 and FEV1 ≥30% but <50% predicted
IV	Very severe	FEV1/FVC <0.7 and FEV1<30%

GOLD: Global Initiative for chronic obstructive lung disease, COPD: Chronic obstructive pulmonary disease, FEV1: Forced expiratory volume, FVC: Forced vital capacity

according to the "Passed/Clear response" result. Those with the result of "refer/unclear response" were retested. Those with a permanent "refer/unclear response" result was considered to have sensorineural hearing loss.

Statistical Analysis

Statistical data analysis was performed using Statistical Package for the Social Sciences (SPSS) for Windows, version 17 (SPSS Inc., Chicago, IL, USA). The chi-square (χ^2) test was used to compare qualitative data. The Kruskal-Wallis test was employed to compare the groups. Dunn's multiple comparison test and the Tukey's range test were used to compare the subgroups. Results were considered significant for $p<0.05$.

RESULTS

The ages of the 90 participants (60 COPD patients and 30 controls) ranged between 45 and 54 (mean age=46.6) years. Forty-six (51.1%) females and 44 (48.9%) males were included.

There was no statistically significant difference between the groups in terms of demographic characteristics (p=0.765).

In addition, there was no statistically significant difference between male and female genders in terms of hearing loss and OAE results (p=0.976, p=0.464, p<0.05).

Based on FEV1 (% expected) value according to the Global Initiative for COPD, the study groups consisted of patients with Stage 1 (mild) COPD FEV1/FVC <70% and FEV1 ≥80%, Stage 2 (moderate) COPD FEV1/FVC 70% and 50%≤ FEV1 80%, Stage 3 (severe) COPD FEV1/FVC 70% and 30%≤ FEV1 50%, and Stage 4 (very severe) COPD FEV1/FVC 70% and FEV1 30%. Each group consisted of 15 patients. The control group consisted of 30 patients with an FEV1/FVC ratio of >70%.

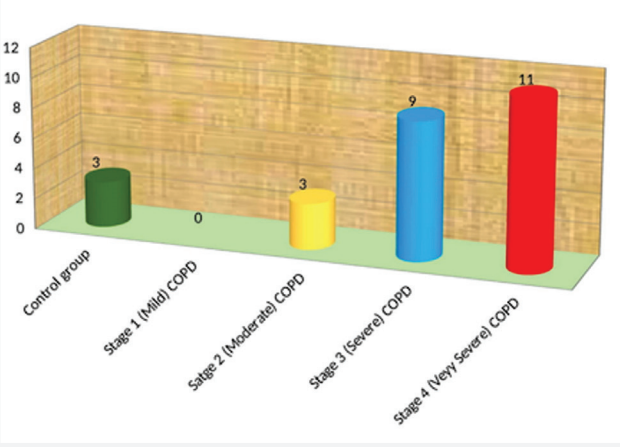

Figure 1. Number of patients with more than 25 dB sensorineural hearing loss in groups

COPD: Chronic obstructive pulmonary disease

The mean ages for the COPD groups including 15 patients with Stage 1 (mild), 15 patients with Stage 2 (moderate), 15 patients with Stage 3 (severe) and 15 patients with Stage 4 (very severe) COPD and the control group including 30 patients were 45.86±8.6, 46.20±8.7, 44.60±8.1, 45.13±7.6, and 45.56±7.7, respectively.

Three of the 30 patients in the control group, none of the 15 patients with Stage 1 (mild) COPD, 3 of the 15 patients with Stage 2 (moderate) COPD, 9 of the 15 patients with Stage 3 (severe) COPD, and 11 of the 15 patients with Stage 4 (very severe) COPD had sensorineural hearing loss (the hearing levels were greater than 25 dB) (Figure 1).

The mean hearing thresholds for the control group and mild, moderate, severe and very severe COPD groups were15.73±6.0

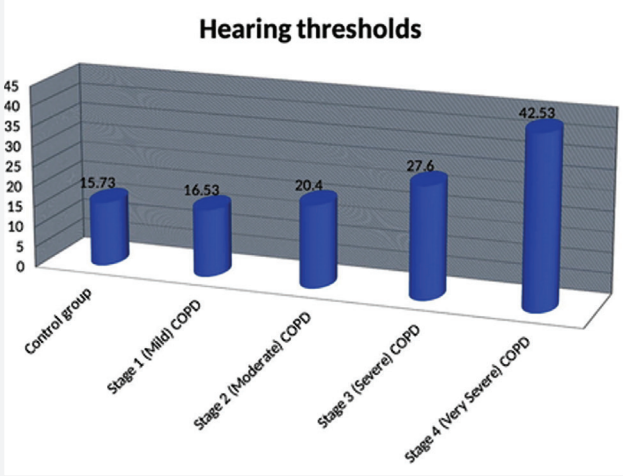

Figure 2. Hearing values in groups

COPD: Chronic obstructive pulmonary disease

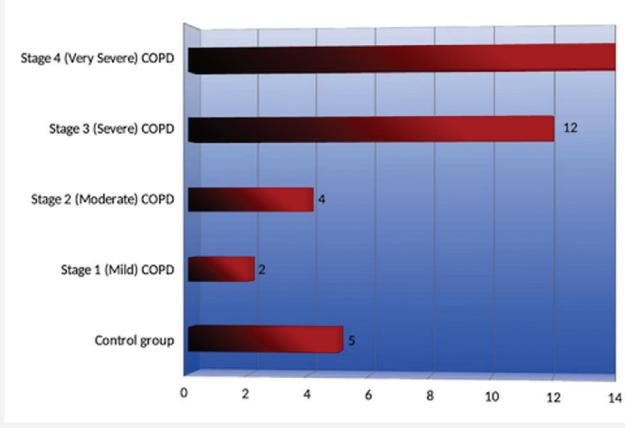

Figure 3. Number of patients who failed the auto acoustic emission test in groups

COPD: Chronic obstructive pulmonary disease

dBHL, 16.53±5.2 dBHL, 20.4±8.9 dBHL, 27.6±9.7 dBHL, and 42.53±17.4 dBHL, respectively (Figure 2). According to the audiological evaluation, the results of the patients in the control group and the patients in the mild and moderate COPD groups were below the expected values, while the results of the patients in the severe and very severe COPD groups were higher than expected. There was a statistically significant difference compared to the control group ($p<0.001$).

In the OAE test results, 5 of the 30 patients in the control group failed in the OAE test, while 2 of the 15 patients with Stage 1 (mild) COPD, 4 of the 15 patients with Stage 2 (moderate) COPD, 12 of the 15 patients with Stage 3 (severe) COPD and 14 of the 15 patients with Stage 4 (very severe) COPD failed in the OAE test (Figure 3).

According to the OAE results of the patients, the results of the patients in the control group and the patients in the mild and moderate COPD groups were below the expected values, while the results of the patients in the severe and very severe COPD groups were higher than expected. There was a statistically significant difference compared to the control group ($p<0.001$).

DISCUSSION

COPD is a multisystemic disease with comorbidities[9]. COPD has significant effects on cognitive functions and verbal memory, especially on the central nervous system[10].

The findings and comorbidities of COPD are the results of systemic "spread" caused by inflammation in the lungs triggered by the chronic hypoxic state of COPD patients and occur by resulting in multiple organ dysfunction[11].

COPD-induced hypoxemia falls into one of two hypoxemic states described in the literature. The first one is characterized by short-interval and recurrent attacks of hypoxemia, as observed in obstructive sleep apnea syndrome patients. The second hypoxemic state is a chronic persistent hypoxemic state, as observed in those living at higher altitudes or patients with COPD. This chronic decrease in oxygen saturation causes oxygen deprivation in tissues over time, resulting in hypoxia and initiating end-organ damage. Thus, it triggers the systemic effects of COPD. There is evidence of systemic inflammation measured by an increase in circulating cytokines, chemokines and acute phase proteins or abnormalities of circulating cells in COPD patients, especially when the disease is severe and during exacerbations[12]. Both systemic inflammation and chronic hypoxia create mechanisms that trigger each other, causing a decrease in blood supply to the peripheral nerve and preventing oxygen supply.

The results of the study conducted in the light of these data indicate that Stage 3 (severe) and Stage 4 (very severe) COPD can result in sensorineural hearing loss. This result is consistent with the previous studies in the literature reporting that COPD-induced hypoxemia may have a negative effect on auditory function[13,14].

In the literature review performed to evaluate the relationship between COPD and the affection of the auditory system, there were studies showing that the arterial transport mechanism in the inner ear was closely associated with the cochlear oxygen reserve and that the decrease in oxygen level greatly affected the inner ear cells[15]. In addition, most studies have shown that reductions in oxygen supply to the cochlea directly affect the functions of the cochlea[16,17]. In addition, decreases have been observed in distortion product OAEs (DPOAEs) and endocochlear potential values based on hypoxia[18,19].

Various studies have found changes in auditory brainstem responses and cortical auditory evoked potentials of experimental animals exposed to hypoxia[20]. The results of the present study showed that patients with Stage 1 (mild) and Stage 2 (moderate) COPD did not have impaired auditory functions, while patients with both Stage 3 (severe) and Stage 4 (very severe) COPD had sensorineural hearing loss. Although our study yielded such a result, there is a need for further studies with greater participation to define the link between FEV1 value and severity of hearing loss in COPD patients.

Study Limitations

The presence of additional risk factors (smoking, diabetes type 2, old age) that negatively affect the auditory pathway in terms of hypoxia in COPD patients can be considered as a limitation of the study. When we use these additional risk factors as exclusion criteria, we would like to state that, as mentioned in the literature, we had to continue working with a population that was 20% of the COPD patient group who did not smoke[21].

CONCLUSION

In conclusion, there is convincing evidence that the risk of advanced dysfunction in the vascular and nervous structures of the body is inevitable for patients suffering from chronic hypoxemia secondary to COPD. The results of the present study have showed that auditory mechanisms may also be affected in patients with severe and very severe COPD. The authors of this study argue that necessary measures should be taken in the early stages of the disease for COPD patients to prevent the negative effects of chronic hypoxemia on the auditory system.

Ethics

Ethics Committee Approval: The study were approved by the Tekirdağ Namık Kemal University of Local Ethics Committee (protocol number: 2015/118/11/01, date: 10/12/2015).

Informed Consent: Additional informed consent was obtained from all patients whose data were included in this article.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: M.D., Design: T.E., Data Collection or Processing: O.B.D., Analysis or Interpretation: T.E., Literature Search: T.E., M.D., Writing: O.B.D., T.E.

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

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ORIGINAL ARTICLE

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Clinicopathological Factors Determining the Pathological Response to Neoadjuvant Therapy in HER2 Positive Breast Cancer

HER2 Pozitif Meme Kanserinde Neoadjuvan Tedaviye Patolojik Yanıtı Belirleyen Klinikopatolojik Faktörler

Abdullah Evren YETİŞİR[1], Semra PAYDAŞ[2]

[1]*Adana City Training and Research Hospital, Clinic of Medical Oncology, Adana, Turkey*
[2]*Çukurova University Faculty of Medicine, Department of Oncology, Adana, Turkey*

ABSTRACT

Aim: In our study, we aimed to determine the clinicopathological factors affecting the pathological response after neoadjuvant chemotherapy in HER2 positive breast cancer.

Materials and Methods: A total of 54 HER2 expression positive cases were included in this study. Neoadjuvant chemotherapy regimen containing trastuzumab was applied to all patients. Patients' age, gender, disease stage, tumor size and lymph node status, estrogen and progesterone receptor status, Ki-67 proliferation index, tumor grade, menopausal status and pathological complete response status after neoadjuvant therapy, neoadjuvant treatment regimen and the relationship between the tumor and histological subtype were examined.

Results: Grade III tumor, hormone receptor negativity, high Ki-67 score, and the presence of T3 or T4 tumor were found to be better associated with pathological complete response (p=0.036, p=0.033, p=0.021, p=0.048, respectively). High tumor grade, hormone receptor negativity and high Ki-67 score were found as independent risk factors determining pathological complete response (p=0.043, p=0.047, p=0.035, respectively).

Conclusion: In this series of 54 cases with HER2 positive breast cancer, the parameters determining pathological complete response after neoadjuvant treatment are high Ki-67 proliferation index, grade III tumor and hormone receptor negativity.

Keywords: Breast cancer, HER2, neoadjuvant, pathological complete response

ÖZ

Amaç: Çalışmamızda HER2 pozitif meme kanserinde neoadjuvan kemoterapi sonrası patolojik yanıtı etkileyen klinikopatolojik faktörleri saptamayı amaçladık.

Gereç ve Yöntem: Bu çalışmaya HER2 ekspresyonu pozitif toplam 54 olgu dahil edildi. Hastaların tamamına trastuzumab içeren neoadjuvan kemoterapi rejimi uygulandı. Hastaların yaşı, cinsiyeti, hastalığın evresi, tümör boyutu ve lenf nodu durumu, östrojen ve progesteron reseptör durumu, Ki-67 proliferasyon indeksi, tümörün grade'i, menopoz durumu ve neoadjuvan tedavi sonrası patolojik tam yanıt durumu, neoadjuvan tedavi rejimi ve tümörün histolojik alt tipi ile arasındaki ilişki incelendi.

Bulgular: Grade III tümör, hormon reseptör negatifliği, Ki-67 skor yüksekliği, T3 veya T4 tümör varlığı daha iyi patolojik tam yanıt ile ilişkili bulundu (sırasıyla p=0,036, p=0,033, p=0,021, p=0,048). Yüksek tümör grade'i, hormon reseptör negatifliği ve yüksek Ki-67 skoru patolojik tam yanıtı belirleyen bağımsız risk faktörleri olarak saptandı (sırasıyla p=0,043, p=0,047, p=0,035).

Sonuç: HER2 pozitif meme kanserli 54 olguluk bu seride neoadjuvan tedavi sonrası patolojik tam yanıtı belirleyen parametreler yüksek Ki-67 proliferasyon indeksi, grade III tümör varlığı ve hormon reseptör negatifliğidir.

Anahtar Kelimeler: Meme kanseri, HER2, neoadjuvan, patolojik tam yanıt

Address for Correspondence: Abdullah Evren YETİŞİR MD, Adana City Training and Research Hospital, Clinic of Medical Oncology, Adana, Turkey
Phone: +90 555 682 86 25 **E-mail:** evrenyetisir@hotmail.com **ORCID ID:** orcid.org/0000-0001-7214-8184
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INTRODUCTION

Breast cancer is the most common cancer seen in women. According to Globocan 2020, 23.9% of cancers seen in women in our country are breast cancer. Breast cancer is the second most common cause of cancer mortality in our country and in the USA[1,2]. Neoadjuvant treatment of breast cancer refers to the systemic treatment of the tumor before surgery. In this way, by shrinking the tumor, breast-conserving surgery can be performed instead of mastectomy, and better cosmetic results can be obtained, and lymphedema that may develop after surgery can be prevented[3,4]. Another important advantage of neoadjuvant therapy is that therapeutic efficacy can be directly observed[5]. It also provides the opportunity for personalized treatment strategies and drug development[6]. Human epidermal growth factor receptor 2 (HER2) is from the family of epidermal growth factor receptors that play a critical role in the activation of subcellular signal transduction pathways which control epithelial cell growth and differentiation[7,8]. Amplification or overexpression of the HER2 oncogene is present in approximately 15% of invasive breast cancers[9]. Since the presence of HER2 expression is a predictive factor in breast cancer, HER2 expression status should be investigated at the time of diagnosis in breast cancer[10]. In this way, agents targeting HER2 receptors can be used in adjuvant or neoadjuvant therapy[11-13]. To determine the response after neoadjuvant therapy, pathological evaluation of the primary tumor and axillary lymph node is performed, except for negative sentinel lymph node before treatment. The absence of breast and axillary tumors in surgical material indicates pathological complete response (pCR) and is associated with better survival[14,15]. Even if HER2-targeting agents are not used in neoadjuvant therapy in HER2 positive breast cancers, they have better pathological response rates than HER2-negative patients[16,17]. Obtaining pCR after the completion of neoadjuvant therapy and surgical resection is associated with improved disease-free survival. This correlation is dependent on the molecular subtype and is evident in patients with triple negative and HER2 positive breast cancer[5].

In our study, we aimed to determine the factors affecting the pathological response after neoadjuvant chemotherapy in HER2 positive breast cancer.

MATERIALS AND METHODS

From a total of 114 stage II and stage III breast cancer women with axillary lymph node involvement, who received neoadjuvant chemotherapy, 54 patients with HER2 expression positive were included. CerbB2 status was determined by immunohistochemical method from the biopsy material of the patients before neoadjuvant chemotherapy. Patients with cerbB2 negative status and 1+ were considered HER2 negative. HER2 expression was evaluated by fluorescent *in situ* hybridization method (FISH) from the tissues of patients with cerbB2 status of 2++, and those who were positive were considered HER2 positive. Patients with cerbB2 status of 3+++ were considered HER2 positive. Neoadjuvant chemotherapy regimen containing trastuzumab was given to all patients who were considered HER2 positive. Those with estrogen or progesterone receptor levels of ≥1% were considered hormone receptor positive, and those with both <1% were considered hormone receptor negative. Stage, tumor size and lymph node evaluation (TN) according to the American Joint Committee on Cancer (AJCC) TNM Staging Classification for breast cancer 8th edition staging system including age, gender, tumor size, lymph node positivity and metastasis status of the patients and estrogen progesterone receptor status, Ki-67 proliferation index, tumor grade, menopausal status and pCR status after neoadjuvant treatment, neoadjuvant treatment regimen that was given, and histological subtype of the tumor were evaluated (Table 1).

