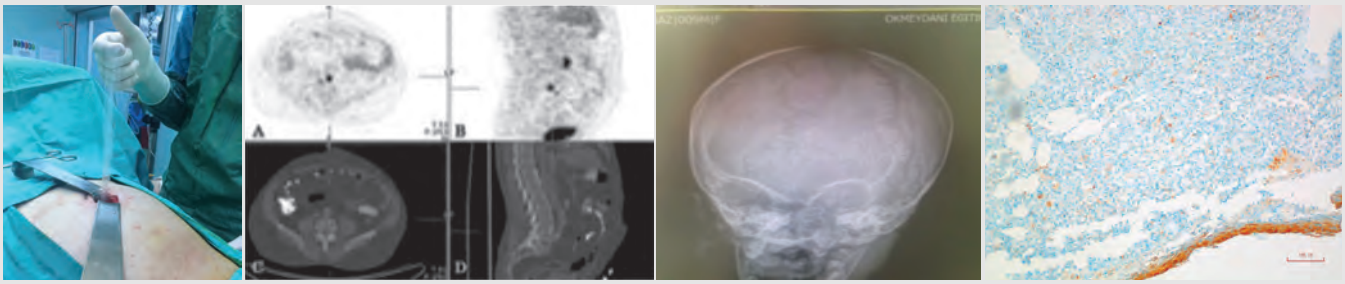
ISSN 2651-3153 • EISSN 2651-3153

European Archives of Medical Research

Formerly Okmeydanı Medical Journal

Volume: 35 • Number: 4 • December 2019



Highlights

galenos yayınevi

Official Journal of Health Sciences University, Okmeydanı Training and Research Hospital

eurarchmedres.org

European Archives of Medical Research

Formerly Okmeydanı Medical Journal

Publisher Contact

Address: Molla Gürani Mah. Kaçamak Sk. No: 21/1 34093 İstanbul, Turkey

Phone: +90 (212) 621 99 25 **Fax:** +90 (212) 621 99 27 **E-mail:** info@galenos.com.tr/yayin@galenos.com.tr **Web:** www.galenos.com.tr

Publisher Certificate Number: 14521

Printing Date: December 2019

ISSN: 2651-3137 **E-ISSN:** 2651-3153

International scientific journal published quarterly.

European Archives of Medical Research

Formerly Okmeydanı Medical Journal

AIMS AND SCOPE

European Archives of Medical Research (Eur Arch Med Res) is the scientific, peer-reviewed, open access publication of Health Sciences University, Okmeydanı Training and Research Hospital. The journal is a quarterly publication, published on March, June, September, December. The publication language of the journal is English.

European Archives of Medical Research aims to contribute to the international literature by publishing original clinical and experimental research articles, case reports, review articles, and letters to the editor on all fields of medicine.

The target audience of the journal includes researchers, general practitioners and specialists from all fields of medicine.

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

European Archives of Medical Research is currently indexed in TUBITAK ULAKBIM TR Index, Türk Medline, Türkiye Atıf Dizini, J-GATE, ROOT INDEXING and EBSCO Academic Search Complate.

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

All expenses of the journal are covered by the Health Sciences University, Okmeydanı Training and Research Hospital. Potential advertisers should contact the Editorial Office. Advertisement images are published only upon the Editor-in-Chief's approval.

Statements or opinions expressed in the manuscripts published in the journal reflect the views of the author(s) and not the opinions of the Health Sciences University, Okmeydanı Training and Research Hospital, editors, editorial board, and/or publisher; the editors, editorial board, and publisher disclaim any responsibility or liability for such materials.

All published content is available online, free of charge at eurarchmedres.org.

Editor in Chief: Tamer Özülker

Address: Department of Nuclear Medicine, University of Health Sciences Okmeydanı Training and Research Hospital, İstanbul, Turkey

Phone: +90 212 314 63 24

E-mail: tozulker@gmail.com

Publishing House: Galenos Yayınevi

Address: Molla Gürani Mah. Kaçamak Sk. No: 21/1 34093 Fındıkzade, İstanbul, Turkey

Phone: +90 (212) 621 99 25

Fax: +90 (212) 621 99 27

E-mail: info@galenos.com.tr/yayin@galenos.com.tr

European Archives of Medical Research

Formerly Okmeydanı Medical Journal

INSTRUCTIONS TO AUTHORS

European Archives of Medical Research (Eur Arch Med Res) is the scientific, peerreviewed, open access publication of Health Sciences University, Okmeydanı Training and Research Hospital. The journal is a quarterly publication, published on March, June, September, December. The publication language of the journal is English.

The aim of the European Archives of Medical Research is to publish original research papers of the highest scientific and clinical value in all medical fields. Eur Arch Med Res also includes reviews, rare case reports, and letters to the editor that are related to recently published articles.

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

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

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

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

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

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

Each individual listed as an author should fulfill the authorship criteria recommended by the International Committee of Medical Journal Editors

(ICMJE - www.icmje.org). The ICMJE recommends that authorship be based on the following 4 criteria:

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

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

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

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

European Archives of Medical Research

Formerly Okmeydanı Medical Journal

INSTRUCTIONS TO AUTHORS

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

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

European Archives of Medical Research requires each submission to be accompanied by a Copyright License Agreement (available for download eurarchmedres.org). When using previously published content, including figures, tables, or any other material in both print and electronic formats, authors must obtain permission from the copyright holder. Legal, financial and criminal liabilities in this regard belong to the author(s). By signing the Copyright License Agreement, authors agree that the article, if accepted for publication by the European Archives of Medical Research, will be licensed under a Creative Commons Attribution-Non Commercial 4.0 International License (CC-BY-NC).

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

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

MANUSCRIPT PREPARATION

The manuscripts should be prepared in accordance with ICMJE-Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals (updated in December 2017 - http://www.icmje.org/icmje-recommendations.pdf). Authors are required to prepare manuscripts in accordance with the CONSORT guidelines for randomized research studies, STROBE guidelines for observational original research studies, STARD guidelines for studies on diagnostic accuracy, PRISMA guidelines for systematic reviews and meta-analysis, ARRIVE guidelines for experimental animal studies, and TREND guidelines for non-randomized public behavior.

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

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

Authors are required to submit the following:

Copyright Licence Agreement, Author Contributions Form, and, ICMJE Potential Conflict of Interest Disclosure Form (should be filled in by all contributing authors) during the initial submission. This form is available for download at eurarchmedres.org.

Preparation of the Manuscript

All manuscripts should be submitted in 12 point Times New Roman type with 2 line spacing.

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

- The full title of the manuscript as well as a short title (running head) of no more than 50 characters,
- Name(s), affiliations, highest academic degree(s), e-mail addresses, and ORCID IDs of the author(s),
- Grant information and detailed information on the other sources of support,
- Name, address, telephone (including the mobile phone number) and fax numbers, and email address of the corresponding author,
- Acknowledgment of the individuals who contributed to the preparation of the manuscript but who do not fulfill the authorship criteria.

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

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

European Archives of Medical Research

Formerly Okmeydanı Medical Journal

INSTRUCTIONS TO AUTHORS

Manuscript Types

Original Articles: This is the most important type of article since it provides new information based on original research. The main text of original articles should be structured with Introduction, Methods, Results, Discussion, and Conclusion subheadings. Please check Table 1 for the limitations for Original Articles.

Statistical analysis to support conclusions is usually necessary. Statistical analyses must be conducted in accordance with international statistical reporting standards (Altman DG, Gore SM, Gardner MJ, Pocock SJ. Statistical guidelines for contributors to medical journals. Br Med J 1983: 7; 1489-93). Information on statistical analyses should be provided with a separate subheading under the Materials and Methods section and the statistical software that was used during the process must be specified.

Units should be prepared in accordance with the International System of Units (SI).

Editorial Comments: Editorial comments aim to provide a brief critical commentary by reviewers with expertise or with high reputation in the topic of the research article published in the journal. Authors are selected and invited by the journal to provide such comments. Abstract, Keywords, and Tables, Figures, Images, and other media are not included.

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

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

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

Tables

Tables should be included in the main document, presented after the reference list, and they should be numbered consecutively in the order they are referred to within the main text. A descriptive title must be placed above the tables.

Abbreviations used in the tables should be defined below the tables by footnotes (even if they are defined within the main text). Tables should be created using the "insert table" command of the word processing software and they should be arranged clearly to provide easy reading. Data presented in the tables should not be a repetition of the data presented within the main text but should be supporting the main text.

Table 1. Limitations for each manuscript type

Type of manuscript	Word limit	Abstract word limit	Reference limit	Table limit	Figure limit
Original Article	3500	250 (Structured)	40	6	7 or total of 15 images
Review Article	5000	250	80	6	10 or total of 20 images
Case Report	1000	200	15	No tables	10 or total of 20 images
Letter to the Editor	500	No abstract	5	No tables	No media

Figures and Figure Legends

Figures, graphics, and photographs should be submitted as separate files (in TIFF or JPEG format) through the submission system. The files should not be embedded in a Word document or the main document. When there are figure subunits, the subunits should not be merged to form a single image. Each subunit should be submitted separately through the submission system. Images should not be labeled (a, b, c, etc.) to indicate figure subunits. Thick and thin arrows, arrowheads, stars, asterisks, and similar marks can be used on the images to support figure legends. Like the rest of the submission, the figures too should be blind. Any information within the images that may indicate an individual or institution should be blinded. The minimum resolution of each submitted figure should be 300 DPI. To prevent delays in the evaluation process, all submitted figures should be clear in resolution and large in size (minimum dimensions: 100 × 100 mm). Figure legends should be listed at the end of the main document.

All acronyms and abbreviations used in the manuscript should be defined at first use, both in the abstract and in the main text. The abbreviation should be provided in parentheses following the definition.

When a drug, product, hardware, or software program is mentioned within the main text, product information, including the name of the product, the producer of the product, and city

INSTRUCTIONS TO AUTHORS

and the country of the company (including the state if in USA), should be provided in parentheses in the following format: "Discovery St PET/CT scanner (General Electric, Milwaukee, WI, USA)"

All references, tables, and figures should be referred to within the main text, and they should be numbered consecutively in the order they are referred to within the main text.

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

References

While citing publications, preference should be given to the latest, most up-to-date publications. If an ahead-of-print publication is cited, the DOI number should be provided. Authors are responsible for the accuracy of references. Journal titles should be abbreviated in accordance with the journal abbreviations in Index Medicus/ MEDLINE/PubMed. When there are six or fewer authors, all authors should be listed. If there are seven or more authors, the first six authors should be listed followed by "et al." In the main text of the manuscript, references should be cited using Arabic numbers in parentheses. The reference styles for different types of publications are presented in the following examples.

Journal Article: Stephane A. Management of Congenital Cholesteatoma with Otoendoscopic Surgery: Case Report. Turkiye Klinikleri J Med Sci 2010;30:803-7.

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

Books with a Single Author: Sweetman SC. Martindale the Complete Drug Reference. 34th ed. London: Pharmaceutical Press;2005.

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

Conference Proceedings: Bengisson S. Sothemin BG. Enforcement of data protection, privacy and security in medical informatics. In: Lun KC, Degoulet P, Piemme TE, Rienhoff O, editors. MEDINFO 92. Proceedings of the 7th World Congress on Medical Informatics; 1992 Sept 6-10; Geneva, Switzerland. Amsterdam: North-Holland;1992. pp.1561-5.

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

Thesis: Yılmaz B. Ankara Üniversitesindeki Öğrencilerin Beslenme Durumları, Fiziksel Aktiviteleri ve Beden Kitle İndeksleri Kan Lipidleri Arasındaki Ilişkiler. H.Ü. Sağlık Bilimleri Enstitüsü, Doktora Tezi. 2007.

Manuscripts Accepted for Publication, Not Published Yet: Slots J. The microflora of black stain on human primary teeth. Scand J Dçent Res. 1974. Epub Ahead of Print Articles: Cai L, Yeh BM, Westphalen AC, Roberts JP, Wang ZJ. Adult living donor liver imaging. Diagn Interv Radiol 2016 Feb 24. doi: 10.5152/dir.2016.15323. [Epub ahead of print].

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

REVISIONS

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

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

Editor in Chief: Tamer Özülker

Address: Department of Nuclear Medicine, University of Health Sciences Okmeydanı Training and Research Hospital, İstanbul, Turkey
Phone: +90 212 314 63 24
E-mail: tozulker@gmail.com

Publishing House: Galenos Yayınevi

Address: Molla Gürani Mah. Kaçamak Sk. No: 21/1 34093 Fındıkzade, İstanbul, Turkey
Phone: +90 (212) 621 99 25
Fax: +90 (212) 621 99 27
E-mail: info@galenos.com.tr/yayin@galenos.com.tr

European Archives of Medical Research

Formerly Okmeydanı Medical Journal

CONTENTS

ORIGINAL ARTICLES

CASE REPORT

ORIGINAL ARTICLE

DOI: 10.4274/eamr.galenos.2018.82687
Eur Arch Med Res 2019; 35 (4):193-6

Effect of Epidermal Growth Factor on Osteosarcoma Cell Proliferation and *Bcl-2* Gene Expression

Ayla Solmaz Avcıkurt[1], Eren Altun[2]

[1]*Balıkesir University Faculty of Medicine, Department of Medical Biology, Balıkesir, Turkey*
[2]*Balıkesir University Faculty of Medicine, Department of Medical Pathology, Balıkesir, Turkey*

Abstract

Objective: The aim of this study was to investigate the effects of epidermal growth factor (EGF) on osteosarcoma cell proliferation and Bcl-2 expression.

Methods: In this study, MTT test was first applied to determine the effect of EGF on cell proliferation. Twenty ng/µL EGF was added to the Saos-2 cell line and the effects on cell proliferation were determined after different incubation intervals. At the next experimental stage, 20 ng/mL EGF was added to the osteosarcoma cell line and the expression of antiapoptotic Bcl-2 was evaluated at 1-, 3-, 24-, 48- and 72-hour.

Results: A statistically significant increase in cell proliferation was observed at 3-, 24- and 72-hour after administration of 20 ng/µL EGF in the osteosarcoma cell line. EGF was observed to stimulate Bcl-2 expression at a dose of 20 ng/mL at 1- and 24-hour.

Conclusion: EGF; at the 3rd, 24th and 72nd hours, a statistically significant increase in cell proliferation was observed. In addition, the expression of antiapoptotic Bcl-2 was evaluated at the 1st, 3rd, 24th, 48th and 72th hours on the EGF osteosarcoma cell line. The expression of Bcl-2 was increased at the end of the 1st and 24th hours of application of EGF at 20 ng/mL dose.

Keywords: Epidermal growth factor, Bcl-2, osteosarcoma

INTRODUCTION

Osteosarcoma (OS) is a malignant mesenchymal tumor associated with the formation of mineralized or non-mineralized bone. OS is the most common primary malignant bone tumor and is mostly seen in young adults and adolescents (1). Apoptosis is a genetically regulated cell death that controls the development of tissues by eliminating physiologically unnecessary abnormal cells (2). Studies focusing on genes and signals that regulate apoptosis play an important role in basic oncology research (3). Chemotherapeutics destroy tumor cells and do so primarily by promoting tumor cell apoptosis (4). Understanding that apoptosis is a gene-directed program has profound effects on developmental biology and tissue homeostasis (5,6). The *Bcl-2* gene family is the key regulator of apoptosis. The gene group containing Bcl-2 and Bcl-xL has anti-apoptotic activity. The second group of proteins such as Bax or Bim promotes cell death and has pro-apoptotic activity (7,8). Epidermal growth factor (EGF) is a 53-amino-acid peptide that is encoded by a 4.8 kb mRNA transcript from a gene that is 110 kb in length, contains 24 exons, and is located on human chromosome 4q25. Like other members of this peptide family, EGF is initially synthesized as a prepropeptide of 1217 amino acids. Members of this family include pre-pro-EGF molecule, a hydrophobic signal peptide, and a transmembrane domain (9). EGF, which acts as a potent mitogenic factor that plays an important role in the growth, proliferation and differentiation of many countless cell types, is a protein that acts by binding to its receptor with high affinity.

The aim of this study was to investigate the effects of EGF on cell proliferation and Bcl-2 expression in the human OS model Saos-2 cell line at different time intervals of 1-, 3-, 24-, 48- and 72-hour.

Address for Correspondence: Ayla Solmaz Avcıkurt, Balıkesir University Faculty of Medicine, Department of Medical Biology, Balıkesir, Turkey
Phone: +90 532 551 40 79 E-mail: aylaavci@balikesir.edu.tr ORCID ID: orcid.org/0000-0002-1521-7152

Cite this article as: Solmaz Avcıkurt A, Altun E. Effect of Epidermal Growth Factor on Osteosarcoma Cell Proliferation and *Bcl-2* Gene Expression. Eur Arch Med Res 2019; 35 (4): 193-6

Received: 13.09.2017
Accepted: 11.09.2018

METHODS

Materials

The human OS cell line Saos-2 was obtained from European Collection of Animal Cell Cultures. EGF was purchased from PeproTech. All cell culture material and materials were purchased from Greiner or Gibco.

Cell Culture and Epidermal Growth Factor Applications

Human OS cell line (Saos-2) cells were grown in a CO_2 oven at 37 °C in an atmosphere of 5% CO_2. Fifteen ml of Dulbecco's Modified Eagle Medium (DMEM) containing 10% heat inactivated fetal calf serum (FCS), 100 units/mL penicillin, 10 µg/mL streptomycin and 0.2 mM L-glutamine were used as food medium. Incubation was performed at 1-, 3-, 24-, 48- and 72-hour with 20 ng/mL administration. Total RNA was isolated from the cells at the end of the period.

Establishment of Cytotoxicity Tests and MTT Test

When the cells grown in a 75 cm^2 flask in 15 mL medium covered the 80-85% of flask surface, the medium was removed and the cells were washed twice with sterile PBS. Four mL of trypsin-EDTA was added to the flask. It was incubated for 5 minutes in a CO_2 incubator. When cells were separated from the surface, medium was added to neutralize trypsin-EDTA. The cells were precipitated by centrifugation at 1000 rpm for 5 minutes, the supernatant was removed and the pellet was thawed with 10 mL of medium. The number of viable cells in the suspension was determined by trypan blue staining and seeded in a 96 well plate with 5000 cells per well. DMEM medium containing 10% FCS was added to each well to a final volume of 200 µL. After cell seeding was completed, the cells were incubated in a CO_2 incubator for 24 hours. At the end of 24 hours, EGF was administered at a dose of 20 ng/mL. MTT test was performed at 1-, 3-, 24-, 48- and 72-hour, and absorbance was taken at 550 nm. The MTT method is based on the ability of viable cells to transform MTT (a tetrazolium salt) into formazan crystals. It is one of the most commonly used methods for measuring cell cytotoxicity, proliferation and viability. According to this method, after the desired incubation period (1-, 3-, 24-, 48- and 72-hour), the stock MTT solution is added to the medium where the final concentration determined as a result of the optimization is 0.5 mg/mL and it is incubated for 4 hours at 37 °C in a medium containing 5% CO_2. At the end of the incubation, the medium containing MTT solution is discarded, the crystals are dissolved with isopropanol containing 0.004 M HCl and absorbance is taken at 550 nm wavelength with UV spectrophotometer.

cDNA Synthesis

Total RNA isolation was performed using RNeasy total RNA isolation kit (Qiagen). cDNA synthesis was performed from the isolated total RNA. Briefly, 1 µL random primer and 1 µg total RNA was mixed. Distilled water was added to complete the final volume to 10 µL. This mixture was incubated for 10 minutes at 70 °C and then on ice, 5 × Moloney murine leukaemia virus (MMLV) buffer, dNTP mixture (10 mM each; dATP, dGTP, dTTP and dCTP), RNase inhibitor (RNasin) and MMLV reverse transcriptase enzyme was added and incubated at 42 °C for 50 minutes. The synthesized cDNAs were stored in the refrigerator at -20 °C.

Realtime Polymerase Chain Reaction

These studies were performed using the applied biosystems 7500 fast instrument. Five µL master mix, 1 µL cDNA, 100 ng/µL 0.5 µL forward and reverse primers, 3 µL distilled H_2O were mixed and final volume was completed to 10 µL. Each cDNA was studied with *Bcl-2* and β-*2-microglobulin* genes for normalization with at least three replicates.

Statistical Analysis

Mean MTT test results and standard deviations were obtained. The absorbance values obtained at each hour were compared with their control group using the Student's t-test. P≤0.05 was considered statistically significant. Livak method was applied to evaluate realtime polymerase chain reaction test results. Each of the CT values obtained for the *Bcl-2* gene was subtracted from the mean of the human β-*2-microglobulin* gene and a base-2 log square was obtained. The results were divided by the control group and obtained multiples of 1 was statistically evaluated by Minitab (One-way ANOVA). P≤0.05 values were considered significant. Ethical permission is not required in this study since as only cell viability was evaluated in cell culture.

Table 1. Mean and standard deviations of absorbance values at 1-, 3-, 24-, 48- and 72-hour of MTT test, and p values (each test group was compared with the control in its own hour interval)

Group	NK (1-h)	EGF (1-h)	NK (3-h)	EGF (3-h)	NK (24-h)	EGF (24-h)	NK (48-h)	EGF (48-h)	NK (72-h)	EGF (72-h)
Mean	0.3451	0.4345	0.2066	0.2581	0.2183	0.357	0.2399	0.3173	0.1988	0.4175
SD	0.0394	0.0316	0.0851	0.0296	0.0384	0.0704	0.0414	0.0656	0.0322	0.0280
p	-	0.040	-	0.409	-	0.040	-	0.172	-	0.01

SD: Standard deviation, EGF: Epidermal growth factor, NK: Naturel killer cell

RESULTS

In this study, firstly, MTT test was used to determine the effect of EGF on cell proliferation. Twenty ng/µL EGF was added to the Saos-2 cell line and its effects on cell proliferation were determined at different incubation intervals. A statistically significant increase in cell proliferation was observed at 3-, 24- and 72-hour (Table 1). At the next experimental stage, 20 ng/mL EGF was added to the OS cell line and expression of antiapoptotic Bcl-2 was evaluated at 1-, 3-, 24-, 48- and 72-hour. It was observed that EGF at a dose of 20 ng/mL increased Bcl-2 expression at 1- and 24-hour (Table 2).