Statistical Analysis

After testing the conformity of the data to the normal distribution, those showing normal distribution of continuous variables were analyzed with the t-test, and those that did not show normal distribution were analyzed with the Mann-Whitney U test. The χ^2 test was used in the analysis of categorical variables. All numerical data were expressed as mean values or ratios. For data that did not show normal distribution, comparisons between pre-post measurements were made using the Wilcoxon test.

Cox regression analysis was used to analyze univariate and multivariate data. Receiver operating characteristic (ROC) curve analysis was used to determine the Ki-67 cut-off value. Results were expressed as mean±standard deviation, median (lower limit and upper limit), number and percentage, and the value of $p<0.05$ was considered statistically significant. Statistical analysis of the data was performed using Statistical Package for the Social Sciences 21.0 software.

This article was approved by Çukurova University Faculty of Medicine Non-Invasive Clinical Research Ethics Committee with the decision number of 54 dated 10.06.2016.

RESULTS

Patient Characteristics

All of the patients participating in the study were women. A total of 114 patients who received neoadjuvant chemotherapy were evaluated. Twenty-eight (24.6%) patients were cerbB2 negative, 4 (3.5%) patients were cerbB2 1+, 35 (30.7%) patients were cerbB2 2++ and 47 (41.2%) patients were cerbB2 3+++.

Table 1. Clinicopathological features of the patients (n:54)

	Number of patients n (%)
Age	
≤50 years	25 (46.3)
>50 years	29 (53.7)
Complete pathological response	
Yes	30 (55.6)
No	24 (44.4)
Menopausal status	
Premenopausal	25 (46.3)
Postmenopausal	29 (53.7)
ER status	
Positive	38 (70.4)
Negative	16 (29.6)
PR status	
Positive	23 (42.6)
Negative	31 (57.4)
Hormone receptor negative	
Yes	16 (29.6)
No	38 (70.4)
CerbB2 status	
2++	7 (13)
3+++	47 (87)
Ki-67 status (%)	
0-10	7 (13)
11-30	17 (31.5)
31-50	10 (18.5)
>50	20 (37)
Tumor grade	
Grade I	1 (1.8)
Grade II	25 (46.3)
Grade III	28 (51.9)
T status	
T1	1 (1.9)
T2	21 (38.8)
T3	7 (13)
T4	25 (46.3)
N status	
N1	9 (16.6)
N2	34 (63)
N3	11 (20.4)
Stage	
Stage II	7 (13)
Stage III	47 (87)
Histological subtype	
IDC	40 (74.1)
ILC	14 (25.9)
Chemotherapy protocol	
AC/P+T	23 (42.6)
TCH	25 (46.3)
Other	6 (11.1)

ER: Estrogen receptor, PR: Progesterone receptor, IDC: Invasive ductal carcinoma, ILC: Invasive lobular carcinoma, AC/P+T: Doxorubicin and cyclophosphamide/paclitaxel+trastuzumab, TCH: Docetaxel, carboplatin and trastuzumab

HER2 expression was detected by FISH method in 7 (6%) of 35 patients with CerbB2 2++. A total of 54 (47.3%) HER2 positive patients were evaluated. The median age of the patients included in the study was 52 years (age range: 34-76 years). The median Ki-67 score was 54% (range 5-90%), 20 (37%) patients had a Ki-67 score >50%, and 16 (29.6%) patients were hormone receptor negative. Approximately half of the patients had grade III tumors (n=28, 51.9%) and 29 (53.7%) patients were in the postmenopausal period. While docetaxel, carboplatin, trastuzumab (TCH) chemotherapy protocol was applied to 25 (46.3%) patients, dose-intensive Doxorubicin and cyclophosphamide/paclitaxel+trastuzumab chemotherapy protocol was applied to 23 (42.6%) patients. Reimbursement for pertuzumab was not available in our country at the time when the patient data were collected. Patients were offered this treatment option, but no patient accepted. Fourty-seven (87%) of the patients had stage III disease and approximately half had T4 tumor (n=25, 46.3%) while two-thirds had N2 (n=34, 63%) disease. When the histological subtypes of the tumors were examined, invasive ductal carcinoma was found in 40 (74.1%) patients.

Table 2. Univariate analysis for complete pathological response

Variable	95% CI	HR	p value
Age	0,812-1,891	1,358	0.215
≤50 years - >50 years			
Menopausal status			
Premenopausal-			
Postmenopausal			0.348
ER status			
Positive-Negative	0,785-1,982	1,485	0.129
PR status			
Positive-Negative	0,914-1,715	1,286	0.132
Hormone receptor negative			
Yes-No	0,658-2,152	1,872	0.033
cerbB2 status			
2++ - 3+++	0,751-2,048	1,463	0.654
Tumor grade			
Grade II - Grade III	1,219-2,652	2,159	0.036
T status			
T1 and T2 - T3 and T4	0,955-1,441	1,186	0.048
N status			
<N3 - N3	0,853-2,125	1,543	0.086
Stage			
Stage II - stage III	0,715-2,037	1,422	0.732
Histological subtype			
IDC - ILC	0,512-2,214	1,725	1.142
Chemotherapy protocol			
AC/P+T - TCH	0,689-2,411	1,642	0.865

HR: Hazard ratio, ER: Estrogen receptor, PR: Progesterone receptor, IDC: Invasive ductal carcinoma, ILC: Invasive lobular carcinoma, AC/P+T: Doxorubicin and cyclophosphamide/paclitaxel+trastuzumab, TCH: Docetaxel, carboplatinum and trastuzumab

Relationship Between Pathological Response and Clinicopathological Data

pCR was obtained in 30 (55.6%) of 54 patients. Clinicopathological data of the patients are shown in Table 1.

When the relationship between pCR and clinicopathological data was examined, no correlation was found among patients' age, menopausal status, estrogen or progesterone receptor positivity, cerbB2 positivity, neoadjuvant chemotherapy protocols, N status and disease stage according to the TNM staging system, and histological subtype of the tumor (p>0.05). Higher rate of pCR was detected in the presence of grade III tumor, hormone receptor negativity, high Ki-67 score, and T3 or T4 tumors (p=0.036, p=0.033, p=0.021 and p=0.048, respectively) (Table 2). In the multivariate analysis performed to determine whether the variables associated with pCR were an independent risk factor, the presence of high tumor grade, negative hormone receptor and high Ki-67 score were found to be independent risk factors determining pCR after neoadjuvant therapy in HER2 positive breast cancer patients (p=0.043, p=0.047, p=0.035, respectively) (Table 3).

The most sensitive and specific values for study variables were determined using ROC curve analysis: The cut-off value for Ki-67 was 27.5% (Figure 1).

Table 3. Multivariate analysis for complete pathological response

Variable	95% CI	HR	p value
Hormone receptor			
Negative			
Yes - No	0.758-2,214	1,758	0.047
Tumor grade			
Grade II - Grade III	1.325-2,712	2,321	0.043
T status			
T1 and T2 - T3 and T4	0,842-1,683	1,385	0.075
HR: Hazard ratio, CI: Confidence interval			

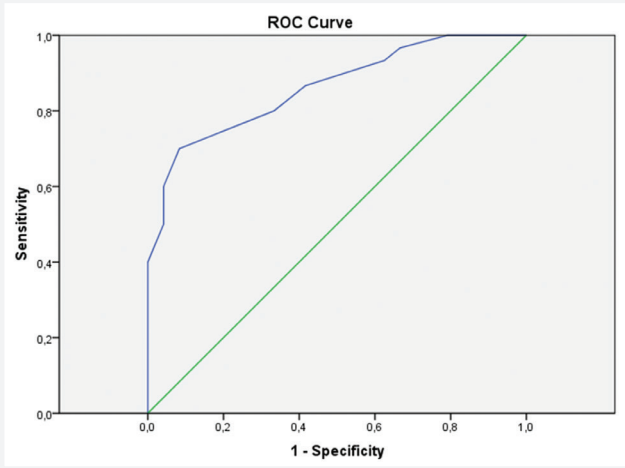

Figure 1. ROC analysis and AUC for Ki-67 sensitivity and specificity. The calculated area under the curve (AUC) is 0.861

ROC: Receiver operating characteristic

DISCUSSION

In our study, we aimed to investigate the factors affecting pCR in patients with HER2-positive breast cancer, and we found that hormone receptor negative, high Ki-67 score and the presence of high-grade tumor were independent risk factors affecting pCR.

Cortazar et al.[16] evaluated 12 international studies on neoadjuvant therapy. They found that HER2 positive patient group had higher pCR than those with hormone receptor negative. In the study of Untch et al.[18], although a higher pCR was shown in hormone receptor negative patients, the hormone receptor status was not statistically significant other than survival. In our study, we found that the hormone receptor negative group had a higher rate of pCR, and we revealed that hormone receptor negativeness was an independent risk factor determining pCR alone in patients with HER2-positive breast cancer (HR: 1,758, 95% CI: 0.758-2,214).

In another study by Cortazar and Geyer[19], it was stated that pCR was lower with neoadjuvant therapy in patients with low-grade tumors. However, it was emphasized in the study that this group was a hormone receptor positive group. In the study of Jarzab et al.[20], tumor grade, Ki-67 and estrogen, and progesterone receptor negativity were determined as pCR-related tumor parameters. The highest chance of pCR was observed in patients with high grade tumor and Ki-67 ≥20%. Tumor grade and estrogen receptor status were predictive for pCR independent of other analyzed parameters. In the study of Spring et al.[21], it was reported that higher pCR rates were observed in patients with grade 3 tumors. In the study of Karatas et al.[22] in our country, no significant relationship was found between pCR and T status, but a significant relationship was found with grade. In our study, we found that a higher rate of pCR was obtained with neoadjuvant therapy in high-grade breast cancer patients independent of hormone receptor status as in the hormone receptor negative group, and tumor grade was an independent risk factor, similar to hormone receptor negativity [hazard ratio (HR): 2,321, 95% confidence interval (CI): 1,325-2,712].

In the study of Silva et al.[23], it was shown that patients with high Ki-67 proliferation index had a better response to neoadjuvant chemotherapy and had a higher rate of clinical complete response. In this study, the cut-off value for Ki-67 was taken as 14% (p=0.005). In the study, different cut-off values in Ki-67 expression were also examined and it was found that with increasing cut-off value for the predictive test, its

sensitivity decreased and its specificity increased. In our study, we showed that a Ki-67 proliferation index higher than 27.5% would provide a higher rate of pCR after neoadjuvant therapy, and we found it to be an independent risk factor.

In the study of Untch et al.[24], in which they evaluated pCR with neoadjuvant therapy in HER2 positive breast cancer patients, no difference was found between patients with tumors larger than 4 cm and those with tumors smaller than 4 cm. In our study, although there was a statistically significant difference in univariate analysis between patients with T1 or T2 (≤5 cm) tumors and patients with T3 or T4 (>5 cm) tumors for pCR, it was not found to be an independent risk factor in multivariate analysis.

Study Limitations

The limitations of our study are the lack of pertuzumab use and the small number of patients. Further studies with more patients treated with new targeted agents are needed.

CONCLUSION

Factors determining pCR after neoadjuvant therapy in HER2 positive breast cancer patients are Ki-67 proliferation index, tumor grade and hormone receptor negativity. Longer disease-free survival can be achieved by obtaining pCR with ideal neoadjuvant therapy in selected patient groups.

Ethics

Ethics Committee Approval: This article was approved by Çukurova University Faculty of Medicine Non-Invasive Clinical Research Ethics Committee with the decision number of 54 dated 10.06.2016.

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: A.E.Y., Concept: A.E.Y., S.P., Design: A.E.Y., S.P., Data Collection or Processing: A.E.Y., S.P., Analysis or Interpretation: A.E.Y., S.P., Literature Search: A.E.Y., S.P., Writing: A.E.Y., S.P.

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

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The Relationship Between Frailty and Treatment Compliance in Diabetic and Geriatric Patients Using Insulin

Diabetes Mellitus Tanılı İnsülin Kullanan Geriatrik Hastalarda Tedaviye Uyum ve Kırılganlık Arasındaki İlişki

Imantay SHAUYET[1], Sema BASAT[1], Gamze GEYİK YÜNOĞLU[1], Rıdvan SİVRİTEPE[2], Selda ÇAKIN ÜNNÜ[1], Mustafa Behçet DEMİRBAŞ[1], Mevlüt KIYAK[1], Okcan BASAT[3]

[1]*University of Health Sciences Turkey, Ümraniye Training and Research Hospital, Clinic of Internal Diseases, İstanbul, Turkey*
[2]*Beykoz State Hospital, Clinic of Internal Diseases, İstanbul, Turkey*
[3]*University of Health Sciences Turkey, Gaziosmanpaşa Taksim Training and Research Hospital, Clinic of Family Medicine, İstanbul, Turkey*

ÖZ

Aim: Understanding and detecting frailty makes convenience to healthcare providers for deciding on appropriate therapy and follow-up strategy. In this study, we tried to determine the relationship between compliance to therapy, hypoglycemia and frailty in the elderly diabetic patients under insulin therapy.

Materials and Methods: One hundred sixty-seven patients diagnosed with diabetes mellitus and under insulin therapy were recruited for the study. Criteria of frailty according to the Cardiovascular Health Study (CHS) were used to determine frailty. According to CHS, patients fulfilling three or more criteria out of five were regarded as frail elderly. Hypoglycemia rates and compliance to treatment were compared between the groups.

Results: 44.3% of our patients were regarded as frail elderly and the rate of hypoglycemia was higher in this group. A dignificant relationship between subgroups of frailty and insulin was detected. Frailty rate was 28.6% in the basal insulin therapy group, 37.1% in the basal-bolus therapy group and 40% in the premixed insulin group. In the frail elderly group, the rate of moderate hypoglycemia was significantly higher than in the non-frail group (40.2% vs 20%). Severe hypoglycemic episodes were observed more frequently in the frail elderly group than in the non-frail group (24.6% vs 12.7%).

Conclusion: Frailty increases the risk of moderate and severe hypoglycemia. Before planning and starting insulin therapy, frailty must be detected and taken into consideration.

Keywords: Diabetes mellitus, frailty, hypoglycemia, elderly

ABSTRACT

Amaç: Geriatrik hastalarda kırılganlığı anlamak ve tespit etmek, hastaları takip etmede ve uygulanacak tedavi seçiminde sağlık ekibine büyük kolaylık sağlayacaktır. Biz bu çalışmada diabetes mellitus tanılı insülin kullanan geriatrik hastalarda tedaviye uyum, hipoglisemi ve frailite arasındaki ilişkiyi belirlemeyi amaçladık.

Gereç ve Yöntem: Çalışmamıza diabetes mellitus tanılı insülin tedavisi kullanan 65 yaş ve üstü toplam 167 hasta alındı. Frailite değerlendirmesi için Kardiyovasküler Sağlık Çalışması'na göre frailite kriterleri kullanıldı. Buna göre 5 özellikten 3 ve daha fazlasını taşıyan hastalar kırılgan yaşlı olarak kabul edildi. Kırılgan hastalarda ise Kanada Sağlık ve Yaşlılık Çalışması'nda önerilen kırılganlık kriterleri ve puanlama kullanıldı.

Bulgular: Hastaların %44,3'ünde frailite olduğunu ve kırılgan olan hastaların hipoglisemi oranının daha yüksek olduğunu saptadık. Çalışmamızda kırılgan grupta daha fazla hipoglisemi olduğu gösterildi. Kırılganlık ile insülin grupları arasında anlamlı farklılık bulundu. Bazal insülin kullanan grupta frailite oranı %28,6, bazal bolus grubunda frailite oranı %37,1, karışım insülin kullanan grupta ise frailite oranı %40 ile daha yüksek saptandı. Orta derece hipoglisemi frailitesi olanlarda olmayanlara göre 2 kat daha yüksekti (%40,2-20). Ağır hipoglisemi kırılgan olmayan grupta %12,7 iken, kırılgan olan grupta %24,6 oranında saptandı.

Address for Correspondence: Rıdvan SİVRİTEPE MD, İstanbul Medipol University Faculty of Medicine, Pendik Hospital, Clinic of Internal Medicine, İstanbul, Turkey
Phone: +90 545 260 59 57 **E-mail:** dr.ridvansivritepe@gmail.com **ORCID ID:** orcid.org/0000-0003-0547-1883
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Sonuç: Frailite varlığının orta ve ağır hipoglisemi riskini belirgin şekilde artırdığını ve insülin tedavisi planlanıyorsa kırılganlığın tespit edilmesi ve tedavi düzenlenirken bu durumun mutlaka göz önüne alınması gerektiğini söyleyebiliriz.

Anahtar Kelimeler: Diabetes mellitus, kırılganlık, hipoglisemi, yaşlılık

INTRODUCTION

The population of our country has been reported as 83 million 154 thousand as of 2020. Looking at the population pyramid, it has a young population. However, as in the whole world, the elderly population is increasing rapidly in our country[1]. According to the Turkish Statistical Institute 2020 data, the elderly population constitutes 9.1% of the country[2]. The World Health Organization states that the number of geriatric people in the world will reach 1.2 billion by 2025[3]. Due to the increase in the geriatric population and the changing lifestyle, chronic diseases are increasing in our country. The increasing elderly population brings with it problems specific to the elderly. One of these problems is the concept of frailty. The American Medical Association used the word "frailty" to describe "the patient group that presents the most complex and challenging problems for healthcare professionals"[4]. While frailty is seen at a rate of 10-25% in people aged 65 and over, it increases to 30-45% in people aged 85 and over[5]. Understanding and knowing the frailty in the geriatric population will provide great convenience to the healthcare team in following up the patients and choosing the treatment to be applied. With this study, we wanted to address frailty and frailty criteria in the geriatric population, while addressing frailty in the diabetic elderly patient group, which is an important problem. Insulin therapy should be carefully planned, especially in the diabetic elderly. Therefore, correct classification of cases is important. Indices used in patient selection exclude the concept of frailty. In this study, we aimed to investigate the relationship between compliance to treatment and frailty (weakness, weight loss, slowness, decreased physical activity, decreased stamina and energy) in patients aged 65 and over using insulin and diagnosed with diabetes mellitus.

MATERIALS AND METHODS

Our study is an analytical cross-sectional study and was conducted with a total of 167 patients aged 65 years and older, who were diagnosed with diabetes mellitus and were using insulin therapy, who applied to Ümraniye Training and Research Hospital Internal Medicine outpatient clinics or were hospitalized for various complications and chronic diseases. Ethics committee approval was obtained from the of Ümraniye Training and Research Hospital Ethics Committee for the study (decision number: 56, date: 24.09.2014). An informed consent form was signed by all patients participating in the study. Geriatric patients aged 65 years and older with diabetes mellitus using insulin were included in the study. Patients with congenital disability and malignancy abd those who did not want to participate in the study were not included.

Frailty Assessment

For the assessment of frailty in the geriatric population, frailty criteria (fatigue, weight loss, slowness, decreased physical activity, decreased stamina and energy) according to the Cardiovascular Health Study (CHS) were used. According to the CHS criteria, patients with 3 or more of 5 characteristics were considered frail elderly. While scoring, frailty criteria and scoring recommended in the Canadian Health and Aging Study were used; classification was made as mild frailty (patients with 1 point), moderate frailty (patients with 2 points), and severe frailty (patients with 3 points).

Metabolic Parameters

Plasma glucose of all patients was measured by enzymatic test; HPLC method was used to measure glycosylated hemoglobin; total cholesterol, high-density lipoprotein cholesterol, calcium, phosphate and triglyceride concentration were measured by enzymatic colorimetric test; and creatinine was measured by the Jaffe' method [Hitachi 747 autoanalyzer (Mito, Ibaragi, Japan)]. Blood samples were taken from the patients between 08:00 and 10:00 on an empty stomach. Blood samples were collected into tubes with SST II, LH PST II and EDTA and analyzed simultaneously.

Statistical Analysis

IBM Statistical Package for the Social Sciences Statistics 22.0 IBM Software program was used for statistical analysis. While evaluating the study data, in addition to descriptive statistical methods (mean, standard deviation), the One-Way ANOVA test was used to compare the parameters with normal distribution between groups in the comparison of quantitative data, and the Tukey HSD test was used to determine the group that caused the difference. Chi-square test was used to compare qualitative data. Spearman correlation analysis was used to examine the relationships between parameters that did not conform to the normal distribution. Significance was evaluated at the $p<0.05$ level.

RESULTS

A total of 167 patients were evaluated. In the sample distribution, 92 (55.1%) of the cases were female and 75

(44.9%) male. The mean age of the cases was detected as 71.83±6.11 years. The majority of the participants were able to meet their bathroom, dressing and toilet needs. While fecal incontinence was present in 28.70% of the patients, it was absent in 71.30% of them. 79.60% of the participants could meet their food needs. The rate of those who could not meet their food needs was 19.80%, the rate of those who did with support was 0.60%. While hypoglycemia was present in 52.70% of the participants, it was absent in 47.30% of the participants. Of the patients participating in the study, 21.6% had heart failure, 83.2% had hypertension, 15% had cerebrovascular accident and dementia, and 17.4% had chronic renal failure. Of the patients using insulin, 41.9% were using basal insulin, 37.1% were using basal+bolus insulin and 2% were using mixed insulin. The number of patients using oral antidiabetic (OAD) was approximately 17% less than those who did not. When all cases were evaluated, the frailty rate was found to be 44.3%. The rate of participants without hypoglycemia was 47.3%. The rate of those with severe hypoglycemia was 16.80%. The proportion of participants with mild hypoglycemia was 8.40%. The total hypoglycemia rate was found to be 52.7%. In the analysis made between daily activities and frailty, a relationship was found between the form of activity and frailty in all activities.

When frailty was evaluated according to age groups, the frailty group was older than the non-frailty group. The comparison of frailty criteria and age in frail and non-frail groups is summarized in Table 1. Heart failure, cerebrovascular disease and dementia were detected more frequently in the frail patient group ($p<0.05$) (Table 2). While the rate of moderate hypoglycemia was 20% in non-frailty patients, this rate was 42.1% in frailty patients and while the rate of severe hypoglycemia was 12.7% in patients without frailty, this rate was found as 24.6% in patients with frailty. Moderate and severe hypoglycemia was 2 times higher in the frail group (Table 3). In the evaluation we made between the frailty groups and the insulin groups, there was 28.6% frailty in the basal insulin group, 37.1% in the basal-bolus group and 40% in the mixed insulin group. The frailty rate was found to be higher in patients using basal-bolus and mixed regimen insulin (Table 4). A significant correlation was found between the HbA1c groups and the insulin groups. While the rate of patients with HbA1c level above 10 was 18.6% in the group using basal insulin, it was 35.5% in the group using basal-bolus insulin and 14.3% in the group using mixed insulin. In the correlation analysis between frailty and age groups, hypoglycemia groups and HbA1c groups; a positive significant correlation was found between frailty and age groups and hypoglycemia.

Table 1. Comparison of frailty criteria and age in frail and non-frail groups

		Frail		Non-frail		p
		n	%	n	%	
Age group	60-70	55	50.5%	14	24.6%	
	70-80	44	40.4%	28	49.1%	
	>80	10	9.2%	15	26.3%	0.01
Bathing	None	4	3.7%	35	61.4%	
	Yes	105	96.3%	3	5.3%	0.01
	With support	0	0.0%	19	33.3%	
Dressing	None	4	3.6%	35	61.4%	
	Yes	106	96.4%	10	17.5%	0.01
	With support	0	0.0%	12	21.1%	
Using the toilet	None	5	4.5%	35	61.4%	
	Yes	105	95.5%	6	10.5%	0.01
	With support	0	0.0%	16	28.1%	
Stool	None	101	91.8%	18	31.6%	
	Yes	9	8.2%	39	68.4%	0.01
Food	None	4	3.6%	29	50.9%	
	Yes	106	96.4%	27	47.4%	0.01
	With support	0	0.0%	1	1.8%	
Incontinence	None	95	86.4%	12	21.1%	
	Yes	15	13.6%	45	78.9%	0.01

Table 2. Comparison of comorbid diseases and frailty conditions

		Frail		None-frail		p
		n	%	n	%	
Heart failure	None	94	85.5%	37	64.9%	
	Yes	16	14.5%	20	35.1%	0.002
Hypertension	None	21	19.1%	7	12.3%	
	Yes	89	80.9%	50	87.7%	0.264
Cerebrovascular event	None	103	93.6%	39	68.4%	
	Yes	7	6.4%	18	31.6%	0.01
Dementia	None	103	93.6%	39	68.4%	
	Yes	7	6.4%	18	31.6%	0.01
Chronic kidney disease	None	95	86.4%	43	75.4%	
	Yes	15	13.6%	14	24.6%	0.077

DISCUSSION

In this study, we found that the rate of frailty was 44.3% in patients over 65 years of age using insulin, and the rate of hypoglycemia was higher in patients with frailty compared to those without. We found a positive correlation between frailty and hypoglycemia. We showed that frailty increased as the degree of hypoglycemia increased. Life expectancy has been prolonged due to many important developments such as the success achieved in the treatment of diseases, the control of

Table 3. Comparison of hypoglycemia and HbA1c levels and frailty status

		Frail		None-frail		p
		n	%	n	%	
Hypoglycemia	Severe	14	12.7%	14	24.6%	
	Moderate	22	20.0%	24	42.1%	
	Mild	9	8.2%	5	8.8%	
	Normal	65	59.1%	14	24.6%	0.01
HbA1c	<6.5	11	10.0%	12	21.1%	
	6.5-8.5	46	41.8%	26	45.6%	
	8.5-10	24	21.8%	8	14.0%	
	>10	29	26.4%	11	19.3%	0.143

Table 4. Comparison of insulin regimes and frailty status

	Basal		Basal-bolus		Mix		p
	n	%	n	%	n	%	
Frail	50	71.4%	39	62.9%	21	60.0%	
None-frail	20	28.6%	23	37.1%	14	40.0%	0.01

infectious diseases, and the improvement of living conditions, and accordingly, the elderly population is increasing rapidly in our country as in the world. Malnutrition, incontinence, inactivity, delirium, depression, falls, dementia, pain, gait disorders, osteoporosis, frailty and pressure sores are the most common problems in the elderly. Frail elderly syndrome has many different definitions. The definition with the greatest consensus is increased susceptibility to external stresses due to age-related physiological reserves, neuromuscular, metabolic, and immune system dysfunction. The frailty rate increases with age and reaches 30% over the age of 90. Frail elderly people are the group that will benefit most from a comprehensive geriatric assessment. By identifying these elderly people and taking the necessary preventive and therapeutic measures in cooperation with their families, the morbidity and mortality rate can be reduced. In the literature, there are not enough studies on frailty in the diabetic elderly patient group. However, when the studies on frailty are examined, we see that these studies are carried out to find the most appropriate method for detecting frailty or to draw attention to frailty and to emphasize the inadequacies in this regard. Considering that studies on the life activities of the elderly are not sufficient in our country, in a study in which 400 patients aged 65 and over were included in a private hospital and their daily living activity indices were examined, the life activity performances of the patients between the ages of 65-70 years (52.5%), 70-75 years (41.2%), 75-80 years (43.8%) and 85-90 years (40%) were found to be "very good", only 50% of patients aged 90-95 were "bad" and it was concluded that a multidisciplinary approach should be taken towards the general care of elderly patients. According to the results of this study, it has been shown that care and treatment approaches change as age increases[6]. In our study, the frail group was found to be older than the non-fragile group. While this rate was 24% for the 60-70 age group, it was 49% for the 70-80 age group. We have shown that frailty increases with age and decreased physical activity is also related to increased frailty, and care and treatment approaches for the elderly population change. In a study it is stated that in elderly patients with diabetes for several years and complications, tight glycemic control reduces the risk of microvascular events, but does not reduce macrovascular events or mortality, better glycemic control is associated with less disability and better organ function, but observation of severe hypoglycemia in advanced age is a risk factor for intensive treatment[7-9]. Again, in the same study, OAD basal-bolus therapy was compared and it was noted that frailty that increases with age is associated with decreased functions and increased mortality, and that elderly diabetic patients tend to be more frail[7]. In people with multiple comorbidities, the high functional variability and limited life expectancy suggest that the benefit of intensive therapy is minimal. The decision to recommend tighter or milder glycemic control is made according to the degree of weakness, and it is noted that the patients with moderate or more severe weakness should not have strict glycemic control because they have a reduced life expectancy, and that when glycemic control is tried to be better in these patients, maybe less pronounced hyperglycemia episodes are seen, but serious episodes of hypoglycemia are more frequent[10]. It is recommended that insulin treatment regimens in the elderly be individualized and determined by considering the safety of the patient. It has been concluded that pre-mixed insulin analogues can be used after meals and are associated with better control compared to basal insulins, but they cause more frequent hypoglycemia and more weight loss[11,12]. Our study showed a positive relationship between frailty and age and hypoglycemia. While severe hypoglycemia was 39.3% in the 60-70 age group, this rate was 50% in the 70-80 age group. No significant relationship was found between age groups and hypoglycemia, but a significant relationship was found in the relationship analysis between frailty groups and insulin groups. The frailty rate was 28.6% in the basal insulin group, 37.1% in the basal bolus group, and it was higher with 40% in the mixed insulin group. We believe that frailty should be taken into account when administering insulin therapy in the elderly patient group and treatment regimens should be adjusted accordingly. The least hypoglycemia was seen in the basal treatment group, and hypoglycemia rates increased significantly in both the mixed and basal bolus groups. In a study by Kim et al.[13], it was stated that geriatric frailty is a syndrome and it affects treatment options. It was pointed out that the development and application of the statement-based frailty index in older adults should be determined and adjusted in pharmacoepidemiological studies, and that more research is needed. In a study by Sourial

et al.[14], it was aimed to evaluate 129 combinations of seven indicators of weakness (cognition, energy, mobility, mood, nutrition, physical activity, and strength) and to determine their predictive accuracy regarding age, gender, and number of chronic diseases. When 129 combinations of weakness indicators from the best model to the worst fit model are compared, it was found that the inclusion of multiple indicators generally improved performance and single markers gave the worst performance result. The results also suggest that frailty as a diagnostic tool, may play a more important role in the 80 and older age group. Although the "CHS model" and the "count model" are among the best models, it was concluded that both were not optimal. Compared to these two studies, our study used frailty criteria (fatigue, weight loss, slowness, decreased physical activity, decreased stamina and energy) according to the CHS to evaluate frailty and adherence to treatment in the geriatric population. In addition, hypoglycemia and other comorbid diseases were also questioned in the diabetic patient population over 65 years of age. While there was no significant relationship between frailty and hypertension and chronic kidney disease, a significant relationship was found between the presence of heart failure, cerebrovascular accident, dementia, hypoglycemia and frailty groups. The frailty rates were 35.1% in those with heart failure, 31.6% in those with cerebrovascular accident, and 31.6% in those with dementia. Both moderate and severe hypoglycemia were observed 2 times more in the frail group. Based on this, we can say that the presence of frailty significantly increases the risk of moderate and severe hypoglycemia, and if insulin therapy is planned, frailty should be detected and this should be taken into account when arranging the treatment. In another study, it was stated that elderly diabetic patients are constantly at high risk of geriatric syndrome, and are at risk for functional impairment, depression, falls, urinary incontinence, malnutrition, and cognitive impairment. It was concluded that the treatment of diabetic patients with geriatric syndrome should focus on a strategy to prevent exacerbation of frailty[15]. In a published guideline, it is recommended that the treatment targets of blood glucose in elderly people with diabetes be individually determined according to age, life expectancy, patient preference, presence of geriatric syndrome, depression, pain, falling, incontinence, polypharmacy, and cognitive impairment[16]. In a study by Gregg et al.[17], which included 6,588 residents aged 60 and over, it was reported that 32% of diabetic women and 15% of men with diabetes did not have the capacity to walk a quarter of a mile, do housework or climb stairs, this rate for those without diabetes was detected as 14% for women and 8% in men. In a study by Amer et al.[18] in which 104 elderly patients were included, similar to our study, it was seen that in physically frail cases the rate of associated chronic diseases, diabetes, ischemic heart diseases, hypertension, stroke, vision and hearing loss was significantly higher than others.

Study Limitations

Our study had some limitations. First, our study was a single-center cross-sectional analysis. Second, frailty was assessed at a single time point. Third, the presence of hypoglycemia was based on one-time measurement.

CONCLUSION

In conclusion, with this study, we wanted to draw attention to frailty in diabetic patients and to examine the effect of hypoglycemia on frailty. In all studies in the literature, it is not considered beneficial to try to keep blood glucose normoglycemic with intensive antidiabetic therapy in elderly diabetic patients[19]. Elderly diabetic patients have many risk factors such as a history of cardiovascular disease and other comorbid diseases. All these risk factors outweigh the benefits of intensive antiglycemic therapy in the elderly diabetic population. In the Action to Control Cardiovascular Risk in Diabetes study; it has been shown that giving intensive antiglycemic therapy and trying to reduce the HbA1c target below 6% increase the risk of death from other causes or cardiovascular causes[8]. Similarly, it was found in the Veterans Affair Diabetes Trial study that excessive glycemic control failed to have benefit in cardiovascular endpoints[20]. In our study, we found that frailty increases the risk of hypoglycemia in the geriatric patient population using insulin. We also showed that other comorbid conditions, such as dementia and Alzheimer, are also associated with increased frailty. This revealed the necessity of considering comorbid conditions apart from activities of daily life when assessing frailty. We observed that fragility increases with increasing age. Considering that the majority of the diabetic patient population will be over the age of 65 in the near future, frailty syndrome will have even more importance. This shows that for elderly diabetic patients, these geriatric syndromes should be evaluated and the treatment should be arranged with this perspective. In addition to the agent to be chosen in the treatment of diabetic patients, attention should be paid to the issue of hypoglycemia. Ultimately, frailty will be beneficial for health improvement only if it is reversible or if adverse outcomes can be changed or at least improved. If the use of insulin is absolutely necessary in elderly individuals, choosing the regimen after considering the frailty will ensure compliance with the treatment and protect the individuals from complications.

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Ethics

Ethics Committee Approval: The study were approved by the Ümraniye Training and Research Hospital Ethics Committee (decision number: 56, date: 24.09.2014).

Informed Consent: An informed consent form was signed by all patients participating in the study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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The Effects of Sodium Fluorescein Dyeing of Metastatic Brain Tumors on Surgical Outcomes Under Microsurgical Operation

Metastatik Beyin Tümörlerinin Mikrocerrahi Yönetiminde Sodyum Fluorescein Boyamanın Cerrahi Sonuçlara Etkileri

Tamer TUNÇKALE, Tezcan ÇALIŞKAN

Tekirdağ Namık Kemal University Faculty of Medicine, Department of Neurosurgery, Tekirdağ, Turkey

ABSTRACT

Aim: We investigated the reflection of tumor dyeing (staining), an auxiliary technique for the resection of metastases, which are the most prevalent group among brain tumors, via microsurgery.