Table 2. Mean, standard deviation and p values of real time polymerase chain reaction

Group	NK	1-hour	3-hour	24-hour	48-hour	72-hour
Mean	1	1.9291	1.289	3.126	0.98	0.8797
SD	0	0.2710	0.4870	0.8393	0.6422	0.1188
p	-	0.04	0.342	0.012	0.952	0.154

NK: Naturel killer cell, SD: Standard deviation

DISCUSSION

Cancer is the leading cause of death in the world. Some molecules in humans are important in the process of cancer. Some molecules increase cancer while others function to reduce it. Apoptosis is an inherited process of cell death specific to multicellular eukaryotic organisms. It plays a critical role in the destruction of cells damaged by infection, chemical damage, oxidative damage or radiation (10). Molecules that contribute to the apoptosis process contribute to cell survival or preparation for programmed death. The gene family involved in apoptosis is the *Bcl-2* gene family. There are members of this family that prevent apoptosis (Bcl-2, BcX2, Mcl-1) and lead to apoptosis (Bax, Bak, Bid, Bim, Noxo, Puma). According to the equilibrium in the expression of these members, the cell is directed to apoptosis. Bcl-2, which is the first anti-apoptotic member of the *Bcl-2* gene family, is one of the molecules that inhibit apoptosis (11). If there is any damage to the mechanism of apoptosis, the cells tend to become cancerous (12). Anti-apoptotic Bcl-2 expression is important in the cancer process (13). EGF acts by binding with EGF receptor (EGFR). Signaling with EGFR leads to cell proliferation and differentiation (14). Chandra et al. (15) showed that EGFR signaling increased proliferation and inhibited apoptosis and was important in maintaining the number of osteoprogenitor cells. In the same study, EGF treatment has been shown to significantly increase the number of osteoblasts by regulating the proportion of proliferative and apoptotic osteoprogenitor cells in the bone (15). This signaling increases the proliferation and survival of osteoprogenitor cells and consequently increases the formation of new bone (15).

CONCLUSION

EGF is thought to increase the progression of various cancers through proliferation, invasion and induction of angiogenesis (16). In this study, the effects of EGF on cell proliferation in Saos-2 cell line which is a model of human OS and the expression of antiapoptotic Bcl-2 were investigated. EGF significantly affected cell proliferation at 3-, 24- and 72-hour. EGF increased Bcl-2 expression in the osteosarcoma cell line at 1- and 24-hour of administration. Regarding our results, EGF-regulated Bcl-2 expression is found to be critical for the survival of neoplastic cells in the OS cell line.

Ethics

Ethics Committee Approval: Not applicable.

Informed Consent: Not applicable.

Peer-review: External and internal peer-reviewed.

Authorship Contributions

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

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

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

REFERENCES

1. Zhang Y, Hu H, Song L, Cai L, Wei R, Jin W. Epirubicin-mediated expression of miR-302b is involved in osteosarcoma apoptosis and cell cycle regulation. Toxicol Lett 2013;222:1-9.
2. Liu K, Liu PC, Liu R, Wu X. Dual AO/EB staining to detect apoptosis in osteosarcoma cells compared with flow cytometry. Med Sci Monit Basic Res 2015;21:15-20.
3. Su Z, Yang Z, Xu Y, Chen Y, Yu Q. Apoptosis, autophagy, necroptosis, and cancer metastasis. Mol Cancer 2015:21;14:48.
4. Taraphdar AK, Roy M, Bhattacharya R. Natural products as inducers of apoptosis: Implication for cancer therapy and prevention. Curr Sci 2001;80:1387-96.
5. Lowe SW, Lin AW. Apoptosis in cancer. Carcinogenesis 2000;21:485-95.
6. Strasser A, Cory S, Adams JM. Deciphering the rules of programmed cell death to improve therapy of cancer and other diseases. EMBO J 2011;30:3667-83.

7. Moon JH, Sohn SK, Lee MH, Jang JH, Kim K, Jung CW, et al. BCL2 gene polymorphism could predict the treatment outcomes in acute myeloid leukemia patients. Leuk Res 2010;34:166-72.

8. Czabotar PE, Lessene G, Strasser A, Adams JM. Control of apoptosis by the BCL-2 protein family: implications for physiology and therapy. Nat Rev Mol Cell Biol 2014;15:49-63.

9. Salomon DS, Brandt R, Ciardiello F, Normanno N. Epidermal growth factor-related peptides and their receptors in human malignancies. Crit Rev Oncol Hematol 1995;19:183-232.

10. Anderson MA, Huang D, Roberts A. Targeting BCL2 for the treatment of lymphoid malignancies. Semin Hematol 2014;51:219-27.

11. Yalçın O, Demir FT, Kılıç AI, Kaya H, Kabukçuoğlu F, Altunay IK. Bcl-2: Is it an Easier Way to Differentiate Psoriasis and Eczema? Okmeydanı Tıp Dergisi 2016;32:190-4.

12. Cory S, Adams JM. The Bcl2 family: regulators of the cellular life-or-death switch. Nature reviews. Cancer 2002;9:647.

13. Sarosiek KA, Letai A. Directly targeting the mitochondrial pathway of apoptosis for cancer therapy using BH3 mimetics–recent successes, current challenges and future promise. FEBS J 2016;283:3523-33.

14. Yarım G F, Kazak F. Epidermal Büyüme Faktörü. Kocatepe Vet J 2016;9:215-25.

15. Chandra A, Lan S, Zhu J, Siclari VA, Qin L. Epidermal growth factor receptor (EGFR) signaling promotes proliferation and survival in osteoprogenitors by increasing early growth response 2 (EGR2) expression. J Biol Chem 2013;288:20488-98.

16. Guillamo JS, de Boüard S, Valable S, Marteau L, Leuraud P, Marie Y, et al. Molecular mechanisms underlying effects of epidermal growth factor receptor inhibition on invasion, proliferation, and angiogenesis in experimental glioma. Clin Cancer Res 2009;15:3697-704.

ORIGINAL ARTICLE

DOI: 10.4274/eamr.galenos.2018.56578
Eur Arch Med Res 2019; 35 (4):197-200

Age-related Differences in Homocysteine and Serological Markers in Patients with Sudden Sensorineural Hearing Loss

Mehmet Burak Aşık[1], Erbil Kılıç[2], Murat Bınar[1]

[1]University of Health Sciences, Gülhane Training and Research Hospital, Clinic of Ear, Nose, Throat, Ankara, Turkey
[2]İstanbul Sultan Abdülhamid Han Training and Research Hospital, Clinic of Ear, Nose, Throat, İstanbul, Turkey

Abstract

Objective: Sudden hearing loss is mostly a unilateral sensorineural hypoacusis with the highest incidence in young adults. The aim of this study was to determine the differences in homocysteine and serological marker levels in sudden sensorineural hearing loss and to define the importance of these markers in diagnosis.

Methods: After audiological examination, 52 patients were considered as having sudden hearing loss. Serological markers (C3, C4, anti-streptolysin-O, C-reactive protein, rheumatoid factor) and homocysteine were examined. The patients were divided into two groups, as patients above and below 40 years of age. Statistical analysis was performed using SPSS version 15 software. P values less than 0.05 were considered statistically significant.

Results: Fifty-two patients were included in the study, and the study group consisted of 33 men and 19 women. The mean age was 43.5 years and the age range was 14-82 years. According to the survey results, 63% of the patients had higher than normal homocysteine levels. It was observed that 60.8% of homocysteine levels were increased in patients below 40 years of age and 69% in patients over 40 years of age. There was no statistically significant difference between the two groups (p=0.49). In the evaluation of immunological serological markers, no parameter was observed except for C3c. Fifty-four percent of all patients were considered to have a C3c parameter below normal limits. C3c levels were decreased in 52% of patients below 40 years of age and 55% of patients over 40 years of age. There was no statistically significant difference between the two groups (p=0.31).

Conclusion: It is thought that microvascular and immunological pathogenesis develops in sudden hearing loss. Homocysteine and C3 levels are valuable but age-related parameters following diagnosis and treatment.

Keywords: Sudden sensorineural hearing loss, homocysteine, serology, prognosis

INTRODUCTION

Sudden hearing loss is a clinical condition that is frequently idiopathic and develops in the last 3 days. It is described audiologically as sensorineural hearing loss of more than 30 dB at least 3 consecutive frequencies (1). The incidence of the disease varies between 5-20 per 100,000 per year and peak incidence occurs between the ages of 41-50 years, with no difference between male and female genders (1-3). In addition, being over 40 years of age has been reported to have a negative effect (4). Vascular, autoimmune, infectious and intracochlear pathological processes have been implicated in the mechanism of the disease (1,2,5,6). Although all of these processes play different roles in the pathophysiology, their effects on the cochlea cannot be determined biochemically and serologically (5). Although vascular, autoimmune, infectious and intracochlear mechanisms have been held responsible, their role in the etiology has not been fully established. Changes in the levels of biochemical and serological markers provide information about the role of these theories in sudden hearing loss. In the literature, lipid profile and homocysteine were thought to play a role in microvascular processes (1). Similarly, in the literature, accompanying autoimmune diseases have been reported in many patients with sudden hearing loss (7,8). The treatment and prognosis of sudden hearing loss depends on the accuracy of the diagnosis of the disease (9). Diagnosis is made by audiometry and clinical criteria in these patients, but it cannot be supported by biochemical and serological tests. The aim of this study was to determine the changes in the levels of immunological

Address for Correspondence: Mehmet Burak Aşık, University of Health Sciences, Gülhane Training and Research Hospital, Clinic of Ear, Nose, Throat, Ankara, Turkey
Phone: +90 505 735 34 89 E-mail: burock312@yahoo.com ORCID ID: orciod.org/0000-0002-1093-841X

Cite this article as: Aşık MB, Kılıç E, Bınar M. Age-related Differences in Homocysteine and Serological Markers in Patients with Sudden Sensorineural Hearing Loss. Eur Arch Med Res 2019; 35 (4): 197-200

Received: 24.04.2017
Accepted: 12.09.2018

markers and acute phase reactants in sudden hearing loss and to determine the diagnostic value of these markers in the etiology of the disease.

METHODS

Serological markers and homocysteine results were evaluated retrospectively in patients who presented to our clinic with audiological and clinical diagnostic criteria of sudden hearing loss between 2011-2013 and had no organic cause of sudden hearing loss. Ethics committee approval was obtained from the Ethics Committee of Gülhane Training and Research Hospital (2012-1491-79-12/1648.3-4674). Written informed consent was obtained from the patients. Homocysteine and C3 parameters were analyzed by High-performance liquid chromatography (Shimadzu, HPLC, Kyoto, Japan). Laboratory reference values were 6-15 mmoL/L for homocysteine and 0.55-1.20 g/L for C3. Exclusion criteria were treatment history for current sudden hearing loss, sudden hearing loss due to other than idiopathic and acoustic trauma, steroid treatment due to comorbidities and other chronic diseases requiring treatment.

After audiological examinations, serological markers [C3, C4, ASO (anti-streptolysin-O), C-reactive protein (CRP), rheumatoid factor (RF)], and homocysteine were examined in patients who were considered as having sudden hearing loss. Steroid treatment was not given to the patients in order not to affect the levels of markers before taking blood for the tests. After obtaining blood, patients were treated according to their age, body weight, comorbidities and clinical conditions. The patients were divided into two groups and the groups were defined as patients above and below 40 years of age, which is considered to be important for prognosis.

Statistical Analysis

Statistical analyses were performed using SPSS version 15.0 (IBM, Chicago, Illinois, USA). The normality of the variables was analyzed by analytical methods. Descriptive analyses were expressed with mean for normally distributed variables. Resulting data were expressed as numbers and percentages. The proportional changes in homocysteine and C3 parameters were compared between groups using chi-square test. P value less than 0.05 was evaluated as statistically significant.

RESULTS

Fifty-two patients were included in the study, and the study group consisted of 33 men and 19 women. The mean age was 43.5 years and the age range was 14-82 years. The age-related data and the ratio of patients according to admission time are presented in Table 1. Table 2 presents the distribution of patients according to audiogram types. There was no history of trauma, co-morbidities, chronic treatment, family history of sudden hearing loss or previous sudden hearing loss. According to the results of the study, 63% of the patients had higher than normal homocysteine values. It was observed that homocysteine levels increased in 60.8% of patients below 40 years of age and in 69% of patients over 40 years of age. There was no statistically significant difference between the two groups (p=0.752) (Table 3). In the evaluation of immunological serological markers, no change was observed in any parameter except C3c. In 54% of all patients, C3c was found to be below normal limits. C3c levels were decreased in 52% of patients below 40 years of age and 55% of patients over 40 years of age. There was no statistically significant difference between the two groups (p=1.00) (Table 4). C3 values according to audiogram types are shown in Table 5.

Table 1. Data about the age and admission times of the patients

	Male (n=33)	Female (n=19)	Total (n=52)
Age			
<40 years	15 (45.4%)	8 (42.1%)	23 (44.2%)
>40 years	18 (54.6%)	11 (57.9%)	29 (56.8%)
Admission			
<7 days	19 (57.5%)	13 (68.4%)	32 (61.5%)
>7 days	14 (42.5%)	6 (31.6%)	20 (38.5%)

Table 2. Data of patients according to audiogram types

	<40 years	>40 years	Total
Low	5 (21.7%)	7 (24.1%)	12 (23%)
Flat	2 (8.6%)	13 (44.8%)	15 (28.9%)
Total	4 (17.3%)	2 (6.8%)	6 (11.5%)
High	12 (52.1%)	7 (24.1%)	19 (36.5%)

Table 3. Changes of homocysteine levels according to audiogram types

Low	11/12	91.6%
Flat	11/15	73.3%
Total	3/6	50%
High	9/19	47%

Table 4. Number of patients with change in C3 level

<40 years	12/23	52%
>40 years	16/29	55%
Total	28/52	54%

Table 5. C3 levels according to audiogram types		
Low	6/12	50%
Flat	8/15	53%
Total	3/6	50%
High	12/19	63.1%

DISCUSSION

The aim of this study was to evaluate the microvascular and autoimmune processes responsible for the ethiopathogenesis of sudden hearing loss by laboratory tests and to evaluate the diagnostic utility of changes in markers. As there are changes in homocysteine and C3 levels in our study, we think that they can be used for diagnostic purposes before treatment in patients with sudden hearing loss.

In the literature, both biochemical and serological acute phase reactants have been questioned in studies investigating autoimmune mechanisms in sudden hearing loss and they have been reported to be useful in diagnosis (2). Greco et al. (7) and Werneck et al. (8) reported that C3 and C4 levels decreased but C3b levels did not decrease. Consistent with the literature, 54% decrease was observed in C3 levels in our study. However, C4 levels did not change. According to Werneck et al. (8), these results support viral etiology. CRP and RF levels in our study contradict the literature. Toubi et al. (10) and Berrocal and Camacho-Ramirez (11) reported that CRP and RF levels increased and that this supported autoimmune etiology. In our study, no significant increase was detected in CRP and RF levels. Although there are sufficient publications in the literature supporting serological markers, these parameters may not be clinical and diagnostic indicators because sudden hearing loss is based on multifactorial mechanisms. Considering the reasons why CRP and RF values were not high in our patient group, it can be due to small number of patients in our study. Of course, although immunological theory is always valid for sudden hearing loss, these markers may not be elevated serologically. This may be due to the fact that patients were not carriers of any additional rheumatological and immunological diseases. Homocysteine is an amino acid that can be monitored in serum and that triggers pathological processes causing prothrombosis and hypercoagulability (7,12). Diabetes, neural diseases and dyslipidemias frequently increase the level of homocysteine (13). The role of homocysteine in prothrombotic processes can be explained by cochlear vascular occlusion (1,13). Plasma activator inhibitor-1 protein, anticardiolipin antibodies, lupus anticoagulants and lipoprotein (a) are also present in the formation of these pathological processes, and the same vascular pathological processes and markers are observed in cardiovascular diseases (12,13). There are studies reporting 3-times higher risk of cardiovascular disease in patients with bilateral sudden hearing loss, and hypercoagulability and thromboembolism were held accountable (13,14). Since 63% of the patients in our study had homocysteine levels above the normal range and homocysteine could be used as a cardiovascular risk test, we think that it can be used for routine controls. In our study, patients with cardiac disease were excluded, and we considered that homocysteine may be an appropriate marker for microvascular ethiopathogenesis in patients with sudden hearing loss due to the absence of statistically significant differences between patients above and below 40 years of age, but more studies should be performed on this issue. The limitations of our study were the low number of groups, the inability to form homogeneous groups when patients were classified according to audiogram types, and the presence of diseases that could change homocysteine and C3 levels but did not become clinically symptomatic. In addition, when we examined the statistical power of the study, the power of the study group consisting of 52 patients was 79% for C3 values and this power was calculated as 52.4% for homocysteine. It is important that the reader takes into account the power analysis results when evaluating the study results.

CONCLUSION

The diagnosis, treatment and follow-up of sudden hearing loss are based on the patient's history, physical examination and audiometric tests. Biochemical and serological markers cannot determine treatment response and prognosis, but are used only as supportive findings. As levels change independent of age after sudden hearing loss, we believe that homocysteine and C3 levels may be valuable parameters in diagnosis and treatment follow-up in sudden hearing loss with microvascular and immunological processes in the ethiopathogenesis. However, larger studies should be conducted on the changes of these parameters with age.

Ethics

Ethics Committee Approval: Ethics committee approval was obtained from the Ethics Committee of Gülhane Training and Research Hospital (2012-1491-79-12/1648.3-4674).

Informed Consent: Written informed consent was obtained from the patients.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: M.B.A., E.K., M.B., Concept: M.B.A., E.K., Design: M.B.A., E.K., Data Collection or Processing:

M.B.A., E.K., Analysis or Interpretation: M.B.A., Literature Search: M.B.A., E.K., M.B., M.B.A., E.K., M.B., Writing: S.G., M.Y.

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

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

REFERENCES

1. Cadoni G, Scorpecci A, Cianfrone F, Giannantonio S, Paludetti G, Lippa S. Serum fatty acids and cardiovascular risk factors in sudden sensorineural hearing loss: a case-control study. Ann Otol Rhinol Laryngol 2010;119:82-8.
2. Enache R, Sarafoleanu C. Prognostic factors in sudden hearing loss. J Med Life 2008;1:343-7.
3. Zhang X, Xu X, Ma W, Zhang Q, Tong B, Yu H, et al. A clinical study of sudden deafness. Acta Otolaryngol 2015;135:1030-5.
4. Wei X, He J. Analysis of prognostic factors for sudden sensorineural hearing loss. Lin Chung Erb Bi Yan Hou Tou Jing Wai Ke Za Zhi 2011;25:599-601.
5. Lasisi AO, Arinola AG. Acute Phase Reactants in Immune-Related Inner Ear Disease. Afr J Biomed Res 2010;13:113-7.
6. Suckfüll M. Perspectives on the Pathophysiology and Treatment of sudden Idiopathic Sensorineural Hearing Loss. Dtsch Arztebl Int 2009;106:669-76.
7. Greco A, Fusconi M, Gallo A, Marinelli C, Macri GF, De Vincentis M. Sudden Sensorineural Hearing Loss : An Autoimmune Disease ?. Autoimmun Rev 2011;756-61.
8. Werneck A, Gurgel L, Mello L, Albuquerque G. Sudden Sensorineural Hearing Loss: A Case Report Supporting the Immunologic Theory. Arq Neuropsiquiatr 2003;61:1018-22.
9. Wilson H, Alderson DJ. Sudden Sensorineural Hearing Loss: When is it Idiopatic?. J Laryngol Otol 2010;124:690-3.
10. Toubi E, Ben-David J, Kessel A, Halas K, Sabo E, Luntz M. Immune-mediated Disorders Associated with Idiopathic Sudden Sensorineural Hearing Loss. Ann Otol Rhinol Laryngol 2004;113:445-9.
11. Berrocal JR., Camacho-Ramirez R. Sudden Sensorineural Hearing Loss: Supporting the Immunologic Theory. Ann Otol Rhinol Laryngol 2002;111:989-97.
12. Ay M, Gürbilen M, Vatansev H. Akut Faz Proteinleri. Gen Tıp Der 1998;8:124-32.
13. Marcucci R, Alessandrello Liotta A, Cellai AP, Rogolino A, Berloco P, Leprini E, et al. Cardiovascular and Thrombophilic Risk Factors for Idiopathic Sudden Sensorineural Hearing Loss. J Thromb Haemost 2005;3:929-34.
14. Oh JH, Park K, Lee SJ, Shin YR, Choung YH. Bilateral versus Unilateral Sudden Sensorineural Hearing Loss. Otolaryngol Head Neck Surg 2007;136:87-91.

ORIGINAL ARTICLE

DOI: 10.4274/eamr.galenos.2018.28199
Eur Arch Med Res 2019; 35 (4):201-4

Prediction of Single Dose Methotrexate Success in Ectopic Pregnancy Treatment

Berna Aslan Çetin[1], Begüm Aydoğan Mathyk[2]

[1]University of Health Sciences, Kanuni Sultan Süleyman Training and Resarch Hospital, Clinic of Obstetrics and Gynecology, İstanbul, Turkey
[2]University of North Carolina Faculty of Medicine, Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology and Infertility, Chapel Hill, North Carolina, USA

Abstract

Objective: To investigate the efficacy of single-dose methotrexate treatment and the factors affecting its success in the treatment of ectopic pregnancy.

Methods: Ectopic pregnancy cases treated with single dose methotrexate treatment between January 2013 and December 2017 were compared as successful and unsuccessful. Demographic characteristics of patients, ectopic mass size, beta human chorionic gonodotropin (β-hCG) values at the beginning of treatment and at 4th and 7th days were evaluated.

Results: A total of 462 patients, who underwent single dose methotrexate treatment as initial ectopic pregnancy treatment, were included in the study. Single dose methotrexate treatment was successful in 350 patients (75.8%) and failed in 112 patients (24.2%). Serum β-hCG levels on the day of methotrexate administration, β-hCG values at 4th day and at 7th day, and ectopic mass size were significantly higher in the failed group ($p<0.001$).

Conclusion: There are a number of factors affecting the success of single dose methotrexate treatment in ectopic pregnancy in hemodynamically stable patients. The most important of these are ectopic mass size and initial β-hCG value.

Keywords: Ectopic pregnancy, single dose, methotrexate

INTRODUCTION

Ectopic pregnancy is the implantation of a blastocyst somewhere other than the endometrial cavity (1). It is one of the most important causes of mortality and morbidity in the first trimester (2). The incidence in all pregnancies varies between 0.5 and 2% (1). The incidence of ectopic pregnancy has increased in recent years due to the increase in the frequency of previous pelvic infections, increased use of intrauterine devices, increased incidence of pelvic inflammatory disease, and increased pregnancy assisted reproductive techniques (3). Early diagnosis and treatment of ectopic pregnancies can be made thanks to the fact that beta human chorionic gonodotropin (β-hCG) values can be routinely examined and transvaginal ultrasonography becomes widespread (4). Medical treatment, surgical treatment or wait and see method are the treatment options. Patients can benefit from medical treatment when the diagnosis can be made while the patients are hemodynamically stable and before rupture (5,6). Medical treatment has many advantages over other treatments; these include less tubal damage, lower cost and preservation of fertility. Single dose methotrexate treatment is an effective and safe medical treatment and success rates vary between 64% and 94% (7). Methotrexate dose may be repeated or surgery may be performed in patients who fail single dose treatment (8-11). In this study, we aimed to compare successful and unsuccessful patients who underwent single-dose methotrexate treatment for ectopic pregnancy in our hospital and to determine the factors affecting success.

METHODS

Our study is a retrospective case-control study involving patients who were treated for ectopic pregnancy in the Clinic of Obstetrics and Gynecology, Kanuni Sultan Süleyman Training

Address for Correspondence: Berna Aslan Çetin, University of Health Sciences, Kanuni Sultan Süleyman Training and Resarch Hospital, Clinic of Obstetrics and Gynecology, İstanbul, Turkey
Phone: +90 533 220 13 14 E-mail: bernaaslan14@hotmail.com ORCID ID: orcid.org/0000-0001-6856-1822

Cite this article as: Aslan Çetin B, Aydoğan Mathyk B. Prediction of Single Dose Methotrexate Success in Ectopic Pregnancy Treatment. Eur Arch Med Res 2019; 35 (4): 201-4

Received: 09.04.2018
Accepted: 27.08.2018

and Research Hospital between January 2013 and December 2017. After the approval of the ethics committee, the records of 461 patients who were hospitalized with ectopic pregnancy and who received single-dose methotrexate treatment were evaluated retrospectively. Diagnosis of ectopic pregnancy was made when serum β-hCG level >1500 mIU/mL and transvaginal ultrasonography failed to show intrauterine gestational sac or succeeded to show ectopic focus, or less than 60% increase in β-hCG levels in 48 hours or plateau when β-hCG level <1500 mIU/mL, or absence of chorionic villus in pathological examination after dilatation curettage and/or patency of elevated β-hCG values despite curettage. The definite contraindications of methotrexate treatment were unstable hemodynamics, active hepatic or renal disease, and relative contraindications were β-hCG >10.000 mIU/mL and fetal cardiac activity. A single dose of 50 mg/m^2 methotrexate was administered intramuscularly. Treatment was considered successful if β-hCG levels decreased by more than 15% between the 4th and 7th days of treatment and the serum β-hCG levels decreased below 5 mIU/mL at weekly follow-up. Patients who had an increase in serum β-hCG concentration, less than 15% decrease on the 7th day of treatment or rupture were treated with a second dose of methotrexate or surgical treatment. Treatment was considered to be unsuccessful in patients who required surgery or second dose methotrexate.

Statistical Analysis

Statistical analyzes were performed using the Statistical Package for Social Sciences 20.0 (SPSS Inc., Chicago, IL, USA) program. The distribution of the data was evaluated by Kolmogorov-Smirnov test. Descriptive statistical methods (mean, standard deviation) as well as independent t-test were used for the comparison of the normally distributed data. Results were evaluated at p<0.05 level of significance. In ROC analysis, serum β-hCG levels and cut-off values, sensitivity and specificity were determined for methotrexate success at the 1st, 4th and 7th days.

RESULTS

A total of 462 patients who were diagnosed as ectopic pregnancy between January 2013 and December 2017 and treated with single dose methotrexate as initial treatment were included in the study. Single dose methotrexate treatment was successful in 350 (75.8%) patients, but failed in 112 (24.2%) patients and the second dose methotrexate or surgical treatment was applied in these patients.

Table 1 shows a comparison of the demographic and clinical characteristics of successful and unsuccessful groups. There was no significant difference between the two groups in terms of age, gravida, parity, hemoglobin and hematocrit values. Serum β-hCG values on the day of methotrexate administration, β-hCG levels at the 4th and 7th day and ectopic mass size were significantly higher in the unsuccessful group (p<0.001). Figure 1 shows ROC analysis for methotrexate success and β-hCG levels and mass size. According to the ROC analysis, when the cut-off value of β-hCG was taken as 2.000 mIU/mL on the day of methotrexate administration, the sensitivity and specificity were 81% and 83%, respectively. When the cut-off value of β-hCG was taken as 2.500 mIU/mL at the 4th day, sensitivity was 82%, specificity was 90%, and when the cut-off value of β-hCG was taken as 2.100 mIU/mL at the 7th day, sensitivity was 83%, specificity was 92%. When the cut-off value for ectopic mass size was calculated as 20 mm, the sensitivity and specificity of predicting methotrexate success were 75% and 56%, respectively.

Table 1. Comparison of demographic and clinical characteristics of the two groups

	Successful (n=350)	Unsuccessful (n=112)	p
Age	29.97±5.62	29.95±5.32	0.963
Gravida	2.90±1.54	2.81±1.60	0.593
Parity	1.19±1.07	1.13±1.08	0.624
Abortus	0.63±0.60	0.58±0.52	0.646
Gestation week	5.88±1.71	6.12±1.79	0.189
Hemoglobin (g/dL)	12.06±1.40	12.04±1.26	0.855
Hematocrit (%)	36.66±3.80	36.70±3.42	0.913
β-hCG 1 (mIU/mL)	1369.67±1013.71	3876.59±3108.21	<0.001
β-hCG 4 (mIU/mL)	1207.55±1157.48	4292.66±2718.72	<0.001
β-hCG 7 (mIU/mL)	866.70±799.39	4067.01±2314.14	<0.001
Mass size (mm)	12.97±11.53	23.35±13.03	<0.001

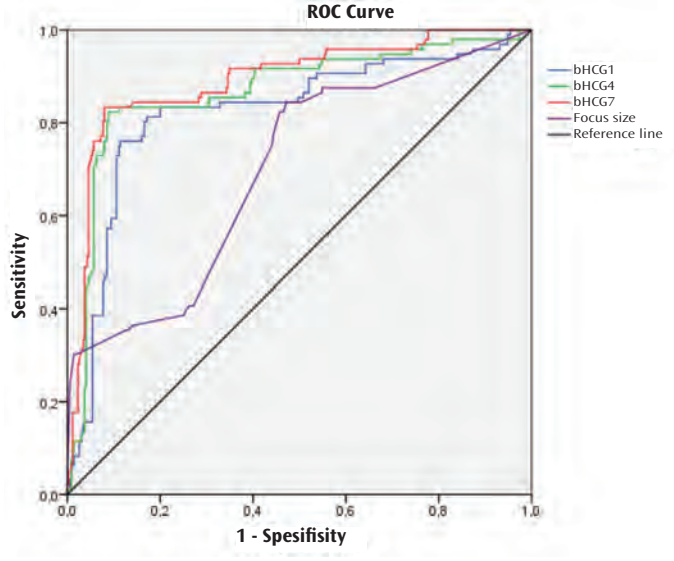


Figure 1. ROC analysis of factors influencing methotrexate success

DISCUSSION

In the treatment of ectopic pregnancy, wait and see method, medical and surgical treatment can be applied. Which of these methods is preferred is decided by considering the clinical and laboratory findings and fertility of the patient (12). In this study, we investigated the factors that affect the success of methotrexate in patients receiving single dose methotrexate treatment. With the early diagnosis of ectopic pregnancy cases, medical treatment options have started to be developed without damaging the tubes. Methotrexate is the most successfully used medical agent for this purpose. Methotrexate is a folic acid antagonist and inhibits the formation of tetrahydrofolate required for DNA, RNA and adenosine triphosphate synthesis (13). In ectopic pregnancies, methotrexate treatment can be administered as single dose or multiple doses, local or systemic. As experiences with methotrexate have increased, single-dose regimens have emerged to simplify treatment, improve compliance, and reduce side effects and cost. Successful results have been obtained with systemic administration of single dose methotrexate (14). In this study, all patients were initially treated with a single dose methotrexate protocol. Methotrexate is used as a medical agent in the treatment of ectopic pregnancy. The success of the drug is related to the clinical and laboratory findings of the patient (15). The definite contraindications of methotrexate treatment are unstable hemodynamics, active hepatic or renal disease, and relative contraindications are β-hCG >10.000 mIU/mL and fetal cardiac activity (16). Success rates of single dose methotrexate use range from 64% to 94% (17). Similar to the literature, the success rate was 75.8% in our study. There are limited publications on the factors that affect the success of single dose methotrexate treatment in ectopic pregnancy. While high serum β-hCG levels were found to be the only effective prognostic factor in a study (17), Kimiaei et al. (18) found that ectopic mass size, in addition to initial β-hCG level, was a factor affecting the success of the single-dose methotrexate treatment. In some studies, in addition to the high initial β-hCG level, the decrease in β-hCG levels at the 4th day after methotrexate treatment was suggested to be the most important prognostic factor in predicting success (19,20). In a study performed by Mungan et al. (21) it was shown that the location of ectopic pregnancy in tubes was important in determining the success of single dose methotrexate treatment. The success of methotrexate has been shown to be better in periampular ectopic pregnancies compared to periistmic location (21). In our study, we determined ectopic mass size and serum β-hCG levels as factors affecting success. We found that treatment success was reduced when the ectopic mass was over 20 mm. Serum β-hCG levels are closely related to medical treatment and success rates. In the study of Lipscomb et al. (22) β-hCG levels were significantly higher in the single-dose methotrexate unsuccessful group. Lipscomb et al. (22) reported that β-hCG at the beginning of treatment was the best prognostic data for predicting methotrexate success. In their study, they determined a success rate of 94% when the initial β-hCG level was below 10.000 and 75% when it was above 10.000. More recent publications have shown that this cut-off value is more effective between 2.000 and 3.000 (19). In our study, we found that treatment success was higher when the initial β-hCG cut-off value was below 2.000.

CONCLUSION

In conclusion, there are many factors affecting the success of single dose methotrexate treatment in ectopic pregnancy. The most important of these are the ectopic mass size and initial ß-hCG level. The patient's fertility status, clinical and laboratory findings, and serum β-hCG follow-up are important considerations in the planning of appropriate treatment.

Ethics

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Sadi Konuk Training and Research Hospital (2017/414).

Informed Consent: Informed consent is not obtained due to the retrospective nature of this study.

Peer-review: External and internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: B.A.Ç., Concept: B.A.Ç., B.A.M., Design: B.A.Ç., B.A.M., Data Collection or Processing: B.A.Ç., Analysis or Interpretation: B.A.M., Literature Search: B.A.M., Writing: B.A.Ç.

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

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

REFERENCES

1. Lozeau AM, Potter B. Diagnosis and management of ectopic pregnancy. Am Fam Physician 2005;72:1707-14.
2. Chang J, Elem-Evans LD, Berg CJ, Herndon J, Flowers L, Seed KA, et al. Pregnancy-related mortality surveillance: United States, 1991-1999. MMWR 2003;52:1-8.
3. Atasü T, Sahmay S (Eds). Jinekoloji, 2. Baski, Nobel Yayınevi, İstanbul 2001:s.547-57.

4. Hajenius PJ, Mol F, Mol BW, Bossuyt PM, Ankum WM, Van der Veen F. Interventions for tubal ectopic pregnancy. Cochrane Database Syst Rev 2007;CD000324.

5. Stovall TG, Ling FW. Single dosemethotrexate: an expanded clinical trial. Am J Obstet Gynecol 1993;168:1759-65.

6. Kirk E, Condous G, Bourne T. The nonsurgical management of ectopic pregnancy. Ultrasound Obstet Gynecol 2006;27:91-100.

7. Fernandez H, Lelaidier C, Thouvenez V, Frydman R. The use of a pretherapeutic, predictive score to determine inclusion criteria for the non-surgical treatment of ectopic pregnancy. Hum Reprod 1991;6:995-8.

8. Canis M, Savary D, Pouly JL, Wattiez A, Mage G. Ectopic pregnancy: criteria to decide betweenmedical and conservative surgical treatment? J Gynecol Obstet Biol Reprod 2003;32:S54-63.

9. Potter MB, Lepine LA, Jamieson DJ. Predictors of success with methotrexate treatment of tubal ectopic pregnancy at Grady Memorial Hospital. Am J Obstet Gynecol 2003;188:1192-4.

10. Nazac A, Gervaise A, Bouyer J, de Tayrac R, Capella-Allouc S, Fernandez H. Predictors of success in methotrexate treatment of women with unruptured tubal pregnancies. Ultrasound Obstet Gynecol 2003;21:181-5.

11. Dilbaz S, Caliskan E, Dilbaz B, Degirmenci O, Haberal A. Predictors of methotrexate treatment failure in ectopic pregnancy. J Reprod Med 2006;51:87-93.

12. Mol F, Mol BW, Ankum WM, van der Veen F, Hajenius PJ. Current evidence on surgery, systemic methotrexate and expectant management in the treatment of tubal ectopic pregnancy: a systematic review and meta-analysis. Hum Reprod Update 2008;14:309-19.

13. Merisio C, Anfuso S, Berretta R, Gualdi M, Pultrone D, et al. Single –dose methotrexate for Ectopic pregnancy treatment: preliminary data Acta Bio Med 2005;76:33-6.

14. Zargar M, Razi T, Barati M. Comparison of single and multidose of methotrexate in medical treatment of ectopic pregnancy. Pak J Med Sci 2008;24:586-9.

15. Gibbs R, Karlan B, Haney A. Danforth's Obstetrics and Gynecology. Baltimore: Lippincott Williams and Wilkins; 2010.

16. The Management of tubal pregnancy. Royal college of obstetricians and gynaecologists guidelines 2004;21:1-10.

17. Tawfiq A, Agameya AF, Claman P. Predictors of treatment failure for ectopic pregnancy treated with single-dose methotrexate. Fertil Steril 2000;74:877-80.

18. Kimiaei P, Khani Z, Marefian A, Ghavamabadi MG, Salimnejad M. The importance of gestational sac size of ectopic pregnancy in response to single- dose methotrexate. ISRN Obstet Gynecol 2013:269425.

19. Ustunyurt E, Duran M, Coskun E, Ustunyurt ÖB, Şimşek H. Role of initial and day 4 human chorionic gonadotropin levels in predicting the outcome of single-dose methotrexate treatment in women with tubal ectopic pregnancy. Arch Gynecol Obstet 2013;288:114952.

20. Çelik E, Türkçüoğlu I, Karaer A, Kırıcı P, Eraslan S. Assessment of early decline in percentage of β-hCG values between days 0 and 4 after methotrexate therapy in ectopic pregnancy for prediction of treatment success. J Turkish German Gynecol Assoc 2013;14:1259.

21. Mungan T, Erdemoğlu E, Guney M. The impact of ultrasonographic impantation site of ectopic pregnancy on methotrexate treatment success and reproductive outcome. J Turkish-German Gynecol Assoc 2007;8:387-91.

22. Lipscomb GH, Puckett KJ, Bran D, Ling FW. Management of separation pain after single-dose methotrexate therapy for ectopic pregnancy. Obstet Gynecol 1999;93:590-3.

ORIGINAL ARTICLE

DOI: 10.4274/eamr.galenos.2018.48344
Eur Arch Med Res 2019; 35 (4):205-10

Comparison of the Effects of Lidocaine and Dexmedetomidine Before Propofol Induction During Laryngeal Mask Airway Insertion

Ali Sait Kavaklı[1], Dilek Kitapçıoğlu[2]

[1]University of Health Sciences, Antalya Training and Research Hospital, Clinic of Anaesthesiology and Reanimation, Antalya, Turkey
[2]Acıbadem Mehmet Ali Aydınlar University Faculty of Medicine, Department of Medical Education, İstanbul, Turkey

Abstract

Objective: Laryngeal mask (LMA) is an airway device that can be used as an alternative to face mask and tracheal intubation. Different drug combinations can be used to ensure suitable conditions during LMA insertion. Lidocaine and dexmedetomidine are drugs that can be used to reduce hemodynamic response and suppress oropharyngeal reflexes during both intubation and LMA insertion. The aim of this study was to investigate the effects of lidocaine and dexmedetomidine on LMA insertion.

Methods: Sixty patients who were scheduled to undergo cystoscopy under general anesthesia with LMA were included in the study. Patients were randomly divided into two groups: Group L (those who received 1.5 mg/kg lidocaine) and Group D (those who received 1 µg/kg dexmedetomidine). Number of attempts of LMA insertion, ease of LMA insertion, mouth opening, laryngospasm, gumming, stomach distention, limb movements and spontaneous breathing and hemodynamic parameters were recorded.

Results: There was no statistically significant difference between the two groups in terms of the number of attempts of LMA insertion. In the first attempt, the LMA insertion success rate was 70% for Group L and 73.3% for Group D. There was no statistically significant difference between the two groups in terms of mouth opening, laryngospasm, ease of LMA insertion, gumming, stomach distension, limb movement and spontaneous breathing. Systolic blood pressure, diastolic blood pressure, mean blood pressure and heart rate were statistically lower in Group D after drug administration. It was determined that the decreases in the other times were statistically significant compared to the baseline values after drug administration in both groups.

Conclusion: Based on the results of the present study, 1 µg/kg dexmedetomidine and 1.5 mg/kg lidocaine used before propofol induction provided similar conditions for LMA insertion. Dexmedetomidine has a more hypotensive effect and provides a greater reduction in heart rate than lidocaine. Furthermore, the use of lidocaine before propofol induction provides better hemodynamic control than dexmedetomidine.

Keywords: Laryngeal mask airway, lidocaine, dexmedetomidine

INTRODUCTION

Laryngeal mask (LMA) is an airway device that can be used as an alternative to facial mask and tracheal intubation. Although there are studies showing that LMA requires less anesthesia compared to endotracheal intubation (1), adequate depth of anesthesia should be provided for proper LMA insertion. It has been reported that the use of propofol in induction provides better conditions for LMA insertion than thiopental because of its higher depressant effects on jaw relaxation and airway reflexes (2,3). However, the use of propofol alone without premedication may be insufficient for LMA insertion. Increasing the dose of propofol to ensure proper conditions increases the incidence of undesirable effects such as cardiac depressant effects (4,5). Lidocaine is an agent that can be used both topically and intravenously because of its dose-dependent suppressing effects on cardiovascular responses and cough reflex due to intubation and LMA insertion (6,7). In addition, lidocaine use has been reported to cause a decrease in the incidence of laryngospasm (8). There are studies showing that dexmedetomidine, a selective α2-receptor agonist, reduces respiratory and cardiovascular responses during intubation and extubation (9,10). In addition, dexmedetomidine has been reported to be a suitable agent for LMA insertion (11). The aim of this study was to compare the

Address for Correspondence: Ali Sait Kavaklı, University of Health Sciences, Antalya Training and Research Hospital, Clinic of Anaesthesiology and Reanimation, Antalya, Turkey
Phone: +90 505 677 51 21 E-mail: alisaitkavakli@hotmail.com ORCID ID: orcid/org.0000-0001-5263-7183

Received: 02.01.2018
Accepted: 16.10.2018

Cite this article as: Kavaklı AS, Kitapçıoğlu D. Comparison of the Effects of Lidocaine and Dexmedetomidine Before Propofol Induction During Laryngeal Mask Airway Insertion. Eur Arch Med Res 2019; 35 (4): 205-10

effects of lidocaine and dexmedetomidine on LMA insertion administered before propofol induction.

METHODS

This study was prepared in accordance with the Declaration of Helsinki. It was approved by the ethics committee and carried out at the Clinic of Anesthesiology and Reanimation, Taksim Training and Research Hospital, Ministry of Health. Written informed consent was obtained from all patients. The primary aim of this study was to investigate the effects of lidocaine and dexmedetomidine administered before induction on LMA insertion quality in patients undergoing cystoscopy. The secondary objective was to investigate the effects of lidocaine and dexmedetomidine on hemodynamic parameters. Sixty American Society of Anaesthesiologists (ASA) I-II patients aged between 40-75 years who were scheduled for cystoscopy under general anesthesia with LMA were included in the study. Patients were randomly divided into two groups: Group L (lidocaine-treated group) and Group D (dexmedetomidine-treated group). Patients with ASA III and above, a history of bleeding diathesis, gastroesophageal reflux disease, a history of previous head and neck surgery, and cooperative impairment were excluded. Standard anesthesia monitoring including pulse oximetry, electrocardiography and noninvasive blood pressure measurement was performed to patients who were taken to the operating room following preoperative routine anesthesia preparation. Peripheral venous access was established with 20 G cannula and 0.09% NaCl infusion was started at 2.0 mL/kg/h. Following preoxygenation, 1.5 mg/kg intravenous (IV) lidocaine diluted with 20 mL saline was administered to Group L and 1 mcg/kg IV dexmedetomidine diluted with 20 mL saline was administered to Group D within 2 minutes. Anesthesia was then induced with 2.5 mg/kg propofol and 1 mcg/kg fentanyl. Neuromuscular blocking agent was not used for muscle relaxation. The first trial was performed for LMA insertion 90 seconds after anesthesia induction. Lubricant gel was used for LMA insertion. Following LMA insertion, the cuff was inflated with the recommended volumes and ventilation was confirmed by end-tidal carbon dioxide measurement. If the first attempt failed, the second trial was performed after 30 seconds of mask ventilation. Patients with three failed attempts were excluded. Anesthesia was maintained with 1.5% sevoflurane in mechanically ventilated patients with 45% O_2-65% N_2O mixture. All LMA insertion procedures were performed by the same person. Evaluations were performed by an anesthesiologist independent of the study. Ease of LMA insertion (easy, difficult, impossible), mouth opening (complete, partial, no), gag reflex (yes, no), stomach distention (yes, no), limb movement (yes, no), spontaneous breathing (yes, no) and laryngospasm (yes, no) were recorded. Laryngospasm was defined as the presence of stridor lasting more than 15 seconds and absence of a capnography wave in the absence of any other upper airway obstruction during LMA insertion. Systolic artery pressure (SAP), diastolic artery pressure (DAP), mean arterial pressure (MAP) and heart rate (HR) values were recorded at baseline, 90 seconds before LMA insertion and 1, 3, 5, 10 and 15 minutes after LMA insertion.