Materials and Methods: Twenty one patients, who were operated under surgical white light, and 27 patients who were operated via sodium fluorescein (FL) dyeing (staining) due to metastatic brain tumors were evaluated retrospectively. The gross total resection (GTR) rates, surgical time, amount of blood loss, and the duration of hospital stay for both groups were compared. The contribution of FL dyeing (staining) to surgery was evaluated for the group with FL dyeing (staining).

Results: The study comprised of 48 patients in total. The median age of patients was 61.5 years (minimum: 20, maximum: 80), the average age was 59.1±11.8 years. There was no difference between the group with FL dyeing and the one without dyeing in terms of gender, age, tumor size, GTR rates and surgical time. Blood loss and duration of hospital stay in the FL used group was significantly less. In the group with FL dyeing (staining) (92.5%), this method contributed to the surgery by giving yellow highlights.

Conclusion: It has been found out that in the surgery of metastatic brain tumors, FL dyeing decreases the blood loss, shortens the surgical time, and aids in the differentiation of tumor glial tissue.

Keywords: Sodium fluorescein, microsurgery, metastatic brain tumors

ÖZ

Amaç: Beyin tümörleri içerisinde en sık görülen grup olan metastazların mikrocerrahi ile çıkartılmasında yardımcı bir teknik olan tümör boyamanın cerrahi sonuçlarımıza yansımasını araştırdık.

Gereç ve Yöntem: Metastatik beyin tümörü nedeni ile mikrocerrahi yöntem ile beyaz ışık altında opere edilen 21 hasta ve sodyum fluorescein (FL) boyama kullanılan 27 hasta retrospektif olarak değerlendirildi. Her iki grubun gross total rezeksiyon (GTR) oranları, cerrahi süreleri, kan kaybı miktarları, hastanede kalış süreleri karşılaştırıldı. FL kullanılan grupta boyamanın cerrahiye yaptığı katkı değerlendirildi.

Bulgular: Çalışmaya toplamda 48 hasta alındı. Hastaların medyan yaşı 61,5 (minimum: 20 maksimum: 80) yıl, ortalama yaşı 59,1±11,8 yıl idi. FL kullanılmayan ve kullanılan gruplarda cinsiyet, yaş, tümör büyüklüğü, GTR oranları ve cerrahi süre açısından fark yoktu. FL kullanılan grupta kan kaybı miktarları ve hastanede kalış süresi belirgin şekilde azdı. FL kullanılan grupta yirmi beş hastada (%92,5) FL sarı röfle vererek cerrahiye katkı sağlamıştır.

Sonuç: Metastatik beyin tümörlerinin cerrahisinde FL boyama kan kaybını ve cerrahi süreleri azaltmakta, tümör glial doku ayrımında cerraha yardımcı olmaktadır.

Anahtar Kelimeler: Sodyum fluorescein, mikrocerrahi, metastatik beyin tümörleri

Address for Correspondence: Tamer TUNÇKALE MD, Tekirdağ Namık Kemal University Faculty of Medicine, Department of Neurosurgery, Tekirdağ, Turkey
Phone: +90 505 373 71 03 **E-mail:** ttunckale@hotmail.com **ORCID ID:** orcid.org/0000-0002-4534-0864
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INTRODUCTION

Cerebral metastases are the most common lesions among all brain tumors and are more common than primary glial masses[1]. Cerebral metastases can be seen in 20-40% of patients with known cancer, while approximately 70% of these patients are symptomatic[2]. As a result of the success in the treatment of the primary disease in recent years, prolonged life expectancy, accessibility to imaging methods, and advances in the techniques of these methods have led to an increase in the incidence of brain metastases[3]. While life expectancy is expressed in months in many patients with brain metastases, it is known that surgery, whole-brain radiotherapy and chemotherapy treatment algorithm, respectively, are the treatment modalities that increase total survival the most in the recent period[4,5]. The aim of surgery, which is the first step of the treatment in these patients, is total resection[6]. The higher the amount of resection, the less the possibility of local recurrence[7]. Recently, many methods such as navigation systems, brain mapping, fluorescein (FL) staining, intraoperative ultrasound and magnetic resonance (MR) have been used to increase the surgical safety and the amount of resection in brain tumors[8,9]. Sodium FL, a FL salt, is a water-soluble organic dye that has been used safely in eye angios for a long time and accumulates in areas where the blood-brain barrier is disrupted[10]. Brain tumors cause contrast enhancement by disrupting the blood-brain barrier where they are located. FL has been used for many years in standard surgical microscopes that give off white light or with the naked eye at high doses of 15-20 mg/kg in order to distinguish tumor glial tissue in contrast-enhancing areas[11].

Side effects, up to anaphylaxis, have been avoided by using low doses of 3-4 mg/kg, thanks to filters that give a yellow highlight to FL at a wavelength of 560 nm, which have recently been installed on surgical microscopes[12]. There are studies showing that the use of FL in brain metastases increases the amount of resection[13,14]. We will also present the results, surgical differences and our experience in patients with brain metastases in which we used and did not use FL staining with a 560 nm filter microscope in surgery.

MATERIALS AND METHODS

In our clinic, 21 patients (group 1) who were operated under white light by two surgeons in 2015-2016 due to metastatic brain tumors and 27 patients (group 2) who were operated between 2017-2019 by performing FL staining were evaluated retrospectively.

Patients with significant contrast enhancement on MR images, aged between 34-75 years, diagnosed with metastatic brain tumor and who underwent surgery were included in the study. Cases that did not have contrast enhancement on MR images and for whom administering contrast agent was contraindicated were excluded from the study.

All patients were operated under general anesthesia. Group 1 patients were operated under white light with a standard surgical microscope. In group 2 patients, 3 mg/kg FL was given as a 10% bolus through the central catheter before the skin incision following the induction of anesthesia. Leica M530 OHX (Wetzlar and Mannheim, Germany) microscope with FL560 fluorescein module was used in the surgery of the patients. The yellow highlights around the tumor tissue under FL560, which could not be clearly differentiated under white light, were excised. Contrast-enhanced control brain MRIs taken at the first month after surgery were evaluated with open source software (Sectra UniView, https://medical.sectra.com/product/sectra-uniview) and residual tumor volumes were calculated. Patients with no contrast enhancement in postoperative MR images were evaluated as gross total resection (GTR), while those with contrast enhancement were considered as subtotal resection. Length of hospital stay, duration of surgery and amount of blood loss were recorded from patient files. NA-FL's aid to surgery was evaluated by examining the surgery notes and surgery videos.

Statistical Analysis

Data were analyzed by entering Statistical Package for the Social Sciences (SPSS) 24.0 (SPSS Inc., Chicago, IL, USA) statistical computer program. Chi-square test for categorical variables (Fisher's Exact test if not applicable) was used to compare the groups using NA-FL and those not using it. In comparison of continuous variables, independent sample t-test was used for those with normal distributions and Mann-Whitney U test for those without normal distribution. The cases where the p value was below 0.05 and the type 1 error level was below 5% were interpreted as statistically significant.

RESULTS

A total of 48 patients were included in the study. The median age of the patients was 61.5 years (minimum: 20, maximum: 80), and the mean age was 59.1±11.8 years. In group 1, 21 patients (female=9 and male=12) were operated without using NA-FL, and in group 2, 27 patients (female=11 and male=16) were operated using NA-FL. There was no difference between the groups in terms of gender (p=0.883). When the mean tumor volumes detected in the contrast-enhanced brain MR images of the patients before the operation were examined, it was seen that it was 13.7±11.4 cm^3 in group 1 and 13.3±13.3 cm^3 in group 2, and both groups were similar in this respect (p=0.540). Considering the blood loss during surgery, there was an average of 434.3 cc of bleeding in group 1, and this average was 320.4 cc in group 2. This amount of bleeding in group 2 was significantly less than group 1 (p=0.001). While the duration of surgery was 275.7±62.8 minutes in group 1,

it was 272.0±60.7 minutes in group 2 and both groups were similar in terms of surgery time (p=0.837). When the control MR images taken after the operation were examined, the GTR rate in group 2 was 92.6%, while it was 71.4% in group 1 (p=0.059) (Figure 1). While the mean hospital stay was 9.6±5.2 in group 2, it was 13.5±7.3 in group 1 and was significantly longer than group 2 (p=0.013). Demographic data and results of both groups are summarized in Table 1, primary focus types are summarized in Table 2 and Table 3. In group 2, it did not appear to be beneficial in two patients with FL, malignant melanoma, and cystic AC adenocarcinoma. In 25 patients (92.5%), FL contributed to the surgery by giving a light yellow highlight (Figure 2). No complications were observed in any of the patients who used FL.

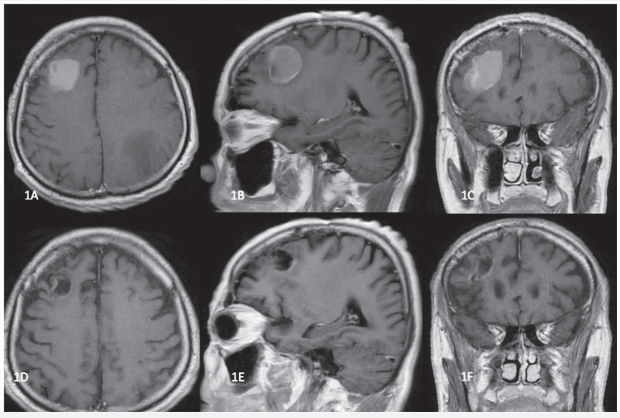

Figure 1. Preoperative and first-month postoperative contrast-enhanced images of the right frontal metastatic mass of a 62-year-old male patient with a diagnosis of AC Ca, for whom fluorescein was used in his surgery; (1A) Preop T1W axial section, (1B) Preop T1W sagittal section, (1C) Preop T1W coronal section, (1D) Postop T1W axial section, (1E) Postop T1W sagittal section, (1F): Postop T1W coronal section

DISCUSSION

The life expectancy of a patient with a metastatic brain tumor is usually quite short, regardless of which organ the primary tumor originates from. Active physical activity, presence of a solitary metastatic mass, no signs of systemic metastasis, and young age (<60-65 years) are generally associated with a good prognosis[15,16]. In addition to these, total resection and mini-

Table 2. Primary tumor distribution of group 1 (none-FL) patients

Cancer type	Incidence	Percentage (%)
AC ca adenocarcinoma	10	47.6
AC ca squamous carcinoma with hc	3	14.3
AC ca carcinoma with small hc	1	4.8
Ewing sarcoma	1	4.8
Colon ca	1	4.8
Breast ca	4	19.0
Rectum ca	1	4.8
Total	21	100.0

Ca: Carcinoma, FL: Fluorescein

Table 3. Primary tumor distribution of group 2 (FL) patients

Cancer type	Incidence	Percentage (%)
AC ca adenocarcinoma	14	51.9
AC ca squamous carcinoma with hc	1	3.7
AC ca carcinoma with small hc	4	14.8
Clear cell carcinoma	1	3.7
Malignant melanoma	1	3.7
Breast ca	5	18.5
Rectum ca	1	3.7
Total	27	100.0

Ca: Carcinoma, FL: Fluorescein

Table 1. Demographic characteristics and surgical results of patients with and without sodium fluorescein

Mean±SD/n		Group 1 (non-FL)		Group 2 (FL)		
		Median	Mean±SD/n	Median	p value	
Age		56.8±14.1	59.0	61.0±9.5	63.0	0.220[t]
Gender	Female	9 (42.9%)		11 (40.7%)		0.883[x2]
	Male	12 (57.1%)		16 (59.3)		
GTR	Yes	15 (71.4%)		25 (92.6)		0.059[f]
	None	6 (28.6%)		2 (7.4)		
Bleeding amount (cc)		434.3±147.8	440.0	320.4±73.7	320.0	0.001[t]
Duration of surgery (min)		275.7±62.8	280.0	272.0±60.7	255.0	0.837[t]
Tm volume (cm^3)		13.7±11.4	12.0	13.3±13.3	9.9	0.540[m]
Duration of hospitalization (day)		13.5±7.3	13.0	9.6±5.2	8.0	0.013[m]

SD: Standard deviation, FL: Fluorescein, GTR: Gross total resection, min: Minute.

[t]t-test, [x2]chi-square test, [f]Fisher's Exact test, [m]Mann-Whitney U test.

*Values with statistical significance are marked in bold.

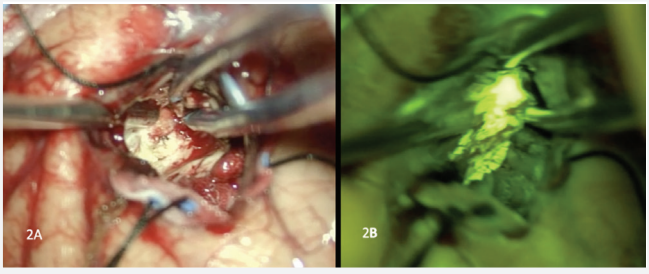

Figure 2. Intraoperative image of the patient whose preoperative and postoperative images are given in Figure 1; (2A) Intra-tumor image following cortical incision under white light, (2B) Tumor tissue appears as bright yellow highlight under FL560 filter

mental testing of the patient, namely memory and cognitive functions. are also associated with good prognosis[13,17]. In brain tumor surgery, the surgeon should ensure that these prognostic factors do not change. The target of the surgery should be total resection, but the patient's motor, memory and cognitive functions should not be affected. For this purpose, in recent years, auxiliary techniques such as intraoperative ultrasound, intraoperative MR, navigation, and brain mapping have been used[8]. In recent years, tumor staining techniques using substances such as 5-ALA, FL and indocyanine green have been a light of hope in distinguishing glial tissue from tumors. High-dose (15-20 mg/kg) FL in brain tumor surgery has been used for many years to increase the amount of resection. More secure and clearer images are obtained at doses of 3-4 mg/kg thanks to special filters that give a yellow highlight with FL at 560 nm, which are recently attached to surgical microscopes[18,19]. Thus, GTR rates have increased in both primary glial and metastatic masses with contrast enhancement[18,20]. Schebesch et al.[14] in their series of 30 patients in which they used low-dose FL with a yellow 560 filter, reported that FL gave bright highlight in 27 patients and was beneficial in surgery, with a GTR rate of 83.3%. They found that FL was not helpful in two patients with AC adenocarcinoma and one patient with malignant melanoma.

Again, Hamamcıoğlu et al.[20] in their series of 23 primary glial tumors and 7 metastatic masses, reported that FL was beneficial in all of their patients, except for one low-grade glial mass. Okuda et al.[21] reported the GTR rate as 86.1% and the local recurrence rate as 19.4% in 36 patients with metastatic brain tumors for whom FL was used. Although there was no statistical significance between the two groups in our series, when we looked at the GTR ratios, it was 71.4% in white light and 92.6% in those using FL (Table 1). We think that GTR ratios should be compared in larger series. The increase in GTR with FL in metastatic brain tumors can be explained by the resection of the yellow highlights on the walls by switching to the filter, especially after cleaning the inside of the lodge with debulking. FL was not found to be beneficial in two cases with a cystic AC adenocarcinoma and a malignant melanoma metastasis.

In retrospect, the patient with AC adenocarcinoma showed very weak thin-edged contrast enhancement around the cyst in preoperative MR images. FL involvement is proportional to impaired blood-brain barrier and intense contrast enhancement[22]. Especially in cystic masses, there is a thin wall enhancement, FL efficiency should be evaluated in larger series in these patients. FL involvement was not observed in the patient with malignant melanoma metastasis, but the black, gray tumor could easily be separated from the brain tissue. There is no study in the literature regarding the amount of bleeding, surgical durations, and hospital stays in surgeries performed with FL yet. In our series, blood loss in the FL group was significantly less than in the other group (p=0.001) (Table 1). We think that the reason for this is that the surgeon acts more controlled and decisively because he distinguishes between tumor and neural tissue while inside or outside the tumor. Again, in the FL group, by minimizing neural tissue damage, patients were discharged from the hospital more rapidly in the postoperative period (p=0.013) (Table 1). The low amount of bleeding in the patients causes the preservation of brain perfusion during surgery. Both the preservation of brain perfusion and the preservation of their motor and cognitive abilities without damaging the glial tissue caused the patients to return to their normal lives more quickly.

Study Limitations

While it would be appropriate to say that the use of FL is an effective method in the surgery of AC Ca metastases, which are the most common in our series of metastatic brain tumors, more cases should be evaluated for this inference in rare metastatic masses and cystic metastases.

CONCLUSION

FL, which has recently been used mainly in the surgery of high-grade glial masses, is also extremely useful in the surgery of contrast-enhancing metastatic brain tumors. It increases GTR rates, reduces blood loss and hospital stay, as a guide to the surgeon in separating the tumor glial tissue border.

Ethics

Ethics Committee Approval: The study were approved by the Tekirdağ Namık Kemal University of Ethics Committee (protokol no: E-39550, date: 28.07.2020.)

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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The Effect of Education on the Compliance of Nurses Working at Surgical Units to Isolation Measures

Cerrahi Birimlerde Çalışan Hemşirelerin İzolasyon Önlemlerine Uyumunda Eğitimin Etkisi

Emine KARAGÜLLE KOZA, Tülin YILDIZ

Tekirdağ Namık Kemal University, Institute of Health Sciences, Department of Nursing, Tekirdağ, Turkey

ÖZ

Aim: This study has been planned and performed as a descriptive and cross-sectional study to evaluate the adaptation of nurses working in surgical departments to the isolation precautions.