Statistical Analysis

SPSS version 15 statistical software (SPSS Inc., Chicago, IL, USA) was used for statistical evaluation of the data. Descriptive statistics (mean, standard deviation, frequency, percentage) were used, and qualitative data were compared using Pearson chi-square and Fisher's exact tests. Mann-Whitney U test was used to compare the quantitative data. The Wilcoxon sign test was used for intragroup comparisons of quantitative data. Results were evaluated at 95% confidence interval and $p<0.05$ level of significance.

RESULTS

Sixty patients who underwent elective cystoscopy under general anesthesia with LMA were included in the study. Surgical procedures were completed with LMA under general anesthesia in all patients. None of the patients had respiratory complications requiring intubation. There was no statistically significant difference between the two groups in terms of age, weight, gender, ASA score and mallampati scores. Demographic data of the patients are presented in Table 1. No statistically

Table 1. Demographic data [values expressed as mean ± standard deviation or number (%)]

	Group L (n=30)	Group D (n=30)	p
Age (years)	56.37±8.93	55.67±9.59	0.824
Weight (kg)	74±12.2	69.87±9.64	0.088
Gender			
Female	8 (26.7)	4 (13.3)	0.197
Male	22 (73.3)	26 (86.7)	
ASA score			
ASA I	13 (43.3)	20 (66.7)	0.069
ASA II	17 (56.7)	10 (33.3)	
Mallampati score			
I	15 (50)	22 (73.3)	0.077
II	12 (40)	8 (26.7)	
III	3 (10)	0 (0)	

ASA: American Society of Anaesthesiologists

significant difference was found between the two groups in terms of LMA insertion trials. LMA insertion success was 70% for Group L and 73.3% for Group D in the first attempt. In Group D, LMA insertion was performed in the third attempt in one patient (3.3%), and in all patients, the first and second trials were successful. For Group L, LMA insertion was performed in all patients in the first and second trials. When mouth opening, laryngospasm, ease of LMA insertion, gagging, gastric distension, limb movement and spontaneous breathing findings were evaluated, there was no statistically significant difference between the two groups (Table 2). While there was no statistically significant difference between the basal values of SAP, DAP and MAP in both groups, values were significantly lower in Group D compared to Group L after drug administration. When SAP, DAP and MAP changes of the groups were compared, it was seen that there were significant decreases in both groups compared to baseline values. Intergroup comparisons revealed a statistically significant decrease in all values measured after drug administration compared to baseline in Group L. Similarly, there was a statistically significant decrease in Group D compared to baseline values after drug administration. Significant increases in SAP, DAP and MAP were observed in both groups 3 minutes after LMA insertion. HRs were significantly lower in Group L at all times than in Group D (Figure 1).

Table 2. Data related to laryngeal mask insertion [values expressed as numbers (%)]

	Group L (n=30)	Group D (n=30)	p
Number of LMA insertion trials			
1	21 (70)	22 (73.3)	0.529
2	9 (30)	7 (23.3)	
3	0 (0)	1 (3.3)	
LMA insertion ease			
Easy	19 (63.3)	24 (80)	0.152
Difficult	11 (36.7)	6 (20)	
Mouth opening			
Complete	17 (56.7)	21 (70)	0.284
Partial	13 (43.3)	9 (30)	
Laryngospasm			
No	29 (96.7)	29 (96.7)	0.754
Yes	1 (3.3)	1 (3.3)	
Gagging			
No	26 (86.7)	29 (96.7)	0.177
Yes	4 (13.3)	1 (3.3)	
Stomach distension			
No	26 (86.7)	27 (90)	0.500
Yes	4 (13.3)	3 (10)	
Limb movement			
No	18 (60)	16 (53.3)	0.602
Yes	12 (40)	14 (46.7)	
Spontaneous breathing			
No	15 (50)	11 (36.7)	0.297
Yes	15 (50)	19 (63.3)	

LAM: Laryngeal mask

DISCUSSION

Our study showed that the administration of lidocaine and dexmedetomidine before induction had similar effects on the quality of LMA insertion in patients undergoing cystoscopy, and that dexmedetomidine had more effect on hemodynamic parameters than lidocaine. Dexmedetomidine is a selective $\alpha 2$ agonist with analgesic and sedative effects. There are studies that dexmedetomidine reduces the respiratory and circulatory stimuli in intubation and extubation. Wei et al. (12) reported that dexmedetomidine at a dose of 1 µg/kg improves intubation conditions in children and suppresses the hemodynamic response due to intubation. It has also been reported that dexmedetomidine at a dose of 0.6 µg/kg significantly reduces hemodynamic responses to laryngoscopy and endotracheal intubation in patients undergoing thyroid surgery (13). It has been shown that dexmedetomidine at a dose of 1 µg/kg given 30 seconds before propofol induction provides optimum conditions for LMA insertion at 90 seconds after induction and that propofol-dexmedetomidine combination is more effective on LMA insertion than the propofol-fentanyl combination (11). In addition, there are also studies showing that dexmedetomidine facilitates LMA insertion and reduces propofol requirement (14). Similarly, there are studies examining the effects of lidocaine on intubation and LMA insertion. Kocamanoglu et al. (15) reported that both IV and topical lidocaine limited the hemodynamic response in laryngoscopy and endotracheal intubation. Hashemian et al. (16) reported that the combination of fentanyl and lidocaine is more effective than the use of fentanyl alone to prevent hemodynamic response due to intubation. In their study examining the effects of lidocaine on LMA insertion, Baik et al. (17) detected that 1.5 mg/kg dose of lidocaine did not make hemodynamic changes, although the incidence of cough, gagging and laryngospasm was found to be decreased compared to the control group. In our study, similar rates were observed in the incidence of gagging and laryngospasm in both groups. These low rates indicate the utility of both agents in LMA insertion. At the same time, less hemodynamic response in the lidocaine group than dexmedetomidine group was consistent with Baik et al. (17) The authors also reported the success rate

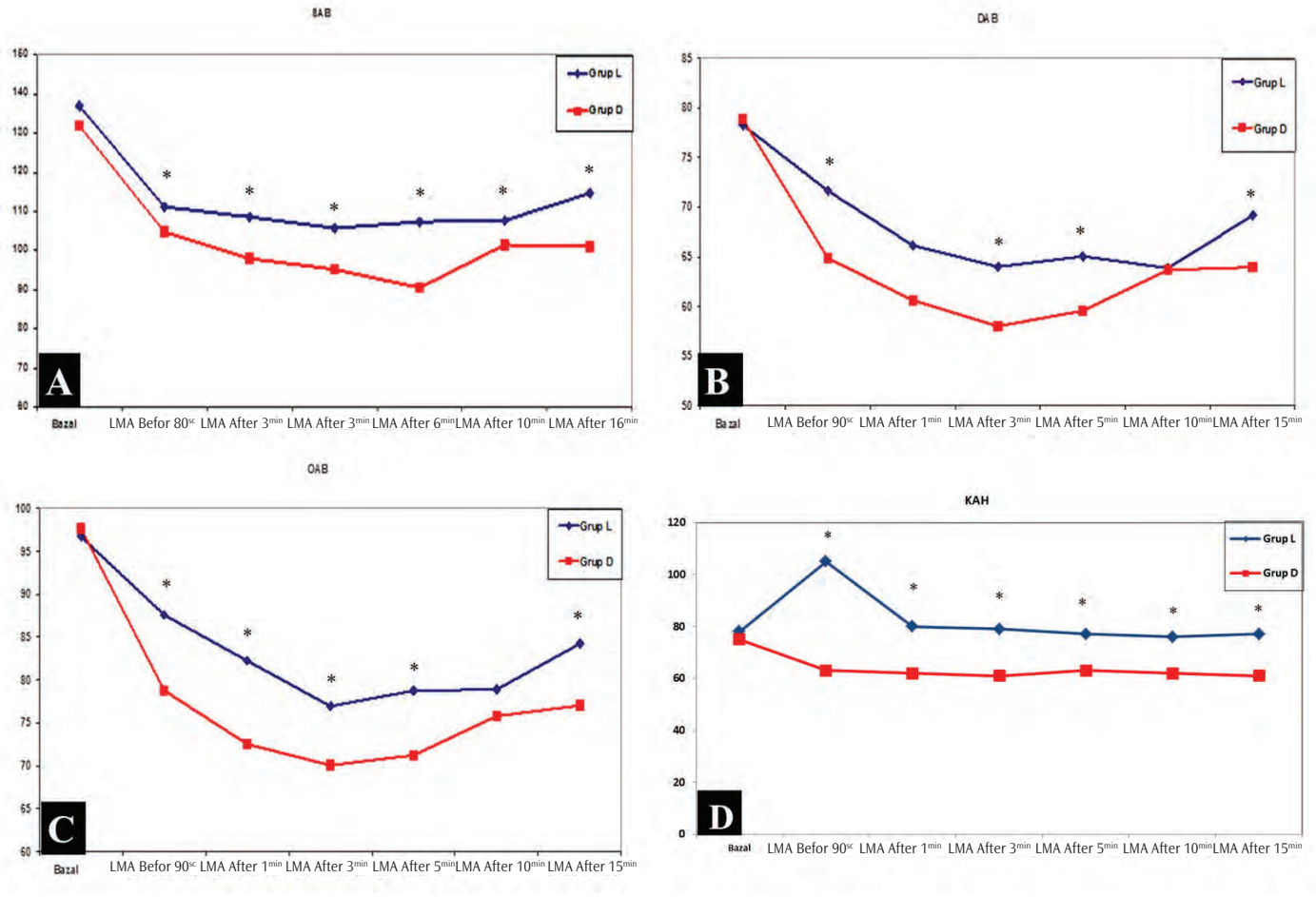


Figure 1. A) Variation of systolic arterial pressure of groups with time, B) Variation of diastolic arterial pressure of groups with time, C) Variation of mean arterial pressure of groups with time, D) Variation of heart rate of groups with time

*p<0.05 comparison between groups

LMA: Laryngeal mask

of LMA insertion to be 97.5% in the lidocaine group versus the 85% success rate in the control group. In our study, the 100% success rate seen in the lidocaine group after a maximum of two trials confirms the results of this study. Although there is no study comparing the effects of both agents on intubation or LMA insertion quality in the literature, there are studies showing that the use of two agents together provides better conditions. Hancı et al. (18) reported that dexmedetomidine-lidocaine-propofol combination provides better intubation conditions than fentanyl-lidocaine-propofol combination. In the study of Yoo et al. (19), 1 mg/kg dose of dexmedetomidine together with 0.5 mg/kg lidocaine reduced propofol requirement by 38%. Similarly, both agents reduced the need for sevoflurane; however, it was reported that patients receiving dexmedetomidine had less need of sevoflurane than those receiving lidocaine (20).

Consistent with all these studies, both lidocaine and dexmedetomidine were found to be effective in LMA insertion in our study. After a maximum of two attempts, 100% success rate was achieved with lidocaine use and this rate was 96.6% for dexmedetomidine. The low incidence of gagging, gastric distension and laryngospasm with both agents indicates that these agents can be used safely in LMA insertion. Although there is no definite conclusion about the appropriate time for LMA insertion after propofol induction, some studies have reported some time periods for jaw relaxation and proper mouth opening. Goyagi et al. (21) studied the effect of the use of fentanyl on LMA insertion and they used lidocaine to prevent injection pain 30 seconds before propofol induction and reported that appropriate conditions were provided for LMA insertion in 90 seconds after propofol induction. Uzumcugil et al. (11) performed induction with propofol 30 seconds after injection of 1 µg/kg dexmedetomidine and reported successful insertion of LMA 90 seconds after induction. Similarly, Baik et al. (17) reported appropriate conditions for LMA insertion were achieved within 90 seconds after lidocaine injection during

anesthesia induction. In all of these studies, it was concluded that 90 seconds is sufficient to provide ideal jaw relaxation and mouth opening for LMA insertion after both dexmedetomidine and lidocaine injection. In our study, the first LMA insertion trial was performed in 90 seconds after propofol in accordance with these studies. Consistent with the previous studies, we found that the jaw relaxation and mouth opening were appropriate for LMA insertion in 90 seconds after both propofol lidocaine and propofol dexmedetomidine combinations. The sympatholytic effect of dexmedetomidine is dominant at low plasma concentrations. It leads to vasodilatation by activation of $\alpha 2$ receptors in the central nervous system and vascular endothelial cells, resulting in a decrease in MAP and HR. At high concentrations, a peripheral vasoconstrictive effect occurs with the effect of $\alpha 2$ adrenoreceptor activation in vascular smooth muscle cells, which may lead to an increase in MAP and a further decrease in HR (22,23). Talke et al. (22) showed that the vasoconstrictive effect of dexmedetomidine continued while the sympatholytic effect decreased under general anesthesia. However, suppressing or reversing the increase in hemodynamic effects by the presence of concomitant comorbidity or the use of dexmedetomidine in combination with drugs remains unclear. In studies conducted on healthy volunteers, it has been reported that there is a 21% and 31% decrease in HR after two minutes dexmedetomidine infusion at a dose of 1-2 µg/kg (24). The same study reports an increase in MAP of 7% and 8% at these doses. In contrast to this finding, in our study, the hypotensive response seen at doses of 1 µg/kg seems to be consistent with the hypothesis of suppressing or reversing the hemodynamic effects associated with concomitant use of dexmedetomidine and propofol. However, although there are studies showing the hypotensive effect of lidocaine (25), there are also studies showing that it is not associated with significant hemodynamic changes after endotracheal intubation (26). In our study, the fact that dexmedetomidine had more hypotensive effect and lower HRs than lidocaine treated group supported the opinion that lidocaine may provide a more stable hemodynamics after dexmedetomidine after LMA insertion. The limitation of our study is the absence of a control group in which propofol is used alone. However, we thought that the use of propofol for LMA insertion alone would have to increase the dose (19-27), which may lead to undesired respiratory and hemodynamic responses.

CONCLUSION

The use of 1 µg/kg dexmedetomidine and 1.5 mg/kg lidocaine before propofol induction creates similar and favorable conditions for LMA insertion. However, dexmedetomidine used before propofol induction has more hypotensive effect than lidocaine and causes more decrease in HR. The use of lidocaine before propofol induction provides better hemodynamic control than dexmedetomidine. Controlled randomized trials with larger patient groups may support the results of our study.

Ethics

Ethics Committee Approval: The study was approved by the Institutional Review Board, the study was conducted in accordance with the principles of the Helsinki Declaration.

Informed Consent: Written informed consent was obtained from all participants

Peer-review: External and internal peer-reviewed.

Authorship Contributions

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

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

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

REFERENCES

1. Wilkins CJ, Cramp PG, Staples J, Stevens WC. Comparison of the anesthetic requirement for tolerance of laryngeal mask airway and endotracheal tube. Anesth Analg 1992;75:794-7.
2. Brown GW, Patel N, Ellis FR. Comparison of propofol and thiopentone for laryngeal mask insertion. Anaesthesia 1991;46:771-2.
3. Scanlon P, Carey M, Power M, Kirby F. Patient response to laryngeal mask insertion after induction of anaesthesia with propofol or thiopentone. Can J Anaesth 1993;40:816-8.
4. Goh PK, Chiu CL, Wang CY, Chan YK, Loo PL. Randomized double-blind comparison of ketamine-propofol, fentanyl-propofol and propofol-saline on haemodynamics and laryngeal mask airway insertion conditions. Anaesth Intensive Care 2005;33:223-8.
5. Siddik-Sayyid SM, Aouad MT, Taha SK, Daaboul DG, Deeb PG, Massouh FM, et al. A comparison of sevoflurane-propofol versus sevoflurane or propofol for laryngeal mask airway insertion in adults. Anesth Analg 2005;100:1204-9.
6. Lee MC, Absalom AR, Menon DK, Smith HL. Awake insertion of the laryngeal mask airway using topical lidocaine and intravenous remifentanil. Anaesthesia 2006;61:32-5.
7. Erb TO, von Ungern-Sternberg BS, Keller K, Frei FJ. The effect of intravenous lidocaine on laryngeal and respiratory reflex responses in anaesthetised children*. Anaesthesia 2013;68:13-20.
8. Koc C, Kocaman F, Aygenc E, Ozdem C, Cekic A. The use of preoperative lidocaine to prevent stridor and laryngospasm after tonsillectomy and adenoidectomy. Otolaryngol Head Neck Surg 1998;118:880-2.

9. Guler G, Akin A, Tosun Z, Eskitascoglu E, Mizrak A, Boyaci A. Single-dose dexmedetomidine attenuates airway and circulatory reflexes during extubation. Acta Anaesthesiol Scand 2005;49:1088-91.

10. Maroof M, Khan RM, Jain D, Ashraf M. Dexmedetomidine is a useful adjunct for awake intubation. Can J Anaesth 2005;52:776-7.

11. Uzumcugil F, Canbay O, Celebi N, Karagoz AH, Ozgen S. Comparison of dexmedetomidine-propofol vs. fentanyl-propofol for laryngeal mask insertion. Eur J Anaesthesiol 2008;25:675-80.

12. Wei L, Deng X, Sui J, Wang L, Liu J. Dexmedetomidine Improves Intubating Conditions Without Muscle Relaxants in Children After Induction With Propofol and Remifentanil. Anesth Analg 2015;121:785-90.

13. Rashmi HD, Komala HK. Clinical evaluation of the effect of intravenous dexmedetomidine on the hemodynamic response to laryngoscopy and endotracheal intubation in patients undergoing thyroid surgeries. Anesth Essays Res 2016;10:483-7.

14. Nellore SS, Waychal AD, Rustagi PS. Comparison of Dexmedetomidine-Propofol versus Fentanyl-Propofol on Insertion Conditions of Proseal Laryngeal Mask Airway. J Clin Diagn Res 2016;10:UC06-UC9.

15. Kocamanoglu IS, Cengel Kurnaz S, Tur A. Effects of lignocaine on pressor response to laryngoscopy and endotracheal intubation during general anaesthesia in rigid suspension laryngoscopy. J Laryngol Otol 2015;129:79-85.

16. Hashemian AM, Zamani Moghadam Doloo H, Saadatfar M, Moallem R, Moradifar M, Faramarzi R, et al. Effects of intravenous administration of fentanyl and lidocaine on hemodynamic responses following endotracheal intubation. Am J Emerg Med 2018;36:197-201.

17. Baik HJ, Kim YJ, Kim JH. Lidocaine given intravenously improves conditions for laryngeal mask airway insertion during propofol target-controlled infusion. Eur J Anaesthesiol 2009;26:377-81.

18. Hanci V, Erdoğan G, Okyay RD, Yurtlu BS, Ayoğlu H, Baydilek Y, Turan IO. Effects of fentanyl-lidocaine-propofol and dexmedetomidine-lidocaine-propofol on tracheal intubation without use of muscle relaxants. Kaohsiung J Med Sci 2010;26:244-50.

19. Yoo JY, Kwak HJ, Kim YB, Park CK, Lee SY, Kim JY. The effect of dexmedetomidine pretreatment on the median effective bolus dose of propofol for facilitating laryngeal mask airway insertion. J Anesth 2017;31:11-7.

20. Harsoor SS, Rani D, Roopa MN, Lathashree S, Sudheesh K, Nethra SS. "Anesthetic Sparing Effect of Intraoperative Lignocaine or Dexmedetomidine Infusion on Sevoflurane during General Anesthesia". Middle East J Anaesthesiol 2015;23:301-7.

21. Goyagi T, Tanaka M, Nishikawa T. Fentanyl decreases propofol requirement for laryngeal mask airway insertion. Acta Anaesthesiol Scand 2003;47:771-4.

22. Talke P, Lobo E, Brown R. Systemically administered alpha2-agonist-induced peripheral vasoconstriction in humans. Anesthesiology 2003;99:65-70.

23. Snapir A, Posti J, Kentala E, Koskenvuo J, Sundell J, Tuunanen H, et al. Effects of low and high plasma concentrations of dexmedetomidine on myocardial perfusion and cardiac function in healthy male subjects. Anesthesiology 2006;105:902-10.

24. Colin PJ, Hannivoort LN, Eleveld DJ, Reyntjens K, Absalom AR, Vereecke HEM, et al. Dexmedetomidine pharmacodynamics in healthy volunteers: 2. Haemodynamic profile. Br J Anaesth 2017;119:211-20.

25. Totonchi Z, Salajegheh S, Mohaghegh MR, Kiaei MM, Shirvani M, Ghorbanlo M. Hemodynamic effect of intravenous lidocaine during aortic cannulation in cardiac surgery. Interv Med Appl Sci 2017;9:56-60.

26. Lin CC, Yu JH, Lin CC, Li WC, Weng YM, Chen SY. Postintubation hemodynamic effects of intravenous lidocaine in severe traumatic brain injury. Am J Emerg Med 2012;30:1782-7.

27. Kodaka M, Okamoto Y, Koyama K, Miyao H. Predicted values of propofol EC50 and sevoflurane concentration for insertion of laryngeal mask Classic and ProSeal. Br J Anaesth 2004;92:242-5.

ORIGINAL ARTICLE

DOI: 10.4274/eamr.galenos.2018.94840
Eur Arch Med Res 2019; 35 (4):211-4

Follicle-stimulating Hormone Induced *In Vitro* Growth of Small Antral Follicles are not Affected by Gonadotropin-releasing Hormone Agonist or Antagonist Treatment in Mice

Yılmaz Güzel

İstanbul Aydın University Faculty of Medicine, Department of Obstetrics and Gynecology, İstanbul, Turkey

Abstract

Objective: In this study, we aimed to investigate if the gonadotropin-releasing hormone (GnRH) agonist and antagonist have any effect on the follicle-stimulating hormone (FSH)-induced-growth of early antral follicles of mice, cultured *in vitro*, and expressing cognate receptors for FSH and GnRH. For this purpose, small antral follicles were isolated from mouse ovaries and randomly assigned to the groups as control, FSH only, FSH + GnRH agonist, and FSH + GnRH antagonist, and they were cultured for five days.

Methods: The ovaries of C57BL/6 mice (n=24), which were 21 days old, were removed after euthanasia. Small antral follicles measuring ~200µ in diameter were mechanically isolated after the enzymatic digestion of the ovaries with collagenase and DNase-I. The expression of FSH and GnRH receptors in these follicles was validated by quantitative real time-polymerase chain reaction. Isolated follicles were randomly assigned into four different groups, each consisting of 20-30 follicles: control, FSH only, FSH + GnRH agonist, and FSH + GnRH antagonist.