Materials and Methods: The target population of the study included 250 nurses working in the surgical departments of University, State and Private hospitals, and the sample of the study included 144 nurses who were volunteer to participate in the study and who met the sampling criteria. The data were obtained through the "Worker's Data Form which was developed in line with the literature and experts' opinion" and "Adaptation Scale To the Isolation Precautions" developed by Tayran (2009), and then these data were evaluated by using appropriate statistical methods within Statistical Package for the Social Sciences 22.00 statistical package programme.

Results: The mean age of the surgical nurses participated in the study was 28.9±8.49 years. Of them, 91% were women, 56.3% were university graduate, 58.3% were in profession for less than five years, 81.9% were in the department for less than five years, 32.6% worked in state hospital, 36.1% in university hospital, 31.3% in private hospitals, 34.7% in the ward, 27.8% in operating room, and 37.52% in intensive care. Immediately after the education, the adaptation score of the nurses to the isolation precautions was calculated as 80.389±7.106 over 90 ($p<0.05$).

Conclusion: The adaptation score of the nurses working in surgical departments was higher than that before the education. It has been suggested that the institution should support the adaptation of the nurses to the isolation precautions, education should be planned considering the professional characteristics of the nurses and the factors of the institution, and this training should be repeated with regular intervals.

Keywords: Surgical nurse, education, hospital infection, isolation precautions, adaptation

ABSTRACT

Amaç: Araştırma, cerrahi birimlerde çalışan hemşirelerin eğitim öncesi (EÖ) ve eğitim sonrası (ES) izolasyon önlemlerine uyumlarını değerlendirmek amacıyla tanımlayıcı ve kesitsel nitelikte planlandı ve uygulandı.

Gereç ve Yöntem: Araştırmanın evrenini; üniversite, devlet ve özel hastanelerin cerrahi kliniklerinde çalışan 250 hemşire, örneklemi ise örneklem seçim kriterlerini karşılayan ve çalışmaya katılmaya gönüllü 144 hemşire oluşturdu. Veriler literatür bilgileri doğrultusunda ve uzman kişilerin görüşleri alınarak araştırmacılar tarafından hazırlanan "Çalışan Veri Formu" ve Tayran tarafından (2009) geliştirilen "İzolasyon Önlemlerine Uyum Ölçeği" ile toplandı. Statistical Package for the Social Sciences 22.00 istatistik paket programında, uygun istatistiksel yöntemler kullanılarak veriler değerlendirildi.

Bulgular: Araştırmaya katılan hemşirelerin yaş ortalamasının 28,9±8,49 yıl, %91'inin kadın, %56,3'ünün lisans mezunu, %58,3'ünün meslekte beş yıldan daha az tecrübesi olduğu, %81,9'unun ise çalışılan birimde beş yıldan daha az çalışma süresine sahip olduğu, %32,6'sının devlet %36,1'inin üniversite, %31,3'ünün özel hastanelerde çalıştıkları, %34,7'sinin servis, %27,8'inin ameliyathane ve %37,52'sinin yoğun bakım birimlerinde çalıştığı belirlendi. Hemşirelerin ES izolasyon önlemlerine uyum düzeyi 80,389±7,106 olarak saptandı ($p<0,05$).

Sonuç: Cerrahi birimde çalışan hemşirelerin izolasyon önlemlerine uyum puanları EÖ'ye göre, ES en yüksek bulunurken, puanın 1. ayda biraz düştüğü 3. ayda ise ES'ye göre daha da düşük olduğu belirlendi. Hemşirelerin izolasyon önlemlerine uyumları konusunda kurumsal desteğin sağlanması, hizmet içi eğitimlerde hemşirelerin mesleki özellikleri ve kurumsal faktörler dikkate alınarak planlamaların yapılması ve eğitimlerin düzenli aralıklarla tekrarlanması önerilmektedir.

Anahtar Kelimeler: Hemşire, hastane enfeksiyonları, izolasyon önlemleri, uyum

Address for Correspondence: Tülin YILDIZ MD, Tekirdağ Namık Kemal Üniversitesi, Sağlık Bilimleri Enstitüsü, Hemşirelik Anabilim Dalı, Tekirdağ, Türkiye
Phone: +90 282 250 31 00 **E-mail:** tyildiz70@hotmail.com **ORCID ID:** orcid.org/0000-0002-4981-6671
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INTRODUCTION

Globally, healthcare-associated infections (HCAI) have become a medical problem in healthcare settings. It is estimated that more than 1.4 million people worldwide experience complications due to transmission in hospitals[1]. HCAI is defined as the most important source of mortality and morbidity by the Centers for Disease Control and Prevention and the World Health Organization (WHO)[2]. WHO states that 20% of HCAI in developed countries and more than 40% in developing countries are preventable[3,4]. HCAI can cause loss of workforce and productivity, decrease in quality of life/increase in morbidity and mortality, functional disorders, sensory stress, and increase in economic cost due to extra diagnosis and treatment methods, prolonged hospitalization, need for isolation, and increased use of drugs (antibiotics)[3,5-7].

The most effective way to control HCAI is to isolate the source of infection and patients with infection, and to take isolation precautions. It is extremely important for nurses, who are among health professionals, to know well in which situations isolation is needed, which isolation to apply and which isolation covers what, and to evaluate their attitudes and behaviors towards isolation measures with the right technique and intervals[8,9]. Urinary system infections (42%) are the most common in HCAI, and followed by surgical site infections (SSI) (40%). Therefore, surgery has an important place in HCAI. Surgical interventions increase the possibility of encountering endogenous and exogenous microorganisms by disrupting the skin integrity of the individual[10]. These infections are not limited to the intervention site, but may lead to more extensive systemic effects. Healthcare professionals should prioritize and adhere to strict infection prevention and control practices for the safety of patients and themselves. In the studies conducted, it is stated that the members of the Infection Control Committee have difficulties in ensuring and maintaining their adaptation to the isolation measures[11,12]. In this context; the research was carried out to evaluate the adaptation of nurses working in surgical units with isolation precautions before training (BT) and after training (AT).

MATERIALS AND METHODS

The research is a descriptive cross-sectional study conducted between April and November 2016 with nurses working in the surgical units of university, public and private hospitals in a province. The population of the study consisted of nurses working in the surgical units of hospitals (n=250). Seventy-six nurses who were on annual leave, maternity leave, military leave and who were not willing to participate in the study were excluded from the study. The sample included 174 nurses working in the surgical unit. A pilot study was conducted with 30 nurses to check the comprehensibility of the questionnaires and scales, and these nurses were excluded from the study, and the sample consisted of 144 nurses. The data were collected by face-to-face interview method using the "Worker's Data Form" and "Adaptation Scale to the Isolation Precautions" (ISIP) prepared by the researchers in line with the literature and by taking the opinions of experts.

Worker's Data Form

The data form prepared by the researcher by taking the opinions of experts and in line with the literature information[4,13] consisted of 17 questions asking about age, gender, educational status, institution, unit of employment, duration of work in the profession, duration of work in the unit, isolation precautions and hospital infections.

Adaptation Scale to the Isolation Precautions

It was developed by Tayran[14] in 2009. In order to measure the adaptation of nurses and physicians with isolation measures, the validity and reliability of the scale consisting of a total of 18 positive and negative items was conducted by Tayran and Ulupınar[15] in 2011. The rating on the scale is 5-point Likert type; 1=I strongly disagree 2=I disagree 3=I have no idea 4=I agree 5=I strongly agree. Negative statements in the scale are the 5th, 7th, 12th and 17th items and are scored as 1=5, 2=4, 3=3, 4=2, 5=1. Other positive items are scored from the smallest to the largest (1, 2, 3, 4, 5). Adaptation to isolation measures is evaluated by calculating the total score. The total score (lowest score 18, highest score 90) or mean (lowest mean 1; highest mean 5) can be used for scoring. The higher the score, the higher the adaptation. The Cronbach's alpha value of the scale was found to be 0.85.

The data in the study were collected in four stages.

1. Stage: Testing the data collection form; In order to evaluate the usability of the forms, a questionnaire was applied to a total of 30 nurses, 10 from each institution, who agreed to participate in the study. It was reviewed whether there were any missing/understandable questions. These nurses were not included in the sample of the study.

2. Stage: Nurses who accepted to participate in the study were given training on isolation measures for 30 minutes on days determined by the institutions. Data were collected by face-to-face interview method using the "Worker's Data Form" and ISIP BT and immediately AT.

3. Stage: Data were collected again 1 month after the training (AT1) using the "Worker's Data Form" and ISIP.

4. Stage: Three months after the training (AT3), the data were collected again using the "Worker's Data Form" and ISIP.

Statistical Analysis

The findings were analyzed using the Statistical Package for the Social Sciences 22.0 package program. The Student's t-test was used for the comparisons of normally distributed variables between two groups, the Mann-Whitney U test for the comparisons of two groups not showing normal distribution, the One-Way ANOVA test for the comparisons of three or more normally distributed groups, and the Bonferroni test for pairwise comparisons. The Kruskal-Wallis test was used for the comparisons of three or more groups not showing normal distribution and Bonferroni-Dunn test was used for pairwise comparisons.

Qualitative variables were compared using the Pearson chi-square test and Fisher Freeman Halton test. In all analyses, a p value of <0.05 was considered statistically significant and the confidence interval was 95%. Continuous variables were reported as mean±standard deviation and categorical variables as percentages and numbers.

RESULTS

According to the results obtained, it was determined that the mean age of the nurses was 28.29±8.49 years, and the majority of them were female, had a bachelor's degree, and worked less than five years both in the profession and in the unit they worked (Table 1). A statistically significant difference was found in the levels of adaptation in the BT evaluation according to the duration of employment in the profession (p=0.030; p<0.05). As a result of the pairwise comparisons made to determine the group that caused the significant difference; the level of adaptation of nurses working in the profession for 6-10 years was found to be higher than those working for 11 years or longer (p=0.025; p<0.05) (Figure 1, Table 2).

A statistically significant difference was found in the levels of adaptation in the AT3 measurement considering the unit where they worked (p=0.031; p<0.05). As a result of the pairwise comparisons made to determine the group that caused the significant difference; the adaptation levels of the nurses working in the intensive care unit were found to be higher than those working in the ward (p=0.033; p<0.05) (Table 3).

It was determined that the majority of the participants answered the question about the most important method in preventing nosocomial infection (NI) as handwashing at the

Table 1. Distribution of demographic and occupational characteristics of nurses by institution

		Institution				p
		Public n=47 (32.6%)	University n=52 (36.1%)	Private n=45 (31.3%)	Total n=144	
Age (year)	Min.-max. (median)	20-58 (36)	18-39 (25)	18-50 (21)	18-58	[a]0.001**
	M±SD	36.04±8.41	25.65±3.71	23.24±6.77	28.9±8.49	
	18-28 years	7 (14.9)	38 (73.1)	40 (88.9)		
	29-38 years	24 (51.1)	13 (25.0)	2 (4.4)		
	≥39 years	16 (34.0)	1 (1.9)	3 (6.7)		
Gender	Female	44 (93.6)	44 (84.6)	43 (95.6)	131 (91)	[b]0.161
	Male	3 (6.4)	8 (15.4)	2 (4.4)	13 (9)	
Educational status	High school	1 (2.1)	6 (11.6)	34 (75.6)	41 (28.5)	[b]0.001**
	Associate degree	14 (29.8)	1 (1.9)	0 (0)	15 (10.4)	
	Undergraduate degree	30 (63.8)	41 (78.8)	10 (22.2)	81 (56.3)	
	Graduate degree	2 (4.3)	4 (7.7)	1 (2.2)	7 (4.9)	
Time of working in the profession	0-5 years	8 (17.0)	36 (69.2)	39 (86.7)	84 (58.3)	[c]0.001**
	6-10 years	13 (27.7)	15 (28.8)	4 (8.9)	32 (22.2)	
	≥11 years	26 (55.3)	1 (1.9)	1 (4.4)	28 (19.5)	
Unit of working	Service	17 (36.2)	14 (26.9)	19 (42.2)	50 (34.7)	[c]0.497
	Operating room	11 (23.4)	18 (34.6)	11 (24.4)	40 (27.8)	
	Intensive care	19 (40.4)	20 (38.5)	15 (33.3)	54 (37.5)	
Time of working in the unit	0-5 years	26 (55.3)	51 (98.1)	41 (91.1)	118 (81.9)	[b]0.001**
	6-10 years	11 (23.4)	1 (1.9)	3 (6.7)	15 (10.4)	
	≥11 years	10 (21.3)	0 (0)	1 (2.2)	11 (7.7)	

[a]One-Way ANOVA test, [b]Fisher-Freeman-Halton test, [c]Pearson chi-square test, Min.: Minimum, Max.: Maximum, M: Mean, SD: Standard deviation.
**p<0.01

highest rate before, during, one month after, and 3 months after the training (Table 4).

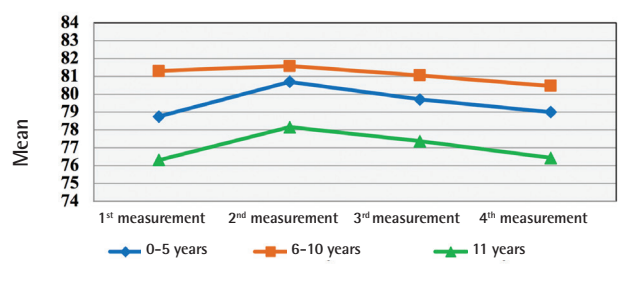

Figure 1. Correlation graph showing the adaptation levels of nurses to isolation precautions by working time in the profession

Compared to pre-training, the level of adaptation to isolation measures was found to be highest right after the training, and it was determined that it decreased 1 month and 3 months after the training (Figure 2).

DISCUSSION

When it is considered that HCAI is preventable and SSIs are in the second place after urinary system infections in HCAI, the roles and responsibilities of surgical nurses in the control and prevention of HCAI and SSIs are even more important. In the study, it was determined that the average age of the nurses and their working time in the profession were effective in their adaptation to isolation measures (p<0.05). In a study on nurses with a mean age of 30.11±6.34 years, which was conducted by

Table 2. Distribution of nurses' adaptation scores to isolation measures by age range

			Adaptation level to isolation measures			
		n	BT	AT	AT1	AT3
Age (year)	[1]18-28 years	85	78.75±6.34 (79)	80.71±5.46 (82)	79.78±5.91 (80)	79.40±6.38 (79)
	[2]29-38 years	39	81.05±5.73 (81)	81.82±5.51 (82)	80.08±6.57 (81)	79.62±5.48 (80)
	[3]≥39 years	20	74.85±11.96 (78)	76.25±12.84 (80)	77.50±9.18 (79)	74.70±13.48 (78)
	[a]p		**0.009****	**0.013***	**0.325**	**0.034***
	[d]p (1-2)		**0.305**	**1.000**	-	1.000
	[d]p (1-3)		**0.093**	**0.032***	-	0.040*
	[d]p (2-3)		**0.006****	**0.012***	-	0.049*
Time of working in the profession (year)	[1]0-5 years	83	78.76±6.42 (79)	80.7±5.57 (82)	79.71±6.11 (80)	78.99±6.26 (79)
	[2]6-10 years	32	81.31±5.83 (82)	81.59±5.58 (82)	81.06±5.81 (82)	80.47±5.59 (81)
	[3]≥11 years	29	76.31±10.4 (78)	78.17±11.2 (81)	77.38±8.34 (78)	76.45±11.92 (78)
	[a]p		**0.030***	**0.334**	**0.089**	**0.117**
	[d]p (1-2)		**0.282**	-	-	-
	[d]p (1-3)		**0.363**	-	-	-
	[d]p (2-3)		**0.025***	-	-	-

[a]One-way ANOVA test, [d]Bonferroni test, BT: Before training, AT: After training

*Age (year): [1]: 18-28 years, [2]: 29-38 years, [3]: ≥39 years; **Time of working in the profession (year) (1-2): [(0-5) years-(6-10) years], (1-3): [(0-5) years-(≥11 years)], (2-3): [(6-10) years-(≥11 years)]; *p<0.05, **p<0.01

Table 3. Distribution of nurses' adaptation scores to isolation measures by unit of working

			Adaptation level to isolation measures			
		n	BT	AT	AT1	AT3
			M±SD (Median)	M±SD (Median)	M±SD (Median)	M±SD (Median)
Unit	[1]Service	50	76.88±9.48 (78.5)	78.66±9.18 (81)	77.82±7.58 (78)	76.56±9.82 (78)
	[2]Operating room	40	79.85±5.67 (81)	81.10±5.14 (82)	80.50±5.72 (81)	79.50±6.44 (78)
	[3]Intensive care	54	79.89±5.99 (79.5)	81.46±5.87 (82)	80.43±6.08 (80)	80.37±5.61 (81)
	[a]p		0.069	0.100	0.074	0.031*
	[d]p (1-2)		0.173	0.314	0.167	0.204
	[d]p (1-3)		0.114	0.133	0.133	0.033*
	[d]p (2-3)		1.000	1.000	1.000	1.000

[a]One-way ANOVA test, [d]Bonferroni test, M: Mean, SD: Standard deviation, BT: Before training, AT: After training

*p<0,05

Table 4. Evaluation of nurses' definitions given to the question about the most important method in preventing nosocomial infection

n (%)		BT	AT	AT1	AT3
		n (%)	n (%)	n (%)	
What is the most important method in preventing NI?	Wearing gloves	39 (27.1)	9 (6.3)	17 (11.8)	22 (15.3)
	UV use	0 (0)	1 (0.7)	0 (0)	1 (0.7)
	Isolation	28 (19.4)	17 (11.8)	32 (22.2)	27 (18.8)
	Hand washing	77 (53.5)	117 (81.3)	95 (66.0)	94 (65.3)

NI: Nosocomial infection, UV: Ultraviolet, BT: Before training, AT: After training

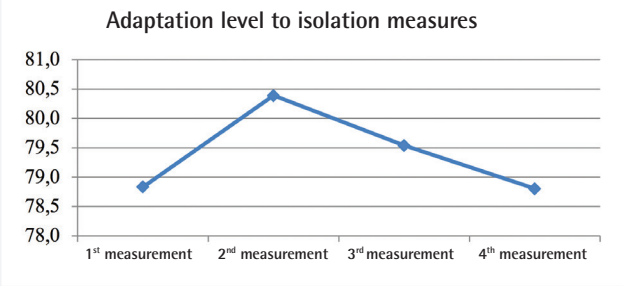

Figure 2. Correlation graph showing nurses' adaptation scores to isolation precautions

1st measurement: before training, 2nd measurement: immediately after training, 3rd measurement: 1 month after training, 4th measurement: 3 months after trainin

Zencir et al.[16], it was determined that factors such as age and working time in the profession affect adaptation to isolation measures. On the other hand, Yüceer et al.[17] found that age and working time increased adaptation to isolation measures. Erden et al.[18] evaluated the adaptation of doctors and nurses working in intensive care units to isolation measures, and found that the mean age did not affect adaptation to isolation measures, but the working time in the unit increased adaptation to isolation measures. In line with these results; except for the mean age in the study of Erden et al.[18], the studies are similar to the results of our research.

Considering that most of the NIs seen in intensive care units can result in death, adaptation to isolation measures is of great importance in the prevention of these infections. In the study, a statistically significant difference was found between the levels of adaptation in the AT3 measurement according to the unit they worked ($p=0.031$; $p<0.05$). In Özden and Özveren's[3] study on the determination of occupational and institutional factors in nurses' adaptation to isolation measures, the adaptation scores of nurses working in intensive care to isolation measures (68.60±10.68), was found to be higher than the nurses working in internal (65.57±13.25) and surgical (66.86±10.79) units. In our study, the adaptation levels of the nurses working in the intensive care unit were similarly higher than those working in the service ($p=0.033$; $p<0.05$).

According to the findings obtained in the study, it was determined that the most effective method was hand washing according to the answers given to the question "What is the most important method in preventing HCAI?" The results suggested that the trainings on handwashing, observations and the continuity of trainings were effective in all units.

Study Limitations

Since the research is limited to the nurses working in the surgical units of university, public and private hospitals, it cannot be generalized to all surgical nurses. The evaluation of the adaptation of nurses working in surgical units to isolation precautions BT and AT is limited to the items in the ISIP and the questions in the Worker's Data Form.

CONCLUSION

According to the findings obtained, it was determined that the adaptation of the nurses working in the surgical units of the hospitals to the isolation measures increased immediately after the training, 1 month and 3 months after the training compared to the pre-training, but the highest increase occurred immediately after the training. It was determined that the longer the time passed after the training, the lower the adaptation scores. It is thought that regular and repetitive in-service trainings affect adaptation to isolation measures, and demographic and occupational characteristics are also effective in adaptation to isolation measures.

Ethics

Ethics Committee Approval: The study were approved by the Tekirdağ Namık Kemal University of Ethics Committee (protocol number: 2016/62/04/11, date: 14/04/2019).

Informed Consent: Consent form was filled out by all participants.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: T.Y., Design: E.K.K., T.Y., Data Collection or Processing: E.K.K., T.Y., Analysis or Interpretation: E.K.K., T.Y., Literature Search: E.K.K., T.Y., Writing: T.Y.

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

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ORIGINAL ARTICLE

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Incidence of Hypophosphatemia after Ferric Carboxymaltose Treatment: Single Center Experience

Demir Karboksimaltoz Tedavisi Sonrası Hipofosfatemi Sıklığı: Tek Merkez Deneyimi

Esra TERZİ DEMİRSOY

University of Health Sciences Turkey, Derince Training and Research Hospital, Clinic of Hematology, Kocaeli, Turkey

ABSTRACT

Aim: The aim of this study was to evaluate the incidence of hypophosphatemia after ferric carboxymaltose (FCM) infusion and the factors affecting hypophosphatemia.

Materials and Methods: Ninety-two patients who received FCM treatment for iron deficiency anemia (IDA) were included in the study. Phosphorus, calcium, magnesium, 25-hydroxy vitamin D, and parathyroid hormone levels before and after FCM infusion were obtained from the medical records. Hypophosphatemia was defined as serum phosphorus level of <2.5 mg/dL and severe hypophosphatemia as <1 mg/dL. The cut-off value for baseline phosphorus in predicting hypophosphatemia was detected as 3.4 mg/mL with the ROC analysis.

Results: Seventy-seven of the patients were women. The mean age was 47.5±15.5 (18-85) years. Thirty of the patients (32.6%) were given 500 mg iron as FCM and 62 patients (67.4%) were given 1000 mg iron as FCM. The level of serum phosphorus measured 10-14 days after FCM was determined lower than the baseline level (2.22±0.57 mg/dL vs 3.34±0.39 mg/dL, p<0.000). Hypophosphatemia was observed in 62 patients (67.4%) after FCM infusion. Severe hypophosphatemia was seen in only 2 patients (2.1%). Patients given a dose of 1000 mg FCM had a higher incidence of hypophosphatemia compared to those given 500 mg FCM (75.8% vs 50%, p=0.013). Baseline phosphorus level of 3.4 mg/dL was associated with an increased risk of developing hypophosphatemia with an odds ratio of 9.2 (p=0.001; 95% confidence interval: 3.41-25.21). On logistic regression analysis, it was found that baseline phosphorus level and a dose of 1000 mg FCM were independent risk factors for the development of hypophosphatemia.

Conclusion: The incidence of hypophosphatemia due to FCM in our study was consistent with the literature. When FCM treatment is given to patients with IDA, the patients should be evaluated according to their baseline phosphorus level, the dose of FCM and frequency of administration, and it should be kept in mind to follow the phosphorus level in patients with risk for hypophosphatemia.

Keywords: Ferric carboxymaltose, iron deficiency anemia, hypophosphatemia

ÖZ

Amaç: Demir karboksimaltoz (DKM) infüzyonu sonrası hipofosfatemi sıklığını ve hipofosfatemiye etki eden faktörleri değerlendirmek amaçlanmıştır.

Gereç ve Yöntem: Demir eksikliği anemisi (DEA) nedeniyle DKM tedavisi alan 92 hasta çalışmaya alındı. DKM öncesi ve sonrası fosfor, kalsiyum, magnezyum, 25-hidroksi vitamin D, parathormon seviyeleri dosya bilgilerinden kaydedildi. Hipofosfatemi serum fosfor düzeyinin <2,5 mg/dL olması, ağır hipofosfatemi de <1 mg/dL olması şeklinde tanımlandı. Hipofosfatemiyi öngörmede bazal fosfor düzeyi için cut-off değeri ROC analizi ile 3,4 mg/dL olarak saptandı.

Bulgular: Hastaların 77'si (%83,6) kadındı. Yaş ortalaması 47,5±15,5 (18-85) yıl idi. Hastaların 30'u (%32,6) 500 mg, 62'si (%67,4) ise 1000 mg dozunda DKM tedavisi aldı. DKM tedavisi sonrası 10.-14. gün bakılan serum fosfor düzeyi bazal değerine göre düşük bulundu (2,22±0,57 mg/dL vs 3,34±0,39 mg/dL, p<0,000). Altmış iki hastada (%67,4) tedavi sonrası hipofosfatemi gözlendi. Ağır hipofosfatemi sadece iki hastada (%2,1) görüldü. 1000 mg DKM alanlarda, 500 mg DKM alanlarla karşılaştırıldığında hipofosfatemi daha sıktı (%75,8 vs %50, p=0,013). Bazal fosfor düzeyinin ≤3,4

Address for Correspondence: Esra TERZİ DEMİRSOY MD, University of Health Sciences Turkey, Derince Training and Research Hospital, Clinic of Hematology, Kocaeli, Turkey
Phone: +90 505 656 54 42 **E-mail:** esraterzi@gmail.com **ORCID ID:** orcid.org/0000-0001-7083-9379
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mg/dL olması 9,2 bir olasılık oranıyla hipofosfatemi geliştirme riskinde artış ile ilişkili bulundu [(p=0,001, %95 güven aralığı: 3,41-25,21). Lojistik regresyon analizinde ise bazal fosfor düzeyi ve 1000 mg DKM dozunun hipofosfatemi gelişimi için bağımsız risk faktörleri olduğu tespit edildi.

Sonuç: Çalışmamızda DKM'ye bağlı hipofosfatemi sıklığı literatür ile uyumlu idi. DEA olan hastalara DKM tedavisi verilirken bazal fosfor düzeyi, DKM dozu ve DKM uygulama sıklığına göre hastalar değerlendirilmeli; hipofosfatemi açısından riskli hastalarda fosfor seviyesinin takip edilmesi akılda tutulmalıdır.

Anahtar Kelimeler: Demir karboksimaltoz, demir eksikliği anemisi, hipofosfatemi

INTRODUCTION

Iron deficiency is the most common nutritional disorder in the world. It is estimated that the majority of pre-school children and pregnant women in developing countries and at least 30-40% in developed countries have iron deficiency[1]. According to the 2011 World Health Organization (WHO) data, the estimated prevalence of anemia in Turkey is 28.2% in pregnant women and 29% in women of reproductive age (15-49 years)[2].

Oral iron preparations are used in the first-line treatment of iron deficiency anemia (IDA) because they are inexpensive and easy to use. The most common side effects of this treatment are nausea, bloating, abdominal pain, diarrhea and constipation[3]. About half of the patients cannot complete their treatment for this reason, and this leads to treatment failure[4]. Parenteral iron therapy is used in cases of intolerance to oral iron therapy or when this therapy is not effective. Iron dextran is a first generation intravenous (iv) agent, and it is the iv agent with the highest risk of anaphylaxis. Second generation agents are ferrous gluconate and iron sucrose. Although anaphylaxis is seen at a lower rate than iron dextran, these agents cannot be administered at high doses at once. Due to administration and anaphylaxis problems, new generation iron compounds have been developed in recent years that allow high amounts of iron to be given with a single dose infusion. Ferric carboxymaltose (FCM), which we use frequently in our clinical practice, is also in this group[5].

Transient hypophosphatemia has been described in the early period in patients receiving iv iron therapy. Hypophosphatemia is more common and longer lasting in FCM compared to other agents[6-8]. It is thought that the transient increase in serum levels of fibroblast growth factor-23 (FGF-23), which stimulates the renal excretion of phosphorus, causes hypophosphatemia[8]. In this study, we aimed to examine the incidence of hypophosphatemia and the factors affecting hypophosphatemia in patients receiving FCM treatment for IDA.

MATERIALS AND METHODS

Patients who received FCM with the diagnosis of IDA in our hematology outpatient clinic between October 2016 and January 2019 were retrospectively screened. Patients whose serum phosphorus levels were reached before and after DCM treatment were included in the study. Exclusion criteria were determined as; being younger than 18 years old, pregnancy, chronic kidney disease, parathyroid disease, presence of osteoporosis, hematological or non-hematological active malignancies, receiving chemotherapy, using active vitamin D, having erythrocyte transfusion in the 1 month before the treatment and receiving oral or parenteral iron therapy.

Ninety-two patients who met the criteria were included in the study. According to the WHO classification, anemia was defined as hemoglobin (Hb) <12 g/dL in women and <13 g/dL in men[9]. If the ferritin value was <30 ng/mL, it was accepted as IDA[10]. Before FCM treatment and between 10-14 days of treatment, complete blood count, serum iron, total iron binding capacity (TIBC), ferritin, phosphorus, calcium, magnesium, 25-hydroxy vitamin D (25-OH Vit D) and parathormone (PTH) values were recorded. Transferrin saturation (TS) was calculated using the formula (serum iron level/TIBC)x100. Hypophosphatemia was defined as serum phosphorus level being <2.5 mg/dL, and severe hypophosphatemia being <1 mg/dL.

Etiological diagnoses were coded using the 10th revision of higher-level classification of the International Statistical Classification of Diseases and Associated Health Problems (International Classification of Diseases-10)[11]. Total iron needs of the patients were calculated according to the doses recommended by the brief product information of FCM (Ferinject® vial 500 mg, Abdi İbrahim-Vifor Pharma). Patients with a total iron need of 500-1000 mg were given 500 mg FCM for the first time, and those with a total iron need of more than 1000 mg were given 1000 mg of FCM for the first time.

Ethics committee approval was obtained by the Üniversity of Health Sicences Turkey, Kocaeli Derince Training and Research Hospital Clinical Research Ethics Committee with the protocol number 2020/82 dated 25.06.2020.

Statistical Analysis

The data were classified and analyzed using the IBM Statistical Package for the Social Sciences version 20 (SPSS Inc., Chicago, IL, USA) program. The Shapiro-Wilk test was used to evaluate whether the distribution of continuous variables was normal. Normally distributed continuous variables were given as mean±standard deviation. Continuous variables that did not show normal distribution were expressed as median-

interquartile range. Percent and number values were given for categorical variables. Paired-samples t-test was used to compare two dependent groups for normally distributed variables and Wilcoxon signed rank test was used for non-normally distributed variables. In the comparison of two independent groups, Independent-samples T test was used for normally distributed variables and Mann-Whitney U test was used for non-normally distributed variables. Pearson chi-square test and Fisher's Exact test were used to evaluate categorical data according to groups. Diagnostic screening tests and ROC analysis were used to calculate the basal phosphorus level cut-off point to determine the development of hypophosphatemia. The cut-off point for basal phosphorus level was determined as ≤3.4 mg/dL by ROC analysis. Logistic regression analysis was used for multivariate analysis of risk factors affecting the development of hypophosphatemia. A p value less than 0.05 was accepted as statistical significance.

RESULTS

Ninety-two patients were included in the study. Of these, 77 (83.6%) were female and 15 (16.4%) were male. The mean age was 47.5±15.5 (18-85) years. All patients received 500 mg (32.6%) or 1000 mg (67.4%) FCM treatment at one time. When evaluated according to etiology, abnormal uterine bleeding was the most common cause in 51 patients (55.4%). Digestive system diseases were seen in 28 patients (30.4%), circulatory system diseases were seen in 7 patients (7.6%), and malignant diseases were seen in 6 patients (6.5%). All of our patients with anemia due to genitourinary system diseases were women and as expected, there were abnormal uterine bleedings such as menorrhagia, metrorrhagia, menometrorrhagia and hypermenorrhea. Of our patients with anemia due to digestive system diseases, 4 had a history of Billroth surgery (due to gastric ulcer), 1 had bariatric surgery, 4 had celiac disease, and 1 had ulcerative colitis. Erosive gastropathy was also detected in the gastroscopic examination of the remaining 18 patients. Circulatory system diseases included coronary artery disease and heart failure. Of the 6 patients with malignancy, 2 had gastric adenocarcinoma, 2 had gastric lymphoma, 1 had esophageal and 1 had colon cancer. Hb, hematocrit, TS, ferritin levels measured between 10-14 days after FCM treatment were higher than before treatment and serum phosphorus level was found to be lower (Table 1).

Hypophosphatemia was observed between 10-14 days after FCM in 62 patients (67.4%). Severe hypophosphatemia was seen in only 2 patients (2.1%), and 2 of them were given 1000 mg FCM. 75.8% of the patients who developed hypophosphatemia received 1000 mg FCM. This rate was 50% (p=0.013) in the group that did not develop hypophosphatemia. The comparison of demographic and baseline laboratory characteristics of groups with and without hypophosphatemia is shown in Table 2.

The cut-off point for basal phosphorus level was determined as ≤3.4 mg/dL by ROC analysis. A basal phosphorus level of ≤3.4 mg/dL was associated with an increased risk of developing hypophosphatemia with a probability ratio of 9.2 [p=0.001, Odds ratio: 9.2 (95% confidence interval: 3.41-25.21)]. In the multivariate logistic regression analysis, when the variables below the p<0.20 value shown in Table 2 (age, basal phosphorus, ferritin, 25-OH Vit D, PTH levels and FCM doses) were evaluated, only the basal phosphorus level (p<0,01) and FCM dose (p<0.05) were statistically significant (Table 3).

Treatment-related pruritus developed in 3 patients (3.2%), myalgia in 2 patients (2.1%), subfebrile fever in 2 patients (2.1%), and nausea in 1 patient (1%). However, it was not necessary to discontinue the drug due to these side effects. Anaphylaxis and injection site complications were not observed in any of the patients.