Results: The FSH treatment significantly enhanced the *in vitro* growth of the follicles compared to those cultured without FSH after five days of the culture period. The antrum formation was markedly enhanced, and cumulus-oophorus complexes were more easily visible in the FSH-treated follicles compared to control follicles. The mean diameters of follicles treated with the FSH + GnRH agonist or the FSH + GnRH antagonist were not significantly different from those treated with FSH only, but they were significantly greater than control follicles.

Conclusion: These results may suggest that the GnRH agonist and antagonist do not appear to adversely affect the FSH-induced proliferation of mitotic non-luteinizing granulosa cells and the growth of early antral follicles of mice *in vitro*.

Keywords: Antral follicle, culture, matrigel, follicle-stimulating hormone, gonadotropin-releasing hormone agonist, gonadotropin-releasing hormone antagonist

INTRODUCTION

Our knowledge about the role of extra-pituitary gonadotropin-releasing hormone (GnRH) and its receptors in ovarian follicle development in humans and other species is very restricted. In the mouse ovary, small antral follicles express the cognate receptors for GnRH and follicle-stimulating hormone (FSH) (1-3). However, their role in FSH driven growth of these follicles is unknown. Previous studies showed that although systemic administration of GnRH agonist (GnRHa) was efficient to disrupt estrus cycles, it failed to inhibit follicular development, irrespective of the doses and injection sites (subcutaneous or intramuscular). Around 20% of healthy growing follicles were still observed during GnRHa treatment, and serum FSH levels were not reduced either by antagonist or agonist treatment, suggesting that GnRHa does not suppress follicular growth even beyond the gonadotropin-dependent stage in mice (4). Also, it might be challenging to interpret the intra-ovarian actions of GnRH analogs when they are administered systemically due to its effect on the hypothalamic-pituitary-ovarian axis. One *in vitro* study showed that GnRHa exerts diverse actions on granulosa cells over the course of follicular growth. One down-regulates granulosa cell proliferation in immature follicles as well as steroidogenesis in mature follicles, and the other upregulates apoptosis of granulosa cells regardless of the stages of follicular growth (5). It is unclear whether *in vitro* GnRH analog treatment has any impact on FSH induced growth in *in vitro* conditions of

Address for Correspondence: Yılmaz Güzel, İstanbul Aydın University Faculty of Medicine, Department of Obstetrics and Gynecology, İstanbul, Turkey
Phone: +90 532 747 90 27 E-mail: yguzel47@gmail.com ORCID ID: orcid.org/0000-0002-5631-8638

Received: 17.10.2018
Accepted: 21.10.2018

Cite this article as: Güzel Y. Follicle-stimulating Hormone Induced *In Vitro* Growth of Small Antral Follicles are not Affected by Gonadotropin-releasing Hormone Agonist or Antagonist Treatment in Mice. Eur Arch Med Res 2019; 35 (4): 211-4

small antral follicles isolated from mice and expressing FSH and GnRH receptors. We aimed to address if GnRHa and antagonist has any effects on FSH induced *in vitro* growth mouse early antral follicles expressing cognate receptors for FSH and GnRH.

METHODS

Study Design

We designed this study to investigate *in vitro* conditions if GnRHa and antagonist treatment have any impact on FSH induced growth of isolated mouse early antral follicles expressing cognate receptors for FSH and GnRH. For this purpose, small antral follicles were isolated from mouse ovaries and randomly assigned to the groups as control, FSH only, FSH + GnRHa, and FSH + GnRH antagonist and cultured for five days.

Follicle Isolations from Mice

All experiments were conducted 21-day-old C57BL/6 mice. The Institutional Animal Care and Use Committee of İstanbul Aydın University Faculty of Medicine approved the protocol (issue number: 2015-20). Animals were euthanized by cervical dislocation, and ovaries were removed, minced into two or three fragments in pre-warmed Dulbecco modified eagle medium-F12 (DMEM-F12) culture medium. Then the pieces were disassociated with collagenase, DNase-I in DMEM-F12 supplemented with 5% bovine serum albumin (BSA) for 30 minutes at 37 °C. Small antral follicles were mechanically isolated under a stereomicroscope (Olympus SZX12, Olympus America Inc., Center Valley, PA, USA) and randomly assigned to the groups to be cultured for five days.

Culture Medium Formulation

Isolated follicles were cultured in (HEPES)-buffered DMEM-F12 culture medium with 10% fetal bovine serum supplemented with and without 100 mIU/mL recombinant FSH, 3 mg/mL BSA, and 1% (v/v) Penicillin-G, streptomycin, amphotericin-B cocktail at 37 °C and 5% CO_2 in air. GnRHa leuprolide acetate (50 ng/mL) and antagonist cetrorelix acetate (5 ng/mL) were used at the concentrations that were previously shown to have *in vitro* activity on granulosa cells and ovarian tissue samples (6).

3D Culture on Matrigel

Growth factor reduced matrigel was used in the study, we described previously (7). In brief, matrigel was thawed at 4 °C slowly, and then diluted with DMEM-F12 medium in a 1:1 ratio and added as 100 uL volume in each well of the 96 well culture plate and kept at 4 °C. Then isolated follicles were placed in one follicle for each well fashion and placed in the incubator. Once the matrigel is solidified 30 minutes after incubation, 100 uL of complete media was added on top of it and replenished every day. The images of the follicle diameter were taken every day using an Olympus IX71 microscope, and follicle diameter was measured using specialized software (Olympus DPS, USA).

Gene Expression Analysis by Quantitative Real Time-Polymerase Chain Reaction

RNA isolation from isolated follicles was performed by Quick-RNA™ MicroPrep (Zymo Research) following the manufacturer's guidelines. RNA quantification was completed by spectrophotometric read at 260 nm by Nanodrop (Thermo Scientific). 1000 ng cDNA synthesis was performed by reverse transcription of RNA using moloney murine leukemia virus reverse transcriptase (Invitrogen). Light Cycler® 480 SYBR Green I Master (Roche) was used to quantify relative mRNA expression levels of GnRH-R and FSH-R genes.

Primers Used

Gene	Sequence	
GnRH-	R	F5'-GGCTGCCTCTTCATCCCCCT-3'
	R	5'-CGTTCTCAGCCGAGCTCTTGGG-3'
FSH-R	F	5'- ACACAACTGTGCATTCAACGG-3'
	R	5'-GACTTGTTGCAAATTGGATGA-3'
GAPDH	F	5'-ACAGTCAAGGCCGATAATGG-3'
	R	5'-TCTCCATGGTGGTGAAGACA-3'

Statistical Analysis

Follicle diameter is continuous data and stated as the mean ± standard deviation. The comparison of the groups was made using ANOVA and multiple comparison Tukey post hoc tests.

RESULTS

We first conducted a validation experiment to assess if the experimental design is appropriate to test the effects of GnRHa and antagonist on *in vitro* growth of early antral follicles in mice. For this purpose, small follicles that were 200-300µ in diameter and had antrum formation were selected for the study. The expression of GnRH and FSH receptor in these follicles were verified with quantitative real time-polymerase chain reaction (Figure 1). Then, isolated follicles were randomly assigned into four groups: no FSH (control), FSH only, FSH + GnRHa, and FSH + GnRH antagonist, each group consisting of 23-25 follicles. The results shown below are the mean values of three independent replicates of the experiments. The initial diameter of the follicles

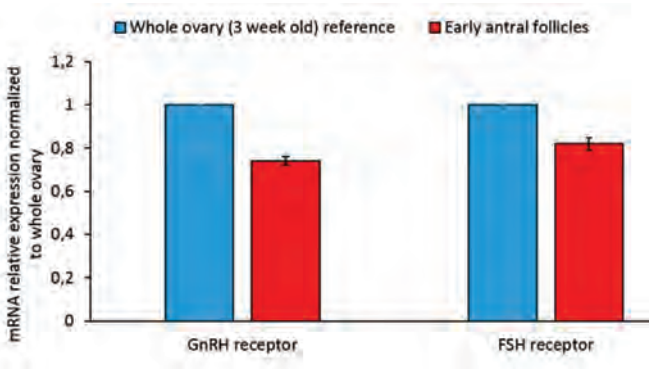


Figure 1. mRNA relative expression normalized to whole ovary
FSH: Follicle-stimulating hormone, GnRH: Gonadotropin-releasing hormone

at the beginning of the experiment was comparable among the groups (Figure 2). At the end of the culture period, the mean follicle size (μ) of the follicles treated with FSH was notably higher than their counterparts incubated without FSH (657±62 vs. 325±30 respectively, p<0.01). Follicles treated with FSH + GnRHa (545±61 vs. 657±62 respectively, p>0.05) or with FSH + GnRH antagonist (615±46 vs. 657±62 respectively, p>0.05) were not different from those treated with FSH only (Figure 2) in the mean of diameter. When the percentage of growth was compared among the groups it appeared that control follicles treated without FSH achieved a growth rate of 61% at the end of 5 day culture period, which was significantly lower compared those treated with FSH (204%, p<0.01), FSH + GnRHa (162%, p<0.01) and FSH + GnRHa (186%, p<0.01) groups. We observed no considerable differences between FSH, FSH + GnRHa, and FSH + GnRH antagonist groups in terms of follicular growth rate.

DISCUSSION

We performed this study to investigate *in vitro* conditions if GnRHa and antagonist treatment have any impact on FSH induced growth of isolated mouse early antral follicles expressing cognate receptors for FSH and GnRH.

Our results suggest that in mice, FSH-driven *in vitro* growth of small antral follicles is not adversely affected by GnRHa or antagonist co-treatment *in vitro*. It has been shown by different groups that mouse ovaries express the GnRH receptor (1-4). In rats, the comparison of GnRH receptor expression at different follicular stages demonstrated that these mRNA levels of the *GnRH-R* gene vary depending on follicles degree of maturation as well as the estrous cycle stage. While follicles in preantral and small antral stages and corpora lutea showed lower expression, Graafian and atretic follicles had the highest level of *GnRH-R* gene expression (3,8,9). In human ovary, GnRH receptors are localized in granulosa cells from the antral stage and in the

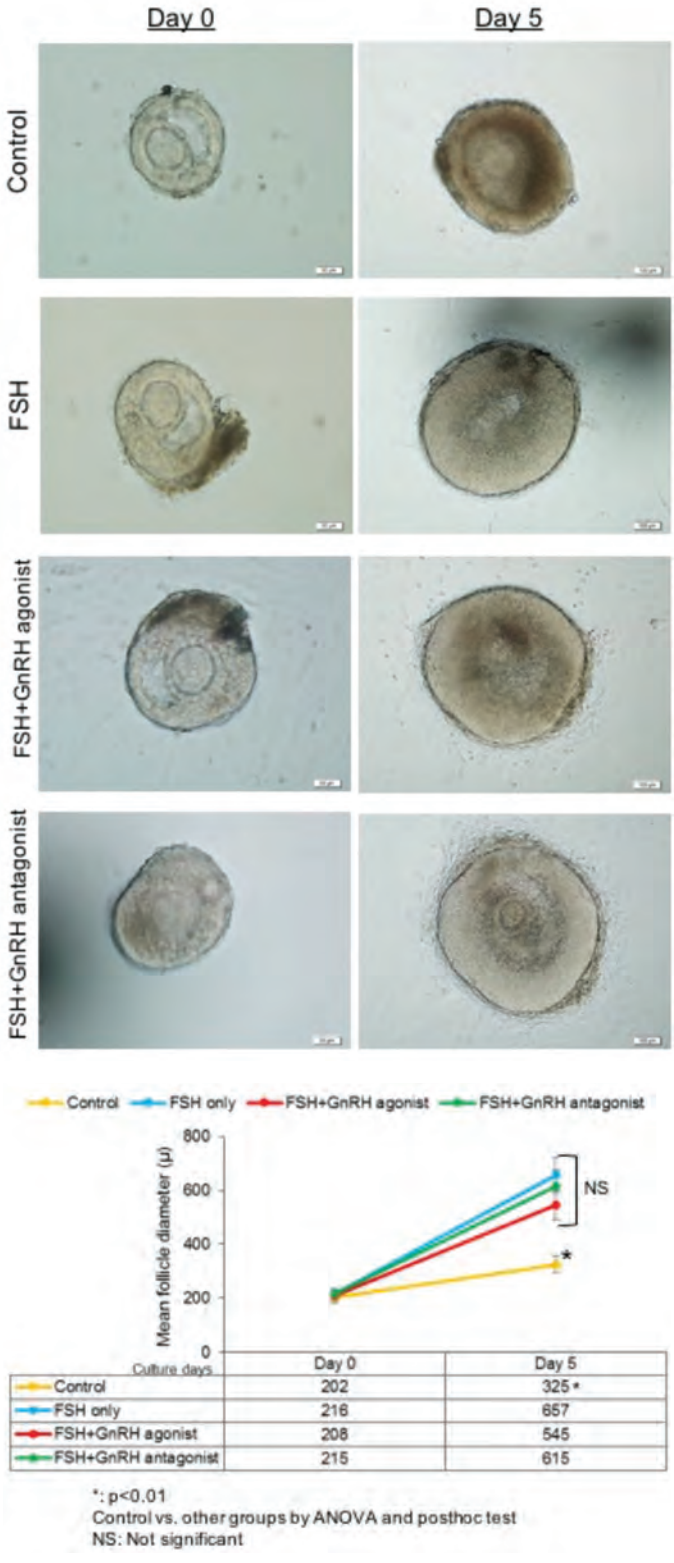


Figure 2. Control vs. other groups by ANOVA and posthoc test
NS: Not significant, FSH: Follicle-stimulating hormone, GnRH: Gonadotropin-releasing hormone

corpus luteum (10). Exploring intra-ovarian actions of GnRH analogs is hampered by the fact that the systemic administration of GnRH analogs affects the hypothalamic-pituitary-ovarian axis,

precluding one from investigating the sole intra-ovarian action of GnRH analogs on folliculogenesis. We, therefore, aimed to investigate the effect of GnRH analogs, namely, GnRHa leuprolide acetate and antagonist cetrorelix acetate on FSH driven *in vitro* growth of small antral follicles in an isolated culture that express FSH and GnRH receptors.

Although not conducted in human antral follicles, our data might be particularly important from the perspective of assisted reproduction in humans because controlled ovarian stimulation is carried out with FSH treatment together either with a GnRHa or antagonist. It is unknown whether GnRH analogs have any effect on the growth kinetics of small antral follicles that have not reached 2-10 mm diameter, therefore, are not visible on ultrasound.

Our study has several limitations as follows: First, this is an *in vitro* study on the individual culture of isolated small antral follicles. Therefore, the paracrine/autocrine interaction of locally produced growth factors in the intra-ovarian environment that may potentially affect or modify the actions of GnRH analogs cannot be assessed using this model. Second, matrigel contains some growth factors such as EGF, FGF, and TGF, in addition to its main components, extracellular matrix proteins collagen, laminin, and entactin, etc. They may change the response of the follicles to FSH with and without GnRH analogs. Third, the competence of these *in vitro* grown follicles for full growth, ovulation, mature oocyte yield, fertilization rate, and embryo development could not be assessed using this experimental methodology. Forth, GnRHa and antagonists were not tested at different concentrations. After a certain threshold level, they may have a different action after they bind to their cognate receptors in the follicle.

Ethics

Ethics Committee Approval: The Institutional Animal Care and Use Committee of İstanbul Aydın University Faculty of Medicine approved the protocol (issue number: 2015-20).

Informed Consent: Not applicable.

Peer-review: External and internal peer-reviewed.

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

REFERENCES

1. Torrealday S, Lalioti MD, Guzeloglu-Kayisli O, Seli E. Characterization of the gonadotropin releasing hormone receptor (GnRHR) expression and activity in the female mouse ovary. Endocrinology 2013;154:3877-87.
2. Mo Y, Peng P, Zhou R, He Z, Huang L, Yang D. Regulation of gonadotropin-releasing hormone (GnRH) receptor-I expression in the pituitary and ovary by a GnRH agonist and antagonist. Reprod Sci 2010;17:68-77.
3. Horicks F, Van Den Steen G, Gervy C, Clarke HJ, Demeestere I. Both in vivo FSH depletion and follicular exposure to Gonadotrophin-releasing hormone analogues in vitro are not effective to prevent follicular depletion during chemotherapy in mice. Mol Hum Reprod 2018;24:221-32.
4. Horicks F, Van Den Steen G, Houben S, Englert Y, Demeestere I. Folliculogenesis Is Not Fully Inhibited during GnRH Analogues Treatment in Mice Challenging Their Efficiency to Preserve the Ovarian Reserve during Chemotherapy in This Model. PLoS One 2015;10:e0137164.
5. Huang J, Wang X, Li Z, Ma R, Xiao W. Effects of GnRH agonists on the expression of developmental follicular anti-mullerian hormone in varying follicular stages in cyclic mice in vivo. Mol Med Rep 2015;12:4305-13.
6. Bildik G, Akin N, Senbabaoglu F, Sahin GN, Karahuseyinoglu S, Ince U, et al. GnRH agonist leuprolide acetate does not confer any protection against ovarian damage induced by chemotherapy and radiation in vitro. Hum Reprod 2015;30:2912-25.
7. Oktem O, Buyuk E, Oktay K. Preantral follicle growth is regulated by c-Jun-N-terminal kinase (JNK) pathway. Reprod Sci 2011;18:269-76.
8. Bauer-Dantoin AC, Jameson JL. Gonadotropin-releasing hormone receptor messenger ribonucleic acid expression in the ovary during the rat estrous cycle. Endocrinology 1995;136:4432-8.
9. Whitelaw PF, Eidne KA, Sellar R, Smyth CD, Hillier SG. Gonadotropin-releasing hormone receptor messenger ribonucleic acid expression in rat ovary. Endocrinology 1995;136:172-9.
10. Maggi R, Cariboni AM, Marelli MM, Moretti RM, Andre V, Marzagalli M, et al. GnRH and GnRH receptors in the pathophysiology of the human female reproductive system. Hum Reprod Update 2016;22:358-81.

ORIGINAL ARTICLE

DOI: 10.4274/eamr.galenos.2018.77486
Eur Arch Med Res 2019; 35 (4):215-9

Intraperitoneal Ventralex™ ST Hernia Patch Application for Ventral Hernia Repair

Birol Ağca, Yalın İşcan

İstanbul Fatih Sultan Mehmet Training and Research Hospital, Clinic of General Surgery, İstanbul, Turkey

Abstract

Objective: Traditional methods commonly used in ventral hernia repair have a high recurrence rate. In patch repair, the recurrence rate is low, but there are many prosthetic materials. Ventralex™ ST hernia patch has small and medium sizes. We aimed to determine the results in ventral hernias.

Methods: A single-center retrospective analysis was performed. Ventral hernias <3 cm were repaired using intraperitoneal Ventralex™ ST hernia patch between January 2015 and March 2017. Demographic characteristics, operative time, analgesic requirement, length of hospital stay, postoperative complications, and recurrences were recorded.

Results: A total of 65 patients with umbilical, epigastric, and trocar site hernia underwent surgery. Thirty-four patients were female, and 31 were male. According to the localization, 43 patients had an umbilical hernia, 16 had an epigastric hernia, and six had trocar site hernia. Hernia size was <2 cm in 35 patients and 2-3 cm in 30 patients. The mean body mass index was 28 kg/m^2. Wound infection was detected in two patients, and one patient had seroma. The mean follow-up was 17 months. No recurrence was observed during follow-up.

Conclusion: Ventralex™ ST hernia patch is a safe prosthetic material that can be applied by open surgical technique with low complication and recurrence rates, especially in small and medium-sized umbilical, epigastric and trocar site hernias.

Keywords: Ventral hernia, umbilical hernia, Ventralex™ hernia patch

INTRODUCTION

The European Hernia Society classifies primary abdominal wall hernias (ventral hernias) by localization and size. Epigastric and umbilical hernias were classified as "midline hernias" and spighelian and lumbar hernias were classified as "lateral hernias". According to hernia size, they classified hernias into three groups as small (<2 cm), medium (≥2-4 cm), and large (≥4 cm) (1). These hernias are most commonly seen in the umbilical region, according to localization (2). Although most do not have any symptoms, umbilical hernias are often symptomatic and require surgical repair due to the risk of incarceration (3,4). Different techniques have been described in surgical repair. The first is the technique of approaching the fascias with interrupted sutures. The second and most commonly used technique is the closure of the fascia in a "double-breasted" fashion (5). Recurrence of 25-55% has been reported in both conventional methods (6-7). However, especially in small hernias, these methods are still preferred (5). In patch repair techniques (hernioplasty), prosthetic materials are placed with or without sutures in the defect area. These materials are placed on the fascia, retromuscular pre-facial area, or pre-peritoneal area (8). Hernia recurrence is reduced to 1% in hernioplasty techniques (7). Ventralex™ ST (C.R.Bard, RI, USA) is a patch placed intraperitoneally by the open surgical method. In the literature, it was reported that this patch was used safely in small and medium-sized abdominal wall hernias, and the recurrence rates were low (0-1%) (9). In this study, early results, complications, and recurrences of Ventralex™ ST hernia patch in small and medium abdominal wall hernias were discussed in the light of the literature.

METHODS

Sixty-five patients with small and medium abdominal wall hernias underwent surgery between January 2015 and March 2017 (Figure 1). Patients under 18 years of age, patients with

Address for Correspondence: Birol Ağca, İstanbul Fatih Sultan Mehmet Training and Research Hospital, Clinic of General Surgery, İstanbul, Turkey
Phone: +90 533 347 27 24 E-mail: birolagca@yahoo.com ORCID ID: orcid.org/0000-0003-0485-476X

Cite this article as: Ağca B, İşcan Y. Intraperitoneal Ventralex™ ST Hernia Patch Application for Ventral Hernia Repair. Eur Arch Med Res 2019; 35 (4): 205-9

Received: 02.07.2018
Accepted: 27.10.2018

a hernia diameter greater than 4 cm, and American Society of Anesthesiologists (ASA) IV patients were excluded from the study. Age and gender characteristics, operative time, body mass index (BMI), postoperative analgesic use, length of hospital stay, postoperative complications, and recurrences were recorded. The study was initiated after approval by the ethics committee of our hospital. All patients in the study were informed by written and verbal explanations, and informed consent forms were obtained. All patients were operated under general anesthesia by two specialists working in the same clinic. Three patients underwent simultaneous laparoscopic cholecystectomy, and one patient underwent inguinal hernia surgery. Ventralex™ ST hernia patches of different sizes (diameter: 4.3 cm-6.4 cm) composed of polypropylene mesh with hydrogel barrier reinforced with double-sided bio-resorbable polyglactin fibers were used as hernia patch.

Surgical Technique

After induction of anesthesia, all patients received prophylaxis with a single dose of intravenous antibiotics (1 g Cefazolin sodium). The hernia sac was reached by transverse skin incision over the hernia, and the sac was exposed (Figure 2). The hernia sac was opened, and the hernia contents were pushed into the abdomen. The diameter of the defect was measured to use the appropriately sized patch. The hernia patch was applied to extend at least 2-2.5 cm in all directions from the intact edges of the defect. The hydrogel barrier-coated side of the patch was placed facing the abdomen. The polypropylene side of the patch was applied facing the anterior wall of the abdomen. Intervening tissues were prevented by pulling up the patch with the help of long strips on the edge of the patch (Figure 3, 4). Each of the hanging strips was sutured to the intact fascia with two 2/0 prolene sutures. The fascia was closed primarily. The surgery was terminated with subcutaneous and skin suturing (Figure 5). Drainage was not applied to any patient. The patients who were discharged were given oral analgesic (paracetamol tablet) treatment for seven days. All patients were called to the outpatient control one week and three weeks after the operation.

Statistical Analysis

Descriptive statistics were used to calculate percentages for categorical variables and mean and standard deviation values for continuous variables. All analyses were performed using the SPSS version 16.0 statistical software package (SPSS Inc., Chicago, IL, USA).

RESULTS

Thirty-four patients were female (52.3%), and 31 were male (47.7%). The mean age was 44.5 years (range=28-74). Of the 65 patients, 43 had an umbilical hernia (66.15%), 16 had an epigastric hernia (24.61%), and six had trocar site hernia (9.23%). Forty-four patients had a BMI of >30 kg/m^2 and 21 patients had a BMI of <30 kg/m^2 (Table 1). The size of the hernia was <2 cm in 35

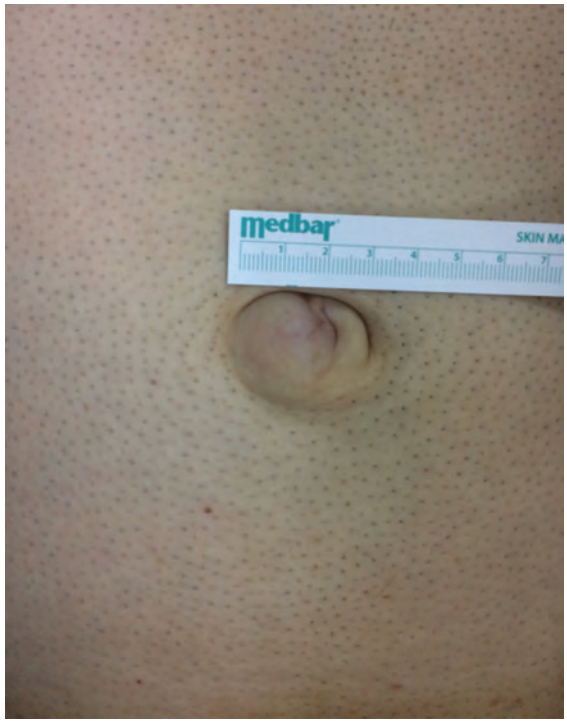


Figure 1. <3 cm umbilical hernia case

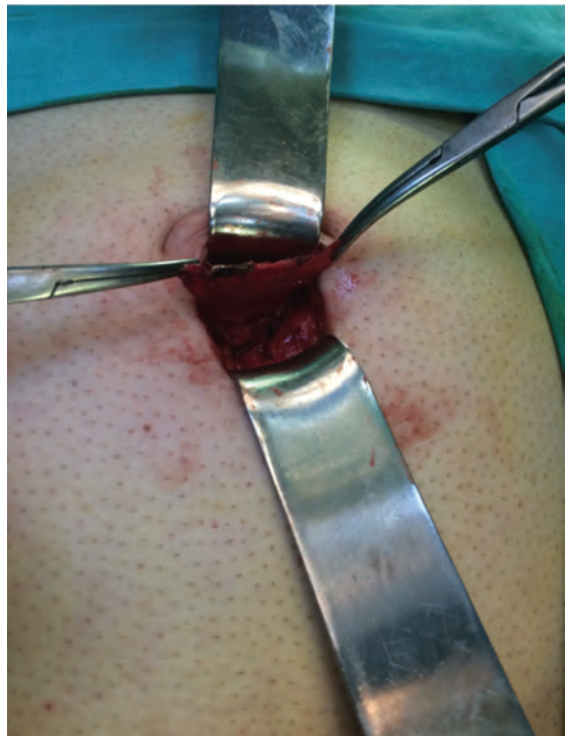


Figure 2. Dissection of the peritoneal sac

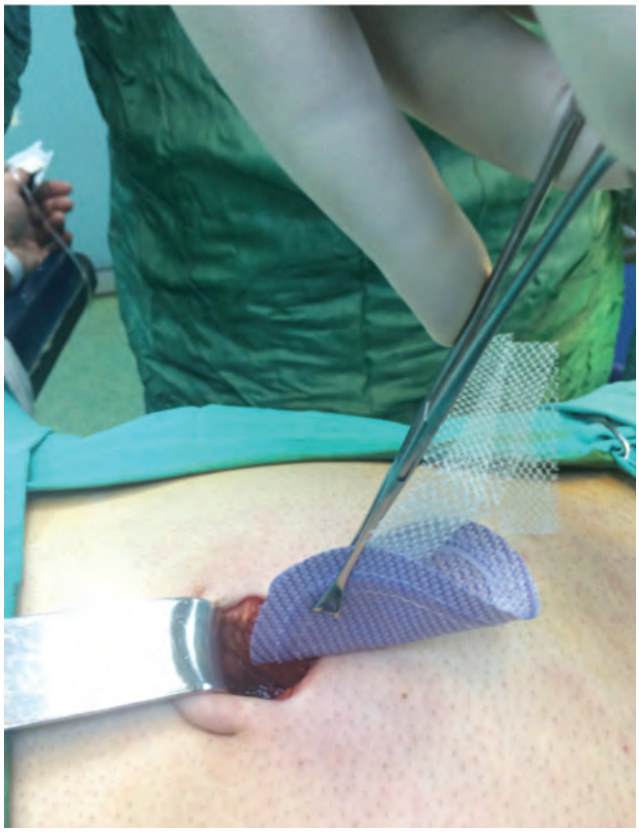


Figure 3. Opening the hernia sac and sending the patch to the intraperitoneal area

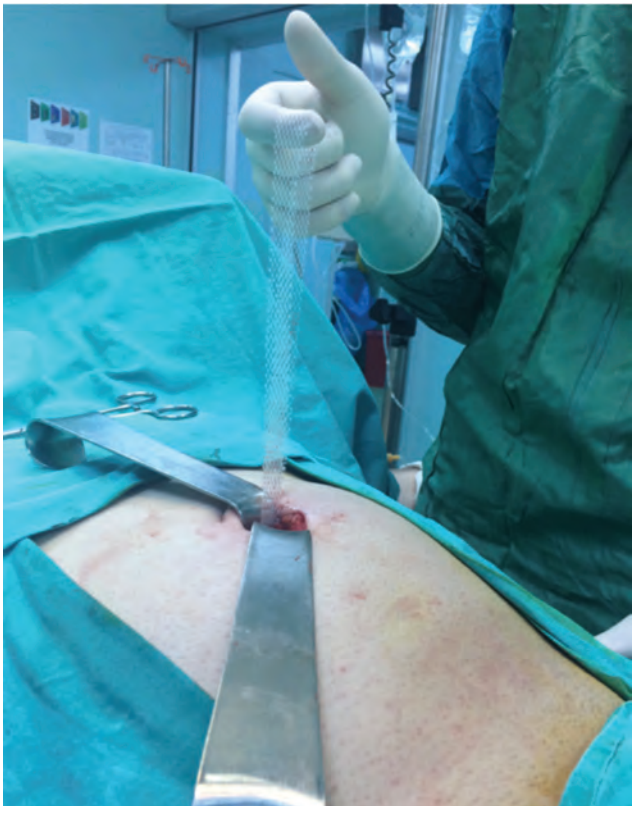


Figure 4. Pulling the patch up from the hangers on both sides

patients and between 2-3 cm in 30 patients. Ventralex™ ST patch was applied in small size (4.3x4.3 cm) in 24 patients and medium size (6.4x6.4 cm) in 41 patients (Table 2). Thirty-five patients had ASA score I, 11 had ASA score II, and 19 had ASA score III. The mean operative time was 37.67 minutes (range=23-67), and the mean hospitalization time was 1.1 days (range=1-4). Four patients were hospitalized for three days for pain control. None of the patients needed narcotic analgesics. The mean follow-up period of the patients was 17.23 months (range=6-30), and no recurrence was observed during the follow-up. Two patients (3.07%) developed a subcutaneous infection during follow-up. These patients received symptomatic treatment (Table 3).

DISCUSSION

The surgical method to be used in umbilical hernias according to defect diameter, which are the most common primary abdominal wall hernias, is controversial. Most surgeons recommend surgery for the treatment of these hernias, even if the defect diameter is small, because of the risk of incarceration and strangulation (10). High recurrence rates (25-55%) have been reported in simple suture techniques, and the Mayo technique (non-patched treatment methods) (11). In a randomized prospective study by Arroya et al. (7), the recurrence rate was reported as 1% when a patch was used in umbilical hernia repair and 11% when suture alone was used, and it was concluded that patch should be used primarily in umbilical hernia repair. In another study with a mean follow-up of 64 months in patients treated with simple sutures, hernia recurrence was reported as 54.5%

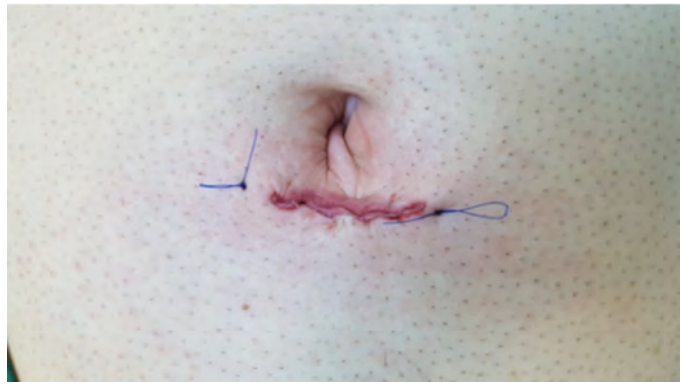


Figure 5. Completed repair

Table 1. Patient characteristics	
Variable	**n**
Total number of patients	65
Female/male	34/31
Mean age (years)	44.5 (28-74)
Mean body mass index (kg/m^2)	32 (18-68)

Table 2. Perioperative data in Ventralex™ ST patch repair		
Variables	**%**	**n**
Hernia size (cm)		
<2 cm	53.84%	35
2-3 cm	46.16%	30
Hernia type		
Umbilical hernia	66.15%	43
Epigastric hernia	24.61%	16
Trocar site hernia	9.23%	6
Ventralex™ ST size		
Small (4.3x4.3 cm)	36.93%	24
Medium (6.4x6.4 cm)	63.07%	41

Table 3. Ventralex™ ST patch repair results		
Variables	**%**	**n**
Complications	-	-
Recurrence	0	0
Seroma	1.53	1
Wound infection	3.07	2
Follow-up period (months)	-	17.23 (minimum-maximum 6-30)

(12). Such high recurrence rates have led to a shift from simple suture techniques to patch repair techniques. Although there is no objective data between umbilical hernia size and treatment, patch repair is recommended, especially in high-risk and obese patients if the defect diameter is >3 cm (3,13). The real question here is what should be the treatment for hernia smaller than 3 cm. The high risk of incarceration and strangulation in this type of hernia has led to the preference of patch repair (7,14,15). In a study of 100 patients with ventral/umbilical hernia smaller than 3 cm in which Wang and Berney (16) applied a Ventralex™ patch, no recurrence was observed during a mean follow-up of 37.9 months. Also, no recurrence was observed in a study by Martin et al. (17), including 88 cases. In our study, no recurrence was observed during the 17-month follow-up period. On the other hand, Berrevoet et al. (18) reported recurrence in five cases treated with intraperitoneal patch and two cases treated with retromuscular patch among 116 patients with umbilical hernia less than 3 cm that were operated with the open method. In a study by Tollens et al. (19) the recurrence rate was reported to be 8.9% at 135 months of follow-up in 135 patients treated with Ventralex™ for ventral hernia. This high recurrence rate was attributed to technical application and differences in the fixation of the polypropylene side to the fascia. In this study of Tollens, it was also observed that all patients had a uniform medium size (6.4x6.4 cm) patch. In our opinion, another reason for high recurrence was the use of a uniform patch regardless of the defect diameter. It has been reported in the literature that the patch to be applied should extend at least ≥2.5 cm from the edges of the defect (18). In our study, this situation was taken into consideration, and appropriate size patches were used by measuring the diameter of the defect. When we look at the complications in the literature, we encounter different types of complications as in recurrences. Complication rates vary between 2-11.8%, and the most common complications are local infections, seroma, and hematoma (20,21). In our study, superficial wound infection was observed in two cases, and seroma was observed in one case. Both cases were controlled with conservative follow-up and antibiotics.

CONCLUSION

In this study, it was concluded that the Ventralex™ ST patch, which can be applied quickly and easily with open surgery method in small and medium-sized abdominal wall hernias, could be used safely due to low complication and recurrence rates. Also, long-term randomized controlled trials with different patches are thought to contribute more to the literature.

Ethics

Ethics Committee Approval: Retrospective study

Informed Consent: This is a retrospective study. Patient data were taken from the files.

Peer-review: External and internal peer-reviewed.

Authorship Contributions

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

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

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

REFERENCES

1. Muysoms FE, Miserez M, Berrevoet F, Campanelli G, Champault GG, Chelala E, et al. Classification of primary and incisional abdominal wall hernias. Hernia 2009;13:407-14.
2. Aguirre DA, Santosa AC, Casola G, Sirlin CB. Abdominal wall hernias:imaging features,complications,and diagnostic pitfalls at multi-detector row CT. Radiographics 2005;25:1501-20.
3. Rodriguez JA, Hinder RA. Surgical management of umbilical hernia. Oper Tech Gen Surg 2004;6:156-64.
4. Ambe P, Meyer A, Köhler L. Repair of small and medium size ventral hernias with a Proceed Ventral Patch: a single center retrospective analysis. Surg Today 2013;43:381-5.

5. Muschaweck U. Umbilical and epigastric hernia repair. Surg Clin North Am 2003;83:1207-21.

6. Sinha SN, Keith T. Mesh plug repair for paraumbilical hernia. Surg J R Coll Edinb Ire 2004;2:99-102.

7. Arroya A, Garcia P, Perez F, Andreu J, Candela F, Calpena R. Randomized clinical trial comparing suture and mesh repair of umbilical hernia in adults. Br J Surg 2001;88:1321-3.

8. Velasco M, Garcia-Ureña MA, Hidalgo M, Vega V, Carnerol F. Current concepts on adult umbilical hernia. Hernia 1999;3:233-9.

9. Vychnevskaia K, Mucci-Hennekinne S, Casa C, Brachet D, Meunier K, Briennon X, et al. Intraperitoneal mesh repair of small ventral abdominal wall hernias with a Ventralex hernia patch. Dig Surg 2010;27:433-5.

10. Sinha SN, Keith T. Mesh plug for paraumblical hernia. Surg J R Coll Edinb Ire 2004,2:99-102.

11. George CD, Ellis H. The results of incisional hernia repair: a 12 years review. Ann R Coll Surg Engl 1986;68:185-87.

12. Schumacher OP, Peiper C, Lörken M, Schumpelick V. Long-term results after Spitzy's umblical hernia repair. Chirurg 2003;74:50-4.

13. Sánchez-Pernaute A, Pérez-Aguirre E, García Botella A, Rodríguez L, Antona EM, Cabeza J, et al. Prophylactic closure of trocar orifices with an intraperitoneal mesh (ventralex) in laparoscopic bariatric surgery. Obes Surg 2008;18:1489-91.

14. Sanjay P, Reid TD, Davies EL, Arumugam PJ, Woodward A. Retrospective comparison of mesh and sutured repair for adult umbilical hernias. Hernia 2005;9:248-51.

15. Arroyo Sebastián A, Pérez F, Serrano P, Costa D, Oliver I, Ferrer R, et al. Is prosthetic umbilical hernia repair bound to replace primary herniorrhaphy in the adult patient? Hernia 2002;6:175-7.

16. Wang K, Berney CR. Prospective Analysis and Technical Recommendations for Repair of Small Ventral/Umbilical Hernias Using the Ventralex Hernia Patch. International Journal of Surgery Research and Practice 2015;2:2-5.

17. Martin DF, Williams RF, Mulrooney T, Voeller GR. Ventralex mesh in umbilical/epigastric hernia repairs: clinical outcomes and complications. Hernia 2008;12:379-83.

18. Berrevoet F, D'Hont F, Rogiers X, Troisi R, de Hemptinne B. Open intraperitoneal versus retromuscular mesh repair for umbilical hernias less than 3 cm diameter. Am J Surg 2011;201:85-90.

19. Tollens T, Den Hondt M, Devroe K, Terry C, Speybroeck S, Aelvoet C, et al. Retrospective analysis of umbilical, epigastric, and small incisional hernia repair using the Ventralex™ hernia patch. Hernia 2011;15:531-40.

20. Vychnevskaia K, Mucci-Hennekinne S, Casa C, Brachet D, Meunier K, Briennon X, et al. Intraperitoneal mesh repair of small ventral abdominal wall hernias with a Ventralex hernia patch. Dig Surg 2010;27:433-5.

21. Hadi HI, Maw A, Sarmah S, Kumar P. Intraperitoneal tension-free repair of small midline ventral abdominal wall hernias with a Ventralex hernia patch: initial experience in 51 patients. Hernia 2006;10:409-13.

ORIGINAL ARTICLE

DOI: 10.4274/eamr.galenos.2018.68077
Eur Arch Med Res 2019; 35 (4): 220-3

Comparison of Wide Awake Local Anaesthesia No Tourniquet Technique with Tourniquet Application under General Anesthesia in Carpal Tunnel Syndrome: A Retrospective Study

Mustafa Çağlar Kır, Mehmet Kürşad Bayraktar, Hakan Gürbüz

İstanbul Okmeydanı Training and Research Hospital, Clinic of Orthopaedics and Traumatology, İstanbul, Turkey

Abstract

Objective: Carpal tunnel syndrome is a common complaint in orthopedic practice and usually treated with surgical intervention. The aim of the study was to compare wide awake local anesthesia no tourniquet (WALANT) technique with general anesthesia and arm tourniquet in Carpal tunnel cases.

Methods: Fifty-two patients who underwent surgery for Carpal tunnel syndrome were retrospectively divided into two groups as WALANT technique (Group W) and general anesthesia with arm tourniquet (Group G). In addition to the demographic data, visual analog scale (VAS), opioid consumption, early complications, duration of stay in operating room (DSOR) and first analgesic need time were evaluated.

Results: When both groups were evaluated, early postoperative VAS scores, opioid consumption and DSOR were significantly lower in Group W than in Group G.

Conclusion: In conclusion, WALANT technique was associated with reduced early postoperative pain, lower DSOR and opioid consumption compared to general anesthesia with tourniquet method for carpal tunnel release.

Keywords: Wide awake local anesthesia no tourniquet, Carpal tunnel syndrome, general anesthesia, pain

INTRODUCTION

Carpal tunnel syndrome, which affects 3% of the population, is an orthopedic condition that often requires surgical approach for permanent treatment (1). In fact, Carpal tunnel surgery is one of the most common surgical wrist procedures in the United States with 300,000 to 600,000 cases per year (2). Anesthesia and surgical techniques may vary according to surgeon preferences and patient-related factors (3). Despite the increasing popularity of local anesthetic methods, general and regional anesthesia techniques are still widely used (4). While elderly patients prefer local methods, younger patients often prefer general anesthesia (5). Adverse effects and complications may occur up to 25% of patients after Carpal tunnel surgery (5,6). Problems that may develop during the surgery or in the very first 24 hours include pain, airway obstruction, bleeding, difficulty in awakening, hypoxia, hypotension, hypertension, nausea and vomiting (5). These all could increase morbidity and reduce patient satisfaction. Tourniquet devices are commonly used in extremity surgery to reduce blood loss and create a blood-free surgical site (7). Though well improved so far, tourniquet techniques have been still complicated with pain, paresthesia, permanent and transient nerve injury, compartment syndrome, lymphedema, and arteriovenous shunt, etc. (8,9). After the misconception that low epinephrine concentrations lead to permanent vasospasm and necrosis in the digits was falsified, wide awake local anesthesia no tourniquet (WALANT) anesthesia technique was started to be applied as it minimizes bleeding without using tourniquet and sedation and eases the surgical intervention (10). Other advantages of a such wide-awake anesthesia technique include no need for perioperative monitoring, early ambulation, reduced costs and the ability to cooperate with the patient for active movement examination during tendon, fracture and ligament procedures (11,12).

Address for Correspondence: Mustafa Çağlar Kır, İstanbul Okmeydanı Training and Research Hospital, Clinic of Orthopaedics and Traumatology, İstanbul, Turkey
Phone: +90 507 485 60 34 E-mail: drcaglarkir@gmail.com ORCID ID: orcid.org/0000-0002-5073-7401

Received: 31.08.2018
Accepted: 28.10.2018

Cite this article as: Kır MÇ, Bayraktar MK, Gürbüz H. Comparison of Wide Awake Local Anaesthesia No Tourniquet Technique with Tourniquet Application under General Anesthesia in Carpal Tunnel Syndrome: A Retrospective Study. Eur Arch Med Res Res 2019; 35 (4): 220-3

This study aimed to compare WALANT technique with tourniquet application under general anesthesia in terms of postoperative complications and function outcomes in cases that underwent open release due to Carpal tunnel syndrome.