Table 1. Comparison of laboratory characteristics before and after ferric carboxymaltose treatment

	Before FCM	After FCM	p value
Hb (gr/dL)	9.99±1.25	11.83±1.05	†<0.000**
Htc (%)	31.69±3.36	36.64±2.74	†<0.000**
TS (%)	6.45 (4.8-9.8)	26.7 (16.8-45)	‡<0.000**
Ferritin (ng/mL)	5.60 (3.7-10.0)	128.2 (34.7-291)	‡<0.000**
Phosphorus (mg/dL)	3.34±0.39	2.22±0.57	†<0.000**
Ca (mg/dL)	9.32±0.40	9.29±0.42	†0.280
Mg (mg/dL)	1.97±0.18	1.99±0.27	†0.589
25-OH Vit D (ng/mL)	13.25 (8.3-21.0)	14.5 (4.2-84.5)	‡0.414
PTH (pg/mL)	73.3±23.24 (21.9-157)	76±24.16 (17.9-153.9)	†0.09

FCM: Ferric carboxymaltose, Hb: Hemoglobin, Htc: Hematocrit, TS: Transferrin saturation, Ca: Calcium, Mg: Magnesium, 25-OH Vit D: 25-hydroxy vitamin D, PTH: parathormone

†Dependent groups t-test; is given as mean±standard deviation.

‡Wilcoxon signed rank test, is given as the median (25-75%)

Table 2. Comparison of groups developing and not developing hypophosphatemia

	Developing hypophosphatemia (n=62)	Not developing hypophosphatemia (n=30)	p value
Age	49.32±17.30	43.80±14.81	[a]0.137
Gender; n (%) Female Male	 51 (66.2) 11 (73.3)	 26 (33.8) 4 (26.7)	[c]0.766
Hb (gr/dL)	9.92±1.26	10.15±1.22	[a]0.393
Hct (%)	31.49±3.57	32.10±2.88	[a]0.423
TS (%)	6.35 (4.3-8.9)	7.10 (4.9-11.4)	[b]0.723
Ferritin (ng/mL)	5.35 (3.3-9.2)	8.00 (40-11.7)	[b]0.008**
Phosphorus (mg/dL)	3.22±0.32	3.57±0.39	[a]0.001**
Ca (mg/dL)	9.32±0.42	9.35±0.39	[a]0.724
Mg (mg/dL)	1.96±0.19	1.98±0.17	[a]0.793
25-OH Vit D (ng/mL)	13.0 (7.2-19.2)	13.85 (8.9-26.9)	[b]0.167
PTH (pg/mL)	72.65 (58.3-89.8)	65.20 (56.3-78.6)	[b]0.184
FCM dose; n (%) 500 mg 1000 mg	 15 (24.2) 47 (75.8)	 15 (50.0) 15 (50.0)	[d]0.013*

Hb: Hemoglobin, Ca: Calcium, Mg: Magnesium, 25-OH Vit D: 25-hydroxy vitamin D, PTH: Parathormone, FCM: Ferric carboxymaltose

[a]Student's t-test; shown as mean±standard deviation.

[b]Mann-Whitney U test, shown as median (25-75%).

[c]Fisher's Exact test, [d]Pearson chi-square test

*p<0.05, *p<0.01

Table 3. Logistic regression analysis of risk factors affecting hypophosphatemia

	p value	Odds ratio	95% confidence interval	
			Lower	Upper
Age (years)	0.217	1.025	0.986	1.066
Basal phosphorus (≤3.4 mg/dL)	0.000**	10.918	3.681	32.383
Basal ferritin	0.351	0.931	0.802	1.081
Basal 25-OH Vit D	0.467	0.979	0.924	1.037
Basal PTH	0.691	0.995	0.969	1.021
FCM dose (1000 mg)	0.013*	4.100	1.353	12.427

25-OH Vit D: 25-hydroxy vitamin D, PTH: Parathormone, FCM: Ferric carboxymaltose

*p<0.05, **p<0.01

DISCUSSION

IDA is a public health problem that mostly occurs in children, premenopausal women and pregnant women[1]. Oral or parenteral iron preparations are used in the treatment of IDA. Discontinuation of oral therapy due to gastrointestinal side effects and the patient's inability to use the drug regularly and for a sufficient time are common[4]. Apart from intolerance to oral iron therapy, parenteral iron therapy should be preferred in inflammatory bowel diseases, absorption disorders such as gastrectomy, and functional iron deficiency (chronic kidney failure, inflammatory diseases, malignancies)[12]. FCM is a new generation parenteral iron preparation. It is administered over a short infusion time of 20-30 minutes and can be given in a single dose of 1000 mg. It has high stability due to its high molecular weight. This makes the side effects of labile iron less common in FCM. Thanks to its neutral pH and physiological osmolarity, complications such as discoloration, extravasation and pain at the injection site are less common. Due to the low immunogenicity of FCM, anaphylactic reactions are extremely rare[5]. Side effects such as nausea, injection site reactions, headache, hypertension, dizziness, vomiting and diarrhea are similar in FCM and other parenteral iron preparations. The rate of severe anaphylactic reaction in FCM is between 0.1% and 0.9%[13]. In our study, mild side effects that did not require discontinuation of treatment were observed in 8 patients after FCM. Application site complications and anaphylaxis did not

occur in any of the patients. Due to these advantages, FCM has been shown to be effective in many conditions such as chronic kidney failure, heart failure, postpartum period, pregnancy, and chronic gastrointestinal bleeding in recent years[5].

Hypophosphatemia due to iv iron therapy is a known side effect. This rate is higher in FCM and the duration of hypophosphatemia is longer. In the literature, the incidence of hypophosphatemia was found to be 0-92.1% in FCM, 0-40% in iron sucrose, 0.4% in ferumoxytol, and 0% in low molecular weight iron dextran, and hypophosphatemia was generally defined as asymptomatic[14]. In the study by Emrich et al.[6], phosphorus values of the patients receiving FCM and iron isomaltozide (IIM) were compared on the 1st, 7th and 35th days. The incidence of hypophosphatemia (p<2 mg/dL) was 75% in FCM and 8% in IIM (p<0.001). The lowest phosphorus value was seen on the 7±2th days and it was observed that the decrease in phosphorus levels continued on the 35th day in patients who received FCM. No change was observed in calcium, PTH, alkaline phosphatase and 25-OH Vit D levels in both groups. In DCM, serum intact FGF-23 (iFGF-23) levels were found to be higher on the first day (p<0.001). In the study conducted by Wolf et al.[8], serum phosphorus levels of patients receiving FCM and ferumoxytol were evaluated at the 1st, 2nd and 5th weeks of treatment, and the rate of hypophosphatemia was higher in FCM patients (50.8% vs 0.9%) (p<0.001) and similarly, the incidence of severe hypophosphatemia (<1.3 mg/dL) was found to be higher (10% vs 0%) (p<0.001). At the end of the fifth week, phosphorus levels of all patients who received ferumoxytol returned to normal, while it was reported that hypophosphatemia continued in 29.1% of those who received FCM (p<0.001). It has been stated that the increase in serum iFGF-23 levels is held responsible for hypophosphatemia.

The incidence of hypophosphatemia in our study was consistent with the literature (67.4%). Severe hypophosphatemia was seen in only 2 patients (2.1%), and this rate was lower than in the literature. This situation may be related to the fact that our study is retrospective so we can evaluate serum phosphorus levels only between 10-14 days. If we had a chance to see serum phosphorus values earlier after treatment, we might have a higher probability of detecting severe hypophosphatemia. In our study, the incidence of hypophosphatemia was higher in those treated with 1000 mg FCM, and we found that basal phosphorus level and FCM dose were independent risk factors for hypophosphatemia. No study comparing 500 mg and 1000 mg FCM doses was found in the literature. However, while hypophosphatemia was found in 26.9% in those receiving 750 mg DCM in studies, this rate was reported as 50.8% in those receiving 1000 mg[7,13]. In a study comparing cumulative doses, hypophosphatemia was found at a higher rate in those who received 2090 mg FCM compared to those who received 1350 mg on average (p=0.04)[15]. The cut-off value of hypophosphatemia and the day of evaluation of serum phosphorus level were specified differently in each study. There is still no standard consensus on whether routine phosphorus levels should be checked in patients undergoing FCM, and if it will be checked on which days after treatment.

FGF-23 is a secretory protein ligand produced mainly by osteoblasts and osteocytes. Only iFGF-23 is biologically active. The C-terminal part of iFGF-23 is cleaved by the proteolytic pathway and becomes inactive. FGF-23 suppresses non-tissue-specific alkaline phosphatase under physiological conditions. It increases urinary phosphate excretion and inhibits phosphate reabsorption. The resulting hypophosphatemia causes inhibition of bone mineralization.

FGF-23, phosphate involved in mineral metabolism and vitamin D are stimulated by PTH. In addition, there is an increase in serum levels in cases of iron deficiency and the release of proinflammatory cytokines[16]. Total serum FGF-23 level is inversely proportional to serum iron concentration in patients without renal dysfunction. Although total FGF-23 levels increase in iron deficiency, iFGF-23 level is usually within normal limits. This is because the stabilizing state of post-translational FGF-23 cleavage ensures that iFGF-23 levels remain normal despite an increase in C-terminal FGF-23 levels. Therefore, hypophosphatemia is not usually seen in IDA[17]. In case of iron deficiency, while FGF-23 transcription increases, iFGF-23 remains within normal limits. When iron dextran is used in treatment, the increase in FGF-23 transcription returns to normal. Increased C-terminal FGF-23 levels normalize, while iFGF-23 levels remain unchanged. When FCM is used, the transcription of FGF-23 regresses to normal, while the production of iFGF-23 protein increases. Although the mechanism of this is not known exactly, it is thought that the carbohydrate sheath of FCM causes the increase of iFGF-23 by inhibiting the proteolytic cleavage of FGF-23. This increase causes hypophosphatemia[13]. The difference in the incidence of hypophosphatemia between FCM and other iv iron preparations is explained by this hypothesis.

Although hypophosphatemia related to FCM has been defined as mostly asymptomatic in randomized controlled studies, cases with severe hypophosphatemia, cramps, dizziness, fatigue, nausea, and requiring long-term hospitalization have been reported in the acute period[14,15,18]. In addition, hypophosphatemic osteomalacia and related fractures have been reported in patients receiving repeated doses of therapy in recent years. In these patients, monthly FCM infusion was administered for two years[19,20]. It has been stated that the risk of osteomalacia and fractures may increase after FCM, especially in those who receive frequent FCM treatment, malnutrition, 25-OH Vit D deficiency, osteoporosis, hyperparathyroidism, and patients with low basal calcium and phosphorus levels[20].

Study Limitations

The limitation of our study is that it is a retrospective study. Therefore, phosphorus levels were only obtained in the range of 10-14 days after FCM treatment. However, the incidence of hypophosphatemia was found to be consistent with the literature.

CONCLUSION

In conclusion, FCM is frequently used in our daily practice for the treatment of IDA. Although its advantages provide us convenience, the incidence and duration of hypophosphatemia is longer than other iv iron preparations. Mostly, hypophosphatemia is asymptomatic. Currently, routine serum phosphorus monitoring is not recommended. However, it has been reported that osteomalacia and fractures due to hypophosphatemia can be seen in the use of FCM at high and frequently repeated doses. Particular attention should be paid to hypophosphatemia in cases with low basal phosphorus levels and high doses of FCM treatment. For this reason, it should be kept in mind to monitor the phosphorus level in risky patients.

Ethics

Ethics Committee Approval: Ethics committee approval was obtained by the Üniversity of Health Sicences Turkey, Kocaeli Derince Training and Research Hospital Clinical Research Ethics Committee with the protocol number 2020/82 dated 25.06.2020.

Informed Consent: Retrospective study.

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ORIGINAL ARTICLE

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Is the Optimal Timing First Complete Remission for Autologous Stem Cell Transplantation in Patients with Peripheral T-Cell Lymphoma?

Periferik T Hücreli Lenfoma Hastarında Otolog Kök Hücre Nakli için Uygun Zamanlama İlk Tam Remisyon mudur?

Bahar UNCU ULU, Derya ŞAHİN, Tuğçe Nur YİĞENOĞLU, Merih KIZIL ÇAKAR, Mehmet Sinan DAL, Fevzi ALTUNTAŞ

University of Health Sciences Turkey, Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital, Clinic of Hematology, Unit of Bone Marrow Transplantation, Ankara, Turkey

ABSTRACT

Aim: Outcome for most patients with peripheral T-cell lymphomas (PTCLs) is poor, with low response rates and short durations of remission. We aim to analyze the outcome of PTCL patients who underwent autologous stem cell transplantation (ASCT) at our center and we researched whether first complete remission (CR) was the optimal timing for ASCT in patients with PTCL.

Materials and Methods: The data of PTCL patients who underwent ASCT between January 2010 and December 2020 at our center were retrospectively analyzed.

Results: Twenty-five patients with PTCL underwent ASCT. The median ASCT age was 44 years (range: 19-68). 68% of the patients were performed upfront ASCT after the first CR; 32% of the patients underwent ASCT after relapse or refractory (R/R) disease. The median follow-up time from diagnosis was 40 months (13-144 months). The five-year PFS and OS were 50.8% and 55.7%, respectively. Five-year OS was 82.4% in the up-front ASCT group, while the five-year OS was 15.6% in the R/R patients (p=0.019). The median OS was 25 months [95% confidence interval (CI): 0-50.4] in patients who had CR2, while it was 13 months (95% CI: 0-35) in patients who had PR before ASCT (p=0.03). According to subtypes of PTCL, OS and PFS were not statistically different in all groups (p=0.96 and p=0.79, respectively).

Conclusion: Even the prognosis is poor in patients with PTCL, Overall survival was significantly longer among the upfront ASCT group. Patients should be consolidated with ASCT in the first CR, and relapse should not be waited to perform ASCT.

Keywords: Peripheral T cell lymphoma, PTCL, autologous stem cell transplantation, upfront ASCT

ÖZ

Amaç: Periferik T hücre lenfomalı (PTHL) çoğu hasta için sonuçlar, düşük yanıt oranları ve kısa remisyon süreleri ile kötü seyreder. Merkezimizde otolog kök hücre transplantasyonu (OKHN) yapılan PTHL hastalarının sonuçlarının analizini ve PTHL'li hastalarda OKHN için ilk tam remisyonun (TR) optimal zamanlama olup olmadığını araştırmayı amaçladık.

Gereç ve Yöntem: Merkezimizde Ocak 2010 ile Aralık 2020 arasında OKHN uygulanan PTHL hastalarının verileri geriye dönük olarak incelendi.

Bulgular: PTHL'li 25 hastaya OKHN uygulandı. Ortanca OKHN yaşı 44 (19-68) yıl olarak saptandı. Hastaların %68'ine ilk TR'den sonra upfront OKHN yapılırken, %32'sine relaps veya refrakter (R/R) hastalıktan sonra OKHN yapıldı. Tanıdan itibaren ortanca takip süresi 40 aydı (13-144 ay). Beş yıllık progresyonsuz sağkalım (PS) ve toplam sağkalım (TS) sırasıyla %50,8 ve %55,7 idi. Beş yıllık TS upfront OKHN grubunda %82,4 iken, R/R hastalarda %15,6 idi (p=0,019). OKHN öncesi TR2'de olan hastalarda ortanca TS 25 ay [%95 güven aralığı (GA): 0-50,4] iken, OKHN öncesi kısmi yanıtı olan hastalarda 13 ay (%95 GA: 0-35) idi (p=0,03). PTHL alt tiplerine göre TS ve PS tüm gruplarda istatistiksel olarak farklı saptanmadı (sırasıyla p=0,96 ve p=0,79).

Address for Correspondence: Bahar UNCU ULU MD, University of Health Sciences Turkey, Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital, Clinic of Hematology, Unit of Bone Marrow Transplantation, Ankara, Turkey
Phone.: +90 542 833 44 30 **E-mail:** baharuncu@gmail.com **ORCID ID:** orcid.org/0000-0002-6230-9519
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Sonuç: PTHL hastalarında prognoz kötü olsa dahi, TS, upfront OKHN yapılan grupta önemli oranda daha uzun saptandı. Hastalar ilk TR'de OKHN ile konsolide edilmeli ve OKHN yapmak için relaps beklenmemelidir.

Anahtar Kelimeler: Periferik T hücreli lenfoma, PTHL, otolog kök hücre nakli, upfront OKHN

INTRODUCTION

The peripheral T cell lymphomas (PTCLs) represent a heterogeneous group of generally aggressive malignancies. They constitute approximately 15% of all non-Hodgkin lymphomas (NHLs) in adults[1,2]. The most common subtype of PTCL is PTCL, not otherwise specified (PTCL, NOS). It accounts for approximately 30% of PTCL and approximately 4% of NHL[3]. The other subtypes of PTCL are anaplastic large cell lymphoma (ALCL), angioimmunoblastic T cell lymphoma (AITCL), extranodal natural killer (NK)/T cell lymphoma, subcutaneous panniculitis-like T cell lymphoma, enteropathy associated T cell lymphoma, and hepatosplenic T cell lymphoma[2,3]. PTCL, NOS is derived from mature T cells[2]. Patients should be classified in this group if they do not meet the criteria for the other specifically defined subtypes of PTCL[2]. The median age at diagnosis is 60 years and there is a male predominance[4,5].

In general, with combination chemotherapy, the 5-year overall survival (OS) has been reported between 49% and 74% among PTCL patients with low or low-intermediate International Prognostic Index (IPI) scores, respectively[6]. On the other hand, the 5-year OS with combination chemotherapy has been reported to be between 6% and 21% among patients with high-intermediate or high IPI scores, respectively[6]. Recent studies have confirmed that, except for anaplastic lymphoma kinase (ALK) positive ALCL, most of the PTCL subtypes have a worse prognosis than most B-cell NHL subtypes[6]. Combination chemotherapy such as cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) or similar are associated with low response rates and short duration of response in the majority of patients with PTCL[7].