METHODS

The study was conducted per ethical standards of the 1964 Helsinki Declaration. Medical records of 93 cases that underwent open release due to Carpal tunnel syndrome between January 2015 and January 2017 was retrospectively reviewed. A total of 41 cases were excluded due to reasons such as revision Carpal tunnel surgery, previous hand-wrist surgery, history of upper extremity malignancy, vascular insufficiency, only undergoing local anesthesia or regional blockade, and missing medical data or regular follow-up. The remaining 52 cases were classified by their applied anesthesia technique as Group W (WALANT anesthesia, n=23) and Group G (general anesthesia plus forearm tourniquet, n=29). All cases in Group G were administered propofol 2-3 mg/kg, fentanyl 2-5 µg/kg, midazolam 0.2 mg/kg and rocuronium 0.6 mg/kg for inducing anesthesia, which was afterwards maintained by N_2O/sevoflurane via laryngeal mask. After a 3 minute arm elevation, an 8 cm forearm tourniquet supported by adequate cotton was applied such that systolic blood pressure was kept above 100 mmHg. Subjects in Group W received a 10 mL subcutaneous injection (27G needle) consisting of 1% lidocaine, 8.4% bicarbonate and buffered 1/100,000 epinephrine, without the use of any sedation or tourniquet. A 2-3 cm palmar longitudinal incision was made between thenar and hypothenar eminence along the axis of the 4th digit. The skin and subcutaneous tissue were passed with sharp dissection, and transverse carpal ligament was thoroughly released with no.: 15 scalpel blade (13). All cases were applied elevation on the first day. Active finger movements were allowed as much as tolerated after two days. The subjects were followed with dressing and elastic bandage until removal of sutures at the end of two weeks, after which they were allowed to resume daily working routine.

Both groups received intravenous paracetamol 15 mg/kg every 8 hours for postoperative analgesia. In case of severe pain [visual analog scale (VAS) >3], tramadol 2 mg/kg was added to the analgesic regimen. Demographic characteristics, early postoperative complications (nausea, vomiting, airway problem, bleeding, hypotension, hypertension, paresthesia, nerve injury), VAS score at hour 24 and 72, duration of stay in operating room (DSOR), postoperative opioid consumption and first analgesic need time (FANT) were recorded.

Statistical Analysis

IBM SPSS Statistics 22 program was used for statistical analysis. The distribution of the data was evaluated by Kolmogorov-Smirnov test. Descriptive statistical methods (mean, standard deviation) were used in the analysis. Independent Samples t-test was utilized for parametric values. Fisher exact test was used for qualitative data. P<0.05 was assessed as statistically significant.

RESULTS

The mean age of the patients was similar between Group W (63.7±6.1 years) and Group G (62.7±6.2 years) (p=0.53). Female patients constituted the majority of cases in both groups, Group W (70%, n=16) and Group G (65.5%, n=19). Although the groups did not significantly differ in terms of operative time [Group W=17.26±4.90 minute (min), Group G=17.04±5.11 min; p=0.87], DSOR was significantly higher in Group G compared to that of Group W (46.48±5.49 min vs. 33.43±6.27 min, respectively; p=0.0001) (Table 1). Analysis of early postoperative pain showed that 24-hour and 72-hour VAS scores were significantly lower in Group W compared to those in Group G (24-hour VAS=3.13±1.79 vs. 4.55±1.62, respectively; p_{24}=0.0042 and 72-hour VAS=2.26±0.92 vs. 3.59±0.87, respectively; p_{72}=0.0001). FANT was longer in Group W than in Group G (282±68.27 min vs. 121.03±31.01 min, respectively; p=0.0001). The opioid consumption due to severe pain was found to be more common

Table 1. Comparison of age, operative time, duration of stay in the operating room, first analgesic need time and visual analog scale scores between the groups

	Group W (Mean ± SD)	Group G (Mean ± SD)	p
Age, years	63.74±6.08	62.66±6.23	0.53
OT, minutes	17.26±4.90	17.04±5.11	0.87
DSOR, minutes	33.43±6.27	46.48±5.49	0.0001*
VAS (24-hour)	3.13±1.79	4.55±1.62	0.0042*
VAS (72-hour)	2.26±0.92	3.59±0.87	0.0001*
FANT, minutes	282±68.27	121.03±31.01	0.0001*

OT: Operative time, DSOR: Duration of stay in the operating room, VAS: Visual analog scale, FANT: First analgesic need time, SD: Standard deviation, Independent Samples t-test, p<0.05*

in Group G than that in Group W (27% vs. 4.3%, respectively; p=0.036) (Table 2).

While two cases (8.6%) developed transient paresthesia in Group W, a total of six cases (20.6%) in Group G experienced early postoperative complications, including two cases with paresthesia, and others with nausea, vomiting, hypertension, and postoperative spasm. The difference in complication rates was not statistically significant (p=0.27).

Table 2. Evaluation of early postoperative complication and opioid consumption rate for Group W and Group G

	Group W		Group G		p
Opioid consumption	n	%	n	%	
	1	4.3	8	27	0.036*
Early complication	2	8.6	6	20.6	0.27

n: Number of patients, Fisher's exact test p<0.05*

DISCUSSION

The most important finding of this study is the superior efficacy of WALANT anesthesia in early postoperative pain over general anesthesia plus forearm tourniquet in cases undergoing Carpal tunnel release procedure. Literature search did not show any direct comparison regarding these modalities. Nevertheless, Iqbal et al. (14) published a study in 2018 in which they randomized Carpal tunnel release cases to undergo either local anesthesia plus adrenaline or local anesthesia plus tourniquet and they found that early pain score was significantly higher in the latter group. Gunasagaran et al. (15) compared WALANT technique to local anesthesia plus tourniquet in 72 cases with minor hand surgery and they reported lower early postoperative pain in subjects undergoing WALANT technique. Consistent with the literature, our findings may be attributed the prolongation of postoperative analgesic time with adrenaline and/or aggravation of postoperative pain level by tourniquet application. In a study consisting of 108 cases, Gibson (16) reported that addition of adrenalin to lidocaine was associated with increased postoperative analgesic time at the expense of relatively low complication rates. Consistent to that, we also detected WALANT technique to prolong the FANT. It may be speculated that epinephrine-induced vasoconstriction in tissues may extend the duration of action of local anesthetic agent. WALANT technique is becoming increasingly popular in hand and wrist surgery. Compared with other anesthesia methods, it is reported as a usually reliable, cost-effective and timesaving procedure (17-19). Other advantages of the technique include avoidance of tourniquet-related pain and other complications and evaluation of perioperative active movements via patient cooperation. Finger fracture fixation, tendon repair or transfer, arthrodesis, arthroplasty, release of median nerve and trigger finger could be performed with this technique (15). In this study, we performed open median nerve release with this technique. Though the duration of the surgery did not alter, DSOR was shorter than that in general anesthesia and tourniquet technique. Carpal tunnel release is one of the most common interventions in hand surgery practice. Despite being a minor surgery, it was reported to be complicated in up to one-fourth of cases. In our study, 20.6% of general anesthesia plus tourniquet group had early postoperative complications, including paresthesia, nausea, vomiting, hypertension, and bronchospasm. On the other hand, it was remarkable that 8.6% of WALANT group had transient paresthesia. The difference between the groups was not significant. Rozanski et al. (5) compared general, regional and local anesthesia in patients undergoing median nerve release and reported that early postoperative complications were found to be more common in local anesthesia than that in general anesthesia. The conflict with our study may be related with the addition of epinephrine to local anesthetic, varying general and local anesthetic techniques, and different levels of preoperative patient counseling about the conditions that may be encountered. Opioids are frequently used for the management of severe postoperative pain. In fact, the use of these drugs has increased in recent years. A study in the United States reported 142% increment of opioid prescriptions in the emergency setting and 42% increment of total opioid use from 2002 to 2009 (20,21). Chapman et al. (20) reported no difference in opioid consumption between WALANT technique and sedation in the study they performed on 277 cases with Carpal tunnel syndrome. In our study, opioid use was found to be less in the WALANT group than in patients undergoing general anesthesia plus tourniquet. It might be partially attributed to be able to establish a better postoperative analgesia with WALANT technique. Our study has several limitations, including a single-center retrospective design, small study sample, and absence of mid- and long-term outcomes and follow-up of perioperative hemodynamic parameters.

CONCLUSION

In conclusion, WALANT technique was associated with reduced early postoperative pain, opioid consumption and DSOR compared to that in general anesthesia plus tourniquet method in cases who underwent open median nerve release.

Ethics

Ethics Committee Approval: Local Editorial Board approval was received from Okmeydanı Training and Research Hospital, Department of Orthopaedic and Traumatology (8060875-E 18634).

Informed Consent: Was obtained.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: M.Ç.K., M.K.B., H.G., Concept: M.Ç.K., M.K.B., H.G., Design: M.Ç.K., M.K.B., Data Collection or Processing: M.Ç.K., Analysis or Interpretation: M.Ç.K., Literature Search: M.Ç.K., M.K.B., Writing: M.Ç.K., M.K.B., H.G .

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

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

REFERENCES

1. Concannon MJ, Brownfield ML, Puckett CL. The incidence of recurrence after endoscopic carpal tunnel release. Plast Reconstr Surg 2000;105:1662-5.
2. Fajardo M, Kim SH, Szabo RM. Incidence of Carpal Tunnel Release: Trends and Implications Within the United States Ambulatory Care Setting. J Hand Surg Am 2012;37:1599-605.
3. Michelotti B, Romanowsky D, Hauck RM. Prospective, randomized evaluation of endoscopic versus open carpal tunnel release in bilateral carpal tunnel syndrome: an interim analysis. Ann Plast Surg 2014;73:157-60.
4. Foster BD, Sivasundaram L, Heckmann N, Cohen JR, Pannell WC, Wang JC, et al. Surgical Approach and Anesthetic Modality for Carpal Tunnel Release. Hand 2017;12:162-7.
5. Rozanski M, Neuhaus V, Thornton E, Becker SJ, Rathmell JP, Ring D. Symptoms During or Shortly After Isolated Carpal Tunnel Release and Problems Within 24 hours After Surgery. J Hand Microsurg 2015;7:30-5.
6. Stutz N, Gohritz A, van Schoonhoven J, Lanz U. Revision surgery after carpal tunnel release--analysis of the pathology in 200 cases during a 2 year period. J Hand Surg Br 2006;31:68-71.
7. Drosos GI, Ververidis A, Stavropoulos NI Mavropoulos R, Tripsianis G, Kazakos K. Silicone ring tourniquet versus pneumatic cuff tourniquet in carpal tunnel release: A randomized comparative study. J Orthop Traumatol 2013;14:131-5.
8. Odinsson A, Finsen V. Tourniquet use and its complications in Norway. J Bone Jt Surg - Br Vol 2006;88:1090-2.
9. Burk RW, Guzman-Stein G, Vasconez LO. Lidocaine and epinephrine levels in tumescent technique liposuction. Plast Reconstr Surg 1996;97:1379-84.
10. Lalonde D, Bell M, Benoit P, Sparkes G, Denkler K, Chang P. A multicenter prospective study of 3,110 consecutive cases of elective epinephrine use in the fingers and hand: The dalhousie project clinical phase. J Hand Surg Am 2005;30:1061-7.
11. Lalonde D. Conceptual origins, current practice, and views of wide awake hand surgery. J Hand Surg Eur Vol 2017;42:886-95.
12. Farhangkhoee H, Lalonde J, Lalonde DH. Teaching medical students and residents how to inject local anesthesia almost painlessly. Can J Plast Surg 2012;20:169-72.
13. Tulipan J, Kim N, Abboudi J Jones C, Liss F, Kirkpatrick W, et al. Open Carpal Tunnel Release Outcomes: Performed Wide Awake versus with Sedation. J Hand Microsurg 2017;9:74-9.
14. Iqbal HJ, Doorgakant A, Rehmatullah NNT, Ramavath AL, Pidikiti P, Lipscombe S. Pain and Outcomes of Carpal Tunnel Release Under Local Anaesthetic with or without a Tourniquet: A Randomized Controlled Trial. J Hand Surg Eur Vol 2018;43:808-12.
15. Gunasagaran J, Sean ES, Shivdas S, Amir S, Ahmad TS. Perceived comfort during minor hand surgeries with wide awake local anaesthesia no tourniquet (WALANT) versus local anaesthesia (LA)/tourniquet. J Orthop Surg (Hong Kong) 2017;25:2309499017739499.
16. Gibson M. Outpatient carpal tunnel decompression without tourniquet: a simple local anaesthetic technique. Ann R Coll Surg Engl 1990;72:408-9.
17. McKee DE, Lalonde DH, Thoma A, Dickson L. Achieving the optimal epinephrine effect in wide awake hand surgery using local anesthesia without a tourniquet. Hand 2015;10:613-5.
18. Codding JL, Bhat SB and Ilyas AM. An economic analysis of MAC versus WALANT: a trigger finger release surgery case study. Hand 2016;12:348-51.
19. Al Youha S, Lalonde DH. Update/review: changing of use of local anesthesia in the hand. Plast Reconstr Surg Glob Open 2014;6:e150.
20. Chapman T, Kim N, Maltenfort M, Ilyas AM. Prospective Evaluation of Opioid Consumption Following Carpal Tunnel Release Surgery. Hand 2017;12:39-42.
21. Volkow ND, McLellan TA, Cotto JH, Karithanom M, Weiss S. Characteristics of opioid prescriptions in 2009. JAMA 2011;305:1299-301.

ORIGINAL ARTICLE

DOI: 10.4274/eamr.galenos.2018.54227
Eur Arch Med Res 2019; 35 (4):224-8

The Awareness of Venous Thromboembolism and Its Prophylaxis: A Survey Study

Nagihan Duran Yakar, Tarkan Mıngır, Nuri Yakar, Cansu Kılınç Berktaş, Namigar Turgut

İstanbul Okmeydanı Training and Research Hospital, Clinic of Anesthesiology and Reanimation, İstanbul, Turkey

Abstract

Objective: The purpose of this questionnaire study was to present the results of venous thromboembolism (VTE) questionnaire and compare the data with the literature by having information about awareness level of VTE in anesthesiology and reanimation specialists and branch surgeons and prophylaxis protocols being performed by them.

Methods: A printed questionnaire consisting of seven questions was used for gathering information from the physicians from anesthesiology and reanimation and relevant surgery clinics. The data were given as percentage in statistical analysis.

Results: One hundred and twenty-three participants were included in the study. According to the study results, the participants stated that VTE was a serious cause of mortality; risk factors were questioned in their clinics; they did not use a questionnaire for risk factor and they did not use a risk factor scoring system in risk factor questioning (84%, 89.1%, 10.9%, 79%, respectively). Eighty-five percent of the participants seemed to perform VTE prophylaxis preoperatively and 76.9% of them preferred to design both pharmacological and mechanical prophylaxis (MP) together. The second most common choice was that they used MP alone (14.6%). Seven point three percent of the participants reported that they gave pharmacological prophylaxis alone and 1.2% of the participants stated that they did not plan any of the prophylaxis methods. The participants used low molecular weight heparin, unfractionated heparin and acetylsalicylic acid among pharmacological methods (70.7%, 8%, 7%, respectively).

Conclusion: Based on data obtained from this study, there is a need for a national risk assessment and thromboprophylaxis policy. Implementation of a change in practice will have the potential to prevent or reduce morbidity and mortality associated with hospital-acquired thrombosis.

Keywords: Prophylaxis, venous thromboembolism, risk

INTRODUCTION

Venous thromboembolism (VTE) is a spectrum of disease comprising deep vein thrombosis (DVT) and pulmonary embolism (PE). Being a silent disease, VTE is the third leading cause of mortality, followed by heart attack and stroke with a common mechanism of cardiovascular diseases. VTE is also responsible for one in four deaths worldwide (1,2). VTE, which is the number one cause of preventable deaths among hospitalized patients, has been one of the well-known risks of surgery for many years. The most effective and inexpensive method to reduce the mortality and morbidity rates due to VTE is prophylaxis. In fact, thromboprophylaxis (TP) begins with appropriate positioning of the patient on the operating table (3,4). Today, even among health professionals working in this field, it is considered that there is little awareness about its conditions, risks, symptoms and preventive applications. The purpose of this survey study was to present the results of VTE questionnaire and to compare the data with the literature by acquiring information about awareness level of VTE in anesthesiology and reanimation specialists and branch surgeons and prophylaxis protocols being performed by them.

METHODS

With the permission of local ethics committee of İstanbul Okmeydanı Training and Research Hospital (17.01.2017/588), a printed questionnaire consisting of seven questions was used for gathering information from the anesthesiology and reanimation clinic and relevant surgery clinics (general surgery, plastic surgery, ear-nose-throat, urology, orthopedics, neurosurgery).

Address for Correspondence: Namigar Turgut, İstanbul Okmeydanı Training and Research Hospital, Clinic of Anesthesiology and Reanimation, İstanbul, Turkey
Phone: +90 533 360 76 59 E-mail: namigarturgut@gmail.com ORCID ID: orcid.org/0000-0003-0252-3377

Cite this article as: Duran Yakar N, Mıngır T, Yakar N, Kılınç Berktaş C, Turgut N. The Awareness of Venous Thromboembolism and Its Prophylaxis: A Survey Study. Eur Arch Med Res Res 2019; 35 (4): 224-8

Received: 18.04.2018
Accepted: 03.12.2018

Data Collection

Anesthesiology and reanimation specialists and physicians from other surgery clinics that participated in the questionnaire study were determined as the sample size and the study aimed to include at least 100 health workers. Anesthesiology and reanimation specialists and physicians from other surgery clinics who agreed to participate in the study were included, and those who did not agree to participate were excluded.

Statistical Analysis

Demographic data, age, gender and years of professional experience were recorded. Questions about awareness, risk factors, scoring systems, time and methods of prophylaxis and pharmacological prophylaxis were directed to the participants. The data were given as percentages (%).

RESULTS

Participants (n=123) were selected from physicians and critical care nurses (84.2% and 15.8% of participants, respectively), including both men and women (67% and 33%, respectively). The age of the participants ranged from 25 to 54 years (mean age=36.6 years). According to the study results, the participants stated that VTE was a serious cause of mortality; risk factors were questioned in their clinics; and they did not use a questionnaire for risk factors (84%, 89.1% and 10.9% of the participants, respectively) (Table 1). The most common risk factors encountered by surgeons were acute risk factors; 39% of the surgeons accepted all risk factors as acute risk factors, while 61% of the surgeons accepted acute risk factors as the following: (a) frequent hospitalization (27%), (b) frequent chemotherapy administration, (c) frequent administration of estrogen, recently started, (d) frequent intravenous catheterization and (e) frequent immobilization. The acute "triggering" risk factors selected by the participants to use prophylaxis are shown in Table 2. Seventy-nine percent of the participants reported that they did not use a scoring system for risk factor questioning; 85% of the participants seemed to start using VTE prophylaxis preoperatively and 76.9% of them preferred to arrange both pharmacological and mechanical prophylaxis together. The second most common use was mechanical prophylaxis alone (14.6%). Seven point three percent of the participants reported that they gave pharmacological prophylaxis alone and 1.2% of the participants stated that they did not plan any prophylaxis methods. With respect to the DVT prophylaxis, the participants used low molecular weight heparin, unfractionated heparin and acetylsalicylic acid among pharmacological methods (70.7%, 8% and 7%, respectively).

Table 1. Characteristics of patients and questionnaire study; questions and answers

	n=123	**Mean**
Female	33%	
Male	67%	
Age, years	25-54	36.6
Answers to questions		
Yes, VTE is a severe condition	84%	
No, VTE is not a serious cause of mortality that much	16%	
Are the risk factors for venous thromboembolism questioned in your clinic?		
Yes	89.1%	
No	10.9%	
Which scoring system do you use/prefer in questioning about risk factors for venous thromboembolism?		
We use Caprini scoring system	21%	
None	79%	
What is your priority choice for a patient whom you decide using venous thromboembolism prophylaxis?		
Pharmacological prophylaxis	7.3%	
Mechanical prophylaxis	14.6%	
Pharmacological + mechanical prophylaxis	76.9%	
Neither	1.2%	

DISCUSSION

With the increasing levels of development of countries, morbidity and mortality due to chronic diseases occur more than contagious diseases. Worldwide deaths due to ischemic heart disease and stroke have increased by 35% and 25%, respectively, since the 90s (5). VTE substantially contributes to the worldwide disease burden. Although VTE-related morbidity and mortality are considerably preventable, global controls reveal a lack of systemic TP use in patients with medium and high risk of VTE (2).

Table 2. Acute "triggering" risk factors of venous thromboembolism

a. Hospitalization
b. Surgical intervention
c. Lower extremity/pelvic trauma or fracture
d. Prolonged travel
e. Estrogen therapy starting a short time ago
f. Intravenous catheterization
g. Chemotherapy
h. Immobility
i. Pregnancy-postpartum period

Cohen et al. (6) described thromboembolism as the "single biggest killer of pregnant women" in their study and in the guidebooks of Royal College of Obstetrics and Gynecologists. Again, DVT occurs in 45% to 51% of patients undergoing orthopedic surgery, unless TP is used. The International Society on Thrombosis and Haemostasis has declared 13th October as "World Thrombosis Day" starting in 2014 in order to increase the global awareness of thrombosis. One of the key activities of that day was to conduct a global survey for measuring global public awareness about thrombosis, specifically VTE, thus evaluating the success of the program through follow-up questionnaires. This survey was designed for collecting data about the degree of awareness on VTE, the extent of recognition of the signs and symptoms of DVT and PE. The aim was to share the general information with the public to raise awareness about VTE and enable patients to engage in discussions with their own surgeons and healthcare providers about their individual risk of VTE and their need for TP. The survey was performed in nine representative countries and included respondents of different ages to assess the differences in awareness between generations. General awareness related to VTE was low, in spite of 7233 respondents. The awareness was 19% for PE, while it was 28% for DVT. The rate of awareness of underlying causes of DVT was moderately high, additionally, only 45% of respondents were aware that many blood clots can be prevented, nevertheless, awareness of VTE was low relative to prostate cancer and breast cancer (7). In our country, Reliance platform was constituted and treatment guides were prepared in order to increase the awareness of VTE on the basis of physicians and also society, to educate physicians and allied health personnel about VTE prophylaxis and treatment, to prevent DVT and PE, thus decreasing the morbidity and mortality caused by them and, to contribute for constitution of a national guide about VTE prophylaxis, diagnosis and treatment (4).

VTE is recognized internationally as a serious health problem. VTE is associated with mortality and morbidity in hospitalized medical patients and imposes a huge economic burden for health service. Although it is well established in the literature that active implementation of a mandatory risk assessment tool and an evidence-based TP policy decreases the incidence of hospital associated thrombosis (HAT), only 26% (n=8/31) of hospitals in Ireland was found to have a local implemented TP policy in a national survey distributed to 40 hospitals throughout Ireland in order to examine the utilization of a VTE risk assessment tool and TP policy. Only six of these eight hospitals had a risk assessment tool in conjunction with the TP policy. Based on data obtained from this survey, a need for a national risk assessment and TP policy in Ireland was reported (8). The results of our study were also similar; 89.1% of the participants reported that they questioned the risk factors in their clinics, but 79% of them reported that they did not use a scoring system. It was also valid for us. In a study of Oh et al. (9) that was conducted among Korean nurses, only 9.3% of the nurses reported having received in service VTE education from their hospital. The findings showed that, beyond prevention practices of VTE, the nurses demonstrated a lack of knowledge about VTE and their self-efficacy level was not highly rated. A training focused on prevention of VTE and risk assessment should be considered as a part of continuing education of nurses. In our study, the most frequent risk factors encountered by the surgeons were acute risk factors; 39% of the surgeons accepted all risk factors as acute, while 61% of the surgeons accepted acute risk factors as the following: (a) frequent hospitalization (27%), (b) frequent chemotherapy administration, (c) frequent administration of estrogen, recently started, (d) frequent intravenous catheterization and (e) frequent immobilization. Seventy nine percent of the participants reported that they did not use a scoring system for risk factor questioning. This indicates that education/training courses on VTE for all healthcare workers should also be arranged in our country. Similar to our study, in a study conducted by Özkaya et al. (10) with plastic and reconstructive surgeons, 36% of the participants reported the time of prophylaxis initiation as preoperative, and many of them reported the time as one day before the operation. Twenty nine percent of the participants reported that prophylaxis was initiated during operation and that the perioperative administration was implemented almost immediately at the beginning of the operation. It is obvious that significant progress has been achieved in recent years, since this rate was 85% in our study. Only 10% of the participants reported using prophylaxis during operation. In their study performed in 292 hospitalized adult patients, Ikama et al. (11) identified risk factors such as age and long-term immobilization in order to identify patients at risk of VTE and evaluate the use of preventive measures. They found that 79% of the surgery candidates received VTE prevention measures, pharmacological prevention was used in 57.4% of them, mechanical prevention in 19.5%, and the two types of prevention in 23.1% of them. In our study, 85% of the participants seemed to start using VTE prophylaxis preoperatively and 76.9% of them preferred to use both pharmacological and mechanical prophylaxis together. The second most common form of use was mechanical prophylaxis alone (14.6%). Seven point three percent of the participants stated that they gave pharmacological prophylaxis alone and 1.2% of the participants stated that they did not plan to implement any of the prophylaxis methods.

CONCLUSION

Nevertheless, these data indicate that certain programs should be arranged for identifying the patients at risk and taking preventive measures besides increasing awareness. Based on the data obtained from this study, there is a need for a national risk assessment and TP policy. Implementation of a change in practice will have the potential of preventing or reducing morbidity and mortality associated with HAT.

Ethics

Ethics Committee Approval: Ethics committee approval was received for this study from the Local Ethics Committee of Okmeydanı Training and Research Hospital (approvel number: 17.01.2017/588).

Informed Consent: A survey study.

Peer-review: External and internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: N.D.Y., T.M., N.Y., C.K.B., N.T., Concept: N.D.Y., N.T., Design: N.D.Y., T.M., N.Y., C.K.B., N.T.,Data Collection or Processing: N.D.Y., T.M., N.Y., C.K.B., Analysis or Interpretation: N.D.Y., N.Y., Literature Search: N.D.Y., T.M., N.Y., Writing: N.D.Y., T.M., N.T.

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

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

Questionnaire of the Awareness of Venous Thromboembolism and its Prophylaxis

Age: Gender: Occupation:

Number of years in your profession:

Your present working place:

Two or five of every 100 people are predicted to experience venous thromboembolism (VTE) at least one time during their lifetime. Approximately 2 millions/year deep vein thrombosis (DVT) and and 600,000/year pulmonary embolism cases are seen in United States, every year. This number is more than the total number of the people lost because of AIDS (Acquired Immune Deficiency syndrome), breast cancer and traffic accidents.

1. Did you find this information convincing?

a. Yes, VTE is a severe condition

b. No, VTE is not a serious reason of mortality that much

2. Are the risk factors for venous thromboembolism questioned in your clinic?

a. Yes

b. No

3. Which scoring systems do you use/prefer in questioning about risk factors for venous thromboembolism?

None

We use ---------- scoring system

4. According to you, which of the following is/are not one of the acute "triggering" risk factors of venous thromboembolism?

a. Hospitalization

b. Surgical intervention

c. Lower extremity/pelvic trauma or fracture

d. Prolonged travel

e. Estrogen therapy starting a short time ago

f. Intravenous catheterization

g. Chemotherapy

h. Immobility

i. Pregnancy-postpartum period

5. According to you, what should be the timing for using venous thromboembolism prophylaxis in a patient?

a. Preoperative

b. At operation

c. Immediately after operation

d. Postoperative at the end of 24th hour

e. No idea

7. What is your priority choice for a patient whom you decide using venous thromboembolism prophylaxis?

a. Pharmacological prophylaxis

b. Mechanical prophylaxis

c. Pharmacological + mechanical prophylaxis

d. Neither

8. What does pharmacological prophylaxis mean for you?

REFERENCES

1. Bartlett MK, Zhang Y, Kreidler N, Sun S, Ardy R, Cao K, Sack L. Global analysis of plasticity in turgor loss point, a key drought tolerance trait. Ecol Lett 2014;17:1580-90.
2. Raskob GE, Silverstein R, Bratzler DW, Heit JA, White RH. Surveillance for deep vein thrombosis and pulmonary embolism: recommendations from a national workshop. Am J Prev Med 2010;38:S502-9.
3. Turpie AG, Leizorovicz A. Prevention of venous thromboembolism in medically ill patients: a clinical update. Postgrad Med J 2006;82:806-9.
4. ULUSAL VENÖZ TROMBOEMBOLİZM PROFİLAKSİ VE TEDAVİ KILAVUZU – 2010 Editörler Prof. Dr. Muzaffer Demir Prof. Dr. BülentErdemli Prof. Dr. Mehmet Kurtoğlu Prof. Dr. GülÖngenYayınlayan cortex iletişim hizmetleri a.ş manolyalı sokak no: 22 Levent, İstanbul ISBN 978-605-5720-05-6 Basım Diasan Basım Form Matbaacılık San. Tic. A.Ş BasımYılı 2010
5. Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 2012;380:2095-128.
6. Cohen A, Botteman M, Stephens J, Ewing M, Collier P, Pashos C, et al. Cost efectiveness of two anticoagulants for the prophylaxis of DVT and subsequent long-term complications (PTS and recurrent VTE) in total hip replacement surgery in the United Kingdom. Poster Presentation. XVIIIth Congress of the International Society on Thrombosis and Haemostasis, Paris, France. ThrombHaemost 2001
7. Wendelboe AM, McCumber M, Hylek EM, Buller H, Weitz JI, Raskob G; ISTH Steering Committee for World Thrombosis Day. Global public awareness of venous thromboembolism. J Thromb Haemost 2015;13:1365-71.
8. Khan MI, O'Leary C, Silvari V, O'Brien A, O'Connor M, Duggan C, et al. Venous Thromboembolism - Risk Assessment Tool and Thromboprophylaxis Policy: A National Survey. Ir Med J 2017;110:499.
9. Oh H, Boo S, Lee JA. Clinical nurses' knowledge and practice of venous thromboembolism risk assessment and prevention in South Korea: a cross-sectional survey. J Clin Nurs 2017;26:427-35.
10. Özkaya Ö, Öztürk MB, Egemen O, Öreroğlu AR, Üsçetin İ, Tasasız K, et al. How Much Do Know About The Venous Thromboembolism? The Approch Of Turkish Plastic Surgeons To The Venous Thromboembolism Prophlaxis And Preferred Methods in Prophylaxis: A Survey Study. Turk Plast Surg 2013;21.
11. Ikama SM, Nkoua MF, Gombet TR, Makani J, Mongo-Ngamami S, Ondze-Kafata LI, et al. Evaluation of venous thromboembolism risk and prophylaxis among hospitalized patients in Brazzaville. J Mal Vasc 2016;41:182-7.

ORIGINAL ARTICLE

DOI: 10.4274/eamr.galenos.2019.07379
Eur Arch Med Res 2019; 35 (4):229-32

Endo-first Approach for Peripheral Vascular Disease: The First Fifty Cases of a New Clinic

Cihan Yücel, Serkan Ketenciler, Mete Gürsoy, İlhan Sanisoğlu, Nihan Kayalar

İstanbul Okmeydanı Training and Research Hospital, Clinic of Cardiovascular Surgery, İstanbul, Turkey

Abstract

Objective: In recent years, endovascular and hybrid therapies have come to the forefront in the treatment of peripheral arterial disease (PAD) instead of open surgical treatment. In this study, we aimed to present the results of endovascular and hybrid treatments performed in our newly established clinic.

Methods: The data of the patients, who were diagnosed as PAD and treated with endovascular and hybrid methods, were retrospectively analyzed and 50 patients were included in the study. Patients were classified according to the Fontaine classification and patients with Fontaine 2B or higher underwent interventional or hybrid treatment.

Results: The majority of the patients were male (96%) and mean age of the patients was 62.89±9.01 years. Twelve patients (24%) had ischemic ulcers (Fontaine 4), 32 patients (64%) had claudication (Fontaine 2B) and six patients (12%) had rest pain (Fontaine 3). Thirty-four patients (68%) underwent endovascular intervention and 16 (32%) underwent hybrid treatment procedures. In 38 patients (76%) with complaints of Fontaine class 2B or 3 before the intervention, symptoms regressed to Fontaine class 1 after the procedure. The mean ankle-brachial index values of the extremities with lesions increased from 0.44±0.30 to 0.85±0.17 on the first postoperative day.

Conclusion: In conclusion, endovascular and hybrid interventions provide satisfactory results in high-risk patients with PAD for both the salvage of extremities and the improvement of symptoms. We believe that endovascular interventions will provide less invasive and safer revascularization in patients with higher comorbidity rates in the future.

Keywords: Peripheral arterial disease, endovascular treatment, hybrid treatment

INTRODUCTION

The prevalence of peripheral arterial disease (PAD) in the population over 50 years of age is 13% and the frequency of symptomatic PAD in the same population is reported to be 5% (1). When its effects on quality of life and pharmacoeconomic criteria are evaluated, the true importance of the disease becomes more evident. The expected 5-year mortality rate in male patients with PAD is similar to prostate and colon cancer (2). This data alone reveals the severity of the disease.

In addition to limiting daily activities and decreasing the quality of life associated with reduced walking range and intermittent claudication, PADs may also lead to mortality and morbidity due to rest pain, ischemic ulcers, prolonged treatment and minor and major amputations. For this reason, early diagnosis of the disease, identification and prevention of risk factors, initiation of appropriate medical treatment, and timely and effective revascularization of symptomatic patients are necessary. The TransAtlantic Inter-Society Consensus II (TASC II) study group has reported that the frequency of amputation in PAD was between 12 and 50 per 100,000 (3). Many studies have demonstrated that the increased rate of revascularization, especially in the last decade of life, creates a significant reduction in the rate of amputation. According to the data from United States, endovascular interventions, which have increased in the last 10 years, reduced the need for amputation and open surgery by about 50% (4). Nowadays, the increasing experience of percutaneous interventional treatments has provided significant benefits for rapid, effective and total revascularization, and therefore many authors now prefer the "endo-first" approach (5). Endovascular interventional treatments offer advantages in symptomatic recovery, graft patency rates, rapid recovery and

Address for Correspondence: Cihan Yücel, İstanbul Okmeydanı Training and Research Hospital, Clinic of Cardiovascular Surgery, İstanbul, Turkey
Phone: +90 506 418 91 08 E-mail: cihanyucell@hotmail.com ORCID ID: orcid.org/0000-0002-1941-0873

Cite this article as: Yücel C, Ketenciler S, Gürsoy M, Sanisoğlu İ, Kayalar N. Endo-first Approach for Peripheral Vascular Disease: The First Fifty Cases of a New Clinic. Eur Arch Med Res Res 2019; 35 (4): 229-32

Received: 17.10.2018
Accepted: 17.01.2019

rapid return to daily life both as a sole treatment and as a part of hybrid approaches.

In this study, our aim was to present early results of our first 50 patients who underwent endovascular or hybrid peripheral vascular interventions at Okmeydanı Training and Research Hospital, Clinic of Cardiovascular between January and July 2018.

METHODS

In this study, data from the clinical database were analyzed retrospectively. Our first 50 patients who have underwent endovascular or hybrid interventions for peripheral vascular disease were evaluated for risk factors, comorbid conditions, early patency, mortality and morbidity. All patients underwent vascular physical examination and ankle-brachial indexes (ABI) were measured at the initial evaluation. For patients with an ABI of less than 0.9, computer-assisted tomographic angiography examinations were performed depending on the severity of arterial Doppler results or symptoms and the preference of the clinician. Patients were classified according to the Fontaine classification and patients with Fontaine 2B or higher underwent interventional or hybrid treatment. Endovascular or hybrid treatment was considered and applied as the first-line intervention for high-risk patients with severe comorbidity. All patients participating in the study were informed about the study and their consents were obtained. Ethics committee approval was not taken since the study was retrospective in design.

Statistical Analysis

Statistical analyses were performed using the SPSS package program (SPSS Inc., Chicago, IL, USA) version 16.0. Continuous variables were expressed as mean ± standard deviation and categorical variables were expressed as number and percentage.

Surgical Technique

Endovascular interventions were carried out under local anesthesia and hybrid interventions were carried out under either general anesthesia or local anesthesia and peripheral nerve block. In cases where a percutaneous approach was performed, arterial puncture site was determined using ultrasonography and punctures were made directly through surgical incision areas in hybrid interventions. Ipsilateral antegrade approach was used preferably where appropriate, and the contralateral approach was also used when necessary. Balloon angioplasty was selected as endovascular technique and atherectomy was used as a combined procedure in cases where this approach was deemed necessary. The angioplasty technique included predilatation with a standard balloon suitable for vessel size and completion of angioplasty using a drug-coated balloon 1 mm larger than the first one. Atherectomy was added to the procedure for patients with total occlusions and diffuse lesions with advanced calcification (>10 cm), and the sequence of the procedure was planned as predilatation, followed by atherectomy and balloon angioplasty. The stenting procedure of arteries was preserved for patients with complications or severe recoil.

RESULTS

This study included 50 patients who had undergone endovascular or hybrid peripheral vascular interventions at Okmeydanı Training and Research Hospital between January and July 2018. The majority of the patients were male (96%) and only two (4%) were female. The age range of the patients was 46-74 years and the mean age was 62.89±9.01 years. The most prevalent risk factor was smoking (88%), followed by diabetes (56%) and hypertension (40%) (Table 1). Twelve patients (24%) had ischemic ulcers (Fontaine 4), 32 (64%) had claudication (Fontaine 2B) and six (12%) had rest pain (Fontaine 3). The mean ABI values of the extremities with lesions were 0.44±0.30. Thirty-four patients (68%) underwent endovascular interventional treatment and 16 patients (32%) underwent hybrid treatment procedures. Detailed data on procedures and arterial sites of intervention are given in Table 2 and 3. In 38 patients (76%) with complaints of Fontaine class 2B or 3 before the intervention, symptoms regressed to Fontaine class 1 after the procedure. Minor amputations were performed in two patients (24%) out of 12 patients with Fontaine 4 classification. The mean ABI of the affected extremities measured on the first postoperative day was 0.85±0.17. During post-operative follow-up, early occlusion compelled the placement of a new graft in one patient with cross-femoral bypass graft. Balloon angioplasty was applied to the superficial femoral artery in the same operation. Due to seroma caused by inguinal incision, this patient required a long-term in-patient follow-up and vacuum assisted closure. No culture positive wound site infection was observed in any patient. After being discharged, one patient underwent primary coronary angioplasty due to inferior myocardial infarction.

Table 1. Preoperative risk factors of patients

Risk factor	Number (n)	Percent (%)
Hypertension	20	40
Diabetes	28	56
Hyperlipidemia	10	20
Coronary artery disease	16	32
Smoking	44	88
Chronic renal failure	4	8

Table 2. Data on endovascular procedures and arterial sites of intervention

Procedure	Number of patients
Left ATA angioplasty	3
Right ATA angioplasty	4
Left SFA angioplasty	6
Right SFA angioplasty	6
Left CIA angioplasty	3
Right CIA angioplasty	3
Right SFA angioplasty + right ATA angioplasty	4
Left SFA angioplasty + left ATA angioplasty	5

ATA: Anterior tibial artery, SFA: Superficial femoral artery, CIA: Common iliac artery,

Table 3. Data on hybrid procedures and arterial sites of intervention

Right femoro-popliteal bypass + right ATA angioplasty	2
Left femoro-popliteal bypass + left ATA angioplasty	4
Left CFA endarterectomy + left SFA angioplasty	2
Left popliteal artery safen vein interpozition + left ATA angioplasty	1
Left CIA angioplasty + cross femoral bypass	2
Left SFA angioplasty + cross femoral bypass	1
Left CIA angioplasty + left femoro-popliteal bypass	2
Right CIA angioplasty + CFA endarterectomy	1
Left axillo-femoral bypass + left ATA angioplasty	1

ATA: Anterior tibial artery, SFA: Superficial femoral artery, CIA: Common iliac artery, CFA: Common femoral artery

No mortality or acute renal insufficiency was observed in any patient in this study. The mean length of hospitalization was 1.8±0.63 days.

DISCUSSION

In parallel to the previously reported researches in the literature, this retrospective data analysis showed that the endovascular interventional and hybrid treatment methods carried out in our clinic provided successful results. Despite not being a prospective trial, we believe that this study is valuable as it reflects the early experiences of a newly established clinic. Throughout the world, the learning process of cardiovascular surgeons for percutaneous angiographic interventions is still going on in parallel to other percutaneous interventional methods, and it can be predicted that this increase in experience will be more successful and will provide more permanent results when combined with experience in vascular surgeries. Hybrid interventions are one of the most important options in the treatment of complex patients and the frequency of such treatments is increasing steadily in our clinic.

It is estimated that over 30 million people in the world suffer from PAD. In the general population, reports verify that the prevalence of PAD is 17% in women and 20% in men over 65 years of age (6).

Along with the developments in technology, endovascular interventions have become widespread in the treatment of arterial diseases. According to TASC II report published in 2007, lesions in the arteries of lower extremities are classified as type A, B, C and D based on the shape and extent of the lesions. According to the current consensus, endovascular interventions are preferred for type A lesions and surgical interventions are preferred for type D lesions. For type B and type C lesions, endovascular or surgical procedures may be selected based on the status of the patient and other concomitant diseases. However, the general recommendation is to prioritize endovascular methods for type B lesions and surgical interventions for type C lesions (7). While the subject of selection of endovascular or surgical interventions for different patients is still a matter of discussion, 2017 European Society for Cardiology/European Society for Vascular Surgery PAD guideline did not employ the TASC II classification and suggested, with a class I indication, endovascular approaches as primary preference for the revascularization of aortoiliac diseases with obstructive lesions shorter than 5 cm. On the other hand, for patients with aortoiliac occlusions and no severe comorbidities, aorto-bi-femoral bypass is recommended as class I indication. It is also emphasized that endovascular interventions should be used as first strategy and as class I indications for patients with serious comorbidities and long and/or bilateral aortoiliac lesions. For aortoiliac lesions that extend to the common femoral artery, hybrid approach (iliac stenting + femoral endarterectomy/bypass) was recommended with class IIa indication for the first time (8). For femoropopliteal arterial obstructions/occlusions with short lesions (<25 cm), endovascular treatment was suggested with class I indication, and surgical treatments with autologous vein grafts were suggested as class I indication for long (≥25 cm) lesions (8). Primary and secondary patency rates of angioplasty and stenting procedures in aortoiliac regions are high. In short lesions (<5 cm), post-angioplasty primary patency was 64.5% and secondary patency was 81.8% (9). There are various publications that report 85% prevalence rate of critical leg ischemia in patients with below the knee lesions. In these series, leg salvage rates with angioplasty were found to be between 80-90% (10). These data suggest that the most up-to-date approach seems to be “endo-first” for PADs that require interventions.

Left common iliac artery (CIA) angioplasty was performed in two patients with severe bilateral iliac disease, followed by a cross-femoral bypass because only one iliac artery was suitable for angioplasty. Due to severe comorbid conditions, this hybrid approach was preferred in these cases instead of aortobifemoral bypass grafting. Since three patients had aortoiliac disease along with long segment (>25 cm) femoro-popliteal disease, two patients had femoro-popliteal bypass after aortoiliac stenting and one patient had a cross femoral bypass in conjunction with femoro-popliteal angioplasty. In the other six patients who had distal disease besides femoro-popliteal lesions, the hybrid approach included femoro-popliteal bypass grafting and distal angioplasty in order to increase both inflow and outflow to the distal extremity. The improved symptoms and ABI levels of these patients suggest that endovascular interventions are effective in complex peripheral vascular disease and that they may be used in conjunction with open surgery in cases that sole endovascular approaches are not sufficient.

Endovascular interventions were performed alone in 34 patients (68%). Isolated angioplasty was applied to the anterior tibial artery in seven patients (14%), superficial femoral artery in 12 patients (24%) and CIA in six patients (12%). Simultaneous angioplasties to both superficial femoral and anterior tibial arteries were performed in nine patients (18%). The procedural failure was observed in four patients (11.76%), and endovascular intervention was re-applied and became successful in three of these patients. In the remaining patient, repeat revascularization was not considered and he was followed-up medically. These results show that procedural success rates of endovascular procedures are sufficient even in high-risk patients and repeat procedures may prove successful results in most patients.

CONCLUSION

In conclusion, endovascular and hybrid interventions provide satisfactory results in high-risk patients with PAD for both the salvage of extremities and the improvement of symptoms. In cases where endovascular approaches alone are not enough, hybrid approaches may help to provide complete revascularization of affected extremities. We believe that the increased angiography experience of cardiovascular surgeons in the following years will help improve endovascular interventions to provide less invasive and safer revascularization in patients with higher rates of comorbidity.

Ethics

Ethics Committee Approval: Retrospective study.

Informed Consent: Retrospective study.

Peer-review: External and interal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: C.Y., Concept: N.K., Design