The poor outcomes with combination chemotherapy have led to an interest in more aggressive strategies such as the role and the timing of autologous hematopoietic stem cell transplantation (ASCT). ASCT is used as consolidation therapy in patients with PTCL, who have a chemosensitive response after induction or salvage therapy. However, the precise role of upfront ASCT remains largely unknown. PTCL is a rare hematological malignancy; therefore, the number of prospective randomized studies and patients enrolled in the studies are limited. In this study, we aimed to analyze the outcome of PTCL patients who underwent ASCT at our center and we researched whether first complete remission (CR) was the optimal timing for ASCT in patients with PTCL.

MATERIALS AND METHODS

The data of PTCL patients who underwent ASCT between January 2010 and December 2020 at Üniversity of Health Sicences Turkey, Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital, Department of Bone Marrow Transplantation Center were retrospectively analyzed. The subtypes of PTCL including PTCL NOS, ALCL, AITCL, and extranodal NK/T cell lymphoma were included. The data about the age, gender, Ann Arbor stage, initial treatment and response to first-line treatment, disease response before ASCT, conditioning regimens patients received before ASCT, progression-free survival (PFS), and OS after ASCT were analyzed. ^{18}F- fluorodeoxyglucose positron emission tomography imaging was performed in all patients at the time of diagnosis, at the end of chemotherapy, and in the third month of ASCT to evaluate the response. The Lugano Modification of Ann Arbor Staging 2014 criteria were used for assessing response after chemotherapy and ASCT[8]. At our center, the patients who achieved CR1 after first-line treatment underwent ASCT for upfront consolidation. The patients who had residual disease after induction were administered a salvage treatment and after that, they underwent ASCT.

Among ASCT, the engraftment definition for neutrophil was defined as the first day when the absolute neutrophil count was >500/mm^3 or 1000/mm^3 for three consecutive days, and thrombocyte engraftment was defined as the first day when thrombocyte count was >20000/mm^3 for three consecutive days without transfusion. All patients received weight-adapted G-CSF before the neutrophil engraftment. OS was calculated from the date of ASCT to death or the last date of follow-up. PFS was calculated from the date of ASCT to disease progress, death, or date of the latest follow-up for progression-free patients, whichever occurred first. Transplantation-related mortality (TRM) was defined as death within the first 100 days after ASCT[9].

The study were approved by the Üniversity of Health Sicences Turkey, Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital of Local Ethics Committee (protocol number: 2020-12/909, date: 09.12.2020).

All procedures performed in the study, involving human participants, were in accordance with the national research committee's ethical standards and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Statistical Analysis

All statistical analyses were conducted using Statistical Package for the Social Sciences (SPSS) V21.0 (SPSS Inc., Chicago, IL) software. Descriptive statistics were applied to summarize the data. The categorical data were reported as rates, and numeric data were reported as medians and average±standard deviations. The Kaplan-Meier test was used to analyze PFS and OS.

RESULTS

In total, there were 25 patients with PTCL who underwent ASCT. The median age at the time of ASCT among all patients was 44 years (range: 19-68). The male-to-female ratio was 19/6. 17 patients (68%) were performed upfront ASCT after the first CR whereas 8 (32%) patients were performed ASCT after relapse or refractory (R/R) disease. 13 (52%) patients were PTCL NOS; 8 (32%) patients were ALK-negative ALCL, and 3 (12%) patients were AITCL, and one (4%) patient was extranodal NK/T cell lymphoma. Most of the patients (76%) were at advanced stage (Stage 3-4) at the diagnosis. Thirteen patients had CHOP (Cyclophosphamide, daunorubicin, vincristine, prednisone); eight patients had CHOEP (cyclophosphamide, daunorubicin, vincristine, prednisone, etoposide), two patients had HYPERCVAD (cyclophosphamide, vincristine, doxorubicin, dexamethasone alternating with high-dose methotrexate and cytarabine), one patient had EPOCH (etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin), one patient had SMILE (dexamethasone, methotrexate, ifosfamide asparaginase, etoposide) for induction regimen. The characteristics of the patients are given in Table 1. When we evaluated the response to the induction regimen, the overall response rate (ORR) was 88% among the patients, 12% of patients were refractory after induction chemotherapy.

Before ASCT, of the patients, 17 (68%) were in CR1, four (16%) patients were in CR2, four (16%) patients were in partial remission (PR). In the R/R patients, five patients received two lines of salvage therapy and three patients had one salvage treatment before ASCT. Twenty-one (84%) patients received BEAM (carmustine, cytarabine, melphalan, etoposide), and three (12%) patients received ICE (carboplatin, ifosfamide, etoposide), and one (4%) patient received BuCyE (busulfan, cyclophosphamide, and etoposide) as conditioning regimen. The median time to transplantation was 12 months (range: 6-36 months). Except for one patient, the patients received mobilized peripheral blood as the stem-cell source, one patient harvested for bone marrow stem-cell source. The median neutrophil engraftment duration was 11 (range: 9-18) days, and the median platelet engraftment was 15 (range: 8-19) days. Twenty (80%) patients achieved CR and four (16%) patients had progressive disease three months after ASCT; one patient was not evaluated due to being exitus in the first month of the transplantation. The median follow-up time from diagnosis was 40 months (13-144 months).

After a median follow up of 25 months (range: 1-129 months) from ASCT, two (25%) patients were alive among patients who underwent ASCT after R/R disease; on the other hand, 14 (%82) patients were alive among patients who underwent upfront ASCT after first CR.

The five-year PFS and OS were 50.8% and 55.7%, respectively. The median of both PFS and OS was not reached. When we evaluated considering patients who underwent upfront ASCT consolidation, OS was significantly longer among the upfront

Table 1. Characteristics of the patients

Patients (n)	**25**
Age (median)	44 years (19-68)
Gender (M/F)	19/6
Ann Arbor stage (Lugano 2014)	Stage 1-2: 3 (12%) Stage 3-4: 19 (76%) Unknown: 3 (12%)
Lymphoma subtype	PTCL, NOS: 13 (52%) ALCL: 8 (32%) AITCL: 3 (12%) Ekstranodal/NK-T cell: 1 (4%)
Initial treatment	CHOP: 13 (52%) CHOEP: 8 (32%) HYPERCVAD: 2 (8%) EPOCH: 1 (4%) SMILE: 1 (4%)
Pre-ASCT disease response	CR1: 17 (68%) CR2: 4 (16%) PR: 4 (16%)
Conditioning	BEAM: 21 (84%) ICE: 3 (12%) BuCyE: 1 (4%)
Response after ASCT	CR: 20 (80%) Progression: 4 (16%) Not evaluated: 1 (4%)
Transplantation-related mortality	1 (4%)
Neutrophil engraftment duration (median)	11 days (range: 9-18)
Platelet engraftment duration (median)	15 days (range: 8-19)
Quantity of CD34+ infused (median)	4.8x10^6/kg (range: 3.5x10^6/kg-11.4x10^6/kg)

AITCL: Angioimmunoblastic T cell lymphoma, ALCL: Anaplastic large cell lymphoma, ASCT: Autologous hematopoietic cell transplantation, BEAM, BCNU: Etoposide, cytarabine and melphalan, BuCyE: Busulfan, cyclophosphamide and etoposide, CHOP: Cyclophosphamide, daunorubicin, vincristine, prednisone, CHOEP: Cyclophosphamide, daunorubicin, vincristine, prednisone, etoposide, CR: Complete response, ECOG: Eastern Cooperative Oncology Group, EPOCH: Etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin, F: Female, IPI: International prognostic index, HYPERCVAD: Cyclophosphamide, vincristine, doxorubicin, dexamethasone alternating with high-dose methotrexate and cytarabine, ICE: Ifosfamide, carboplatin, etoposide, M: Male, PR: Partial response, PTCL-NOS: Peripheral T cell lymphoma, not otherwise specified, SMILE: Dexamethasone, methotrexate, ifosfamide asparaginase, etoposide

ASCT group. These patients' five-year OS was 82.4%, while the five-year OS for the patients who underwent ASCT after R/R disease was 15.6% (p=0.019). In the R/R patients, the median

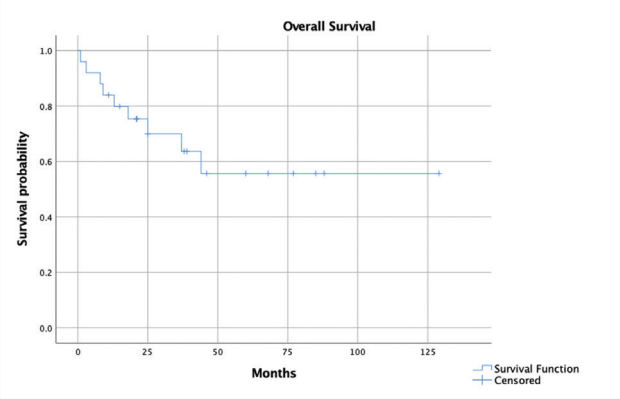

Figure 1. Overall survival

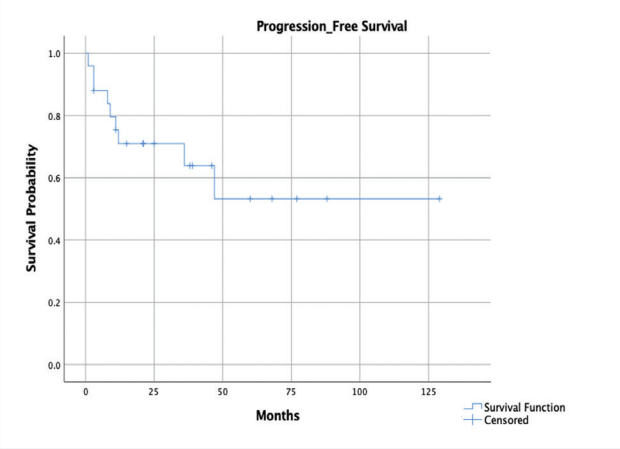

Figure 2. Progression free survival

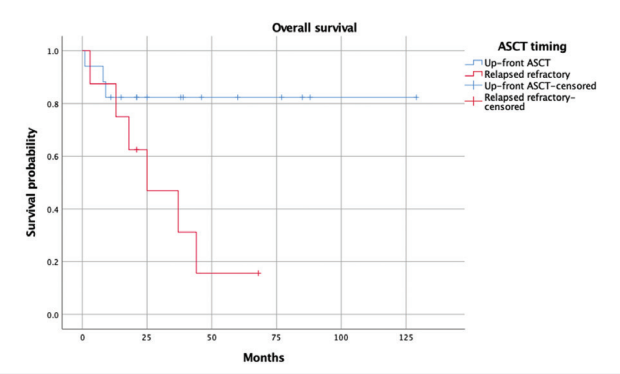

Figure 3. Overall survival according to autologous stem cell transplantation timing

ASCT: Autologous stem cell transplantation

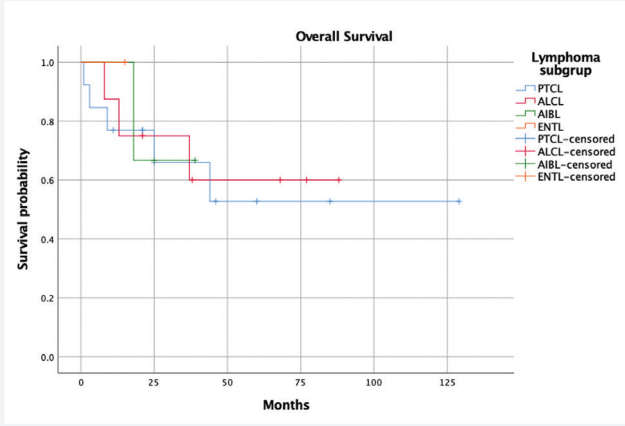

Figure 4. Overall survival according to lymphoma subtype

ASCT: Autologous stem cell transplantation, PTCL: Peripheral T cell lymphomas not otherwise specified, ALCL: Anaplastic large cell lymphoma, AIBL: Angioimmunoblastic T cell lymphoma, ENTL: Extranodal natural killer/T cell lymphoma

OS was 25 months (95% CI: 2.7-47.2), the median PFS was 12 months [95% confidence interval (CI): 1-41.6] after ASCT. However, the median OS and PFS were not reached in the upfront ASCT group. The median OS was 25 months (95% CI: 0-50.4) in patients who had CR2 response before ASCT, while it was 13 months (95% CI: 0-35) in patients who had PR before ASCT (p=0.03). According to the subtypes of PTCL, OS and PFS were not statistically different in all patients (p=0.96 and p=0.79, respectively). The survival curves are given in Figures 1-4. TRM was observed in one patient (due to sepsis).

DISCUSSION

We analyzed the outcome of PTCL patients who underwent ASCT at our center in the current study. We observed that first CR was the optimal timing for ASCT in patients with PTCL. OS was significantly longer among the patients who underwent ASCT in the up-front setting in first CR rather than CR2 or PR response.

Previous studies have demonstrated that most of the PTCL subtypes have a poorer prognosis than most of the B-cell NHL subtypes[4-6]. Currently, CHOP or CHOP-like combination chemotherapies are the standard first-line treatment approaches for PTCLs. However, except for ALK-positive ALCL, the outcome for most patients with PTCL remains dismal, with low response rates and short durations of remission[10].

Savage et al. conducted a study on 199 patients with PTCL and they reported that 5-year OS of patients with PTCL/NOS, ALCL, and AILT was between 35% and 43% with CHOP or CHOP-like chemotherapy[11]. Vose et al.[4] reported that the 5-year failure-free survival and OS of patients with PTCL-NOS, ALK-positive ALCL, ALK-negative ALCL, and AITL were 20% and 32%, 60%

and 70%, 36%, and 49%, and 18% and 32%, respectively.

In the last years, studies have been conducted to evaluate the role of upfront ASCT in patients with PTCL. However, as it is not a common disease, the number of patients enrolled in these studies is limited. In the prospective study by the Nordic Lymphoma Group, ASCT was performed on 115 patients with PTCL after 6 cycles of CHOEP. The TRM was 1.7% and 5-year OS for patients with ALCL, AILT, and PTCL-NOS was 70%, 52%, and 47%, respectively[12]. Reimer et al.[13] reported the outcome of 83 patients with PTCL (excluding ALK-positive ALCL) after induction with CHOP. In their study, patients received total body irradiation and high-dose cyclophosphamide as a conditioning regimen. Only one patient died due to TRM. They found that the median time to relapse following ASCT was 11.5 months and 3-year OS was 48%. In the study conducted by Chen et al.[14], in which they evaluated the outcome of 53 PTCL patients who were performed ASCT, they reported a 5-year PFS and OS as 25% and 48% among all patients. In the same study, the 5-year PFS and OS were 51% and 76% in patients who underwent ASCT in the first remission. In another study, the 5 year OS was 80% in patients who underwent ASCT in CR1 and it was 50% in patients who underwent ASCT in CR2[15]. In the study by Blystad et al.[16], in which researchers evaluated the outcome of 40 patients with PTCL, TRM was 7.5%, and the 3-year event-free survival and OS were 48% and 58%, respectively. In this study, 11 patients were transplanted at CR1 following induction therapy. In a previous study, Ahn et al.[17] demonstrated the outcome of 31 patients with PTCL who underwent upfront ASCT after initial induction chemotherapy and the patients received BuCyE as conditioning regimen[17]. Before ASCT, 23 patients (74.2%) had CR and 8 patients (25.8%) had PR. At a median follow-up of 32.4 months, the 3-year estimated OS and PFS was 64.5±8.6% and TRM was 9.7%.

In our study, the characteristics of the patients were similar to previous studies. Nearly three-quarters of the patients had the advanced stage at diagnosis. Most of the patients received CHOP-based induction and 68% of the patients underwent up-front ASCT. After a median follow-up of 25 months from ASCT, 82% patients were alive among patients who underwent upfront ASCT after first CR, while 25% of R/R patients were alive in the R/R group. We showed that five-year PFS and OS were 50.8% and 55.7% in our cohort, respectively. Among the upfront ASCT group, five-year OS was 82.4%, while it was 15.6% in the R/R group. Our study showed that a significantly longer survival advantage was observed for the PTCL patients by upfront ASCT. Compared to the pre-ASCT responses, the worst survival was seen in the patients who had PR responses before ASCT. Differently, we did not observe survival differences according to lymphoma subtypes.

Study Limitations

The retrospective design of the study and the low number of patients included in our study were our limitations. The variation of the first-line treatment regimens and also ASCT conditioning regimens depending on the clinician's preference was another limiting factor. Randomized-controlled and prospective studies with extended-patients should be conducted to prove the hypothesis.

CONCLUSION

As a result, upfront ASCT is the optimal time for PTCL patients in first CR. The five-year OS was 82.4% in our study. According to our results, the pre-ASCT response is an important and predictive factor for the patients. If the patients could achieve CR1, ASCT may provide a survival advantage rather than CR2 or PR.

Since the remission durations are short with CHOP and CHOP-like chemotherapies and the prognosis is poor in patients with PTCL, the patients should be consolidated with ASCT in the first CR, and relapse should not be waited to perform ASCT.

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Ethics

Ethics Committee Approval: The study were approved by the Üniversity of Health Sicences Turkey, Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital of Local Ethics Committee (protocol number: 2020-12/909, date: 09.12.2020).

Informed Consent: Consent form was filled out by all participants.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: M.K.Ç., M.S.D., F.A., Concept: M.K.Ç., M.S.D., Design: T.N.Y., F.A., Data Collection or Processing: D.Ş., B.U.U., Analysis or Interpretation: B.U.U., F.A., Literature Search: T.N.Y., Writing: B.U.U.

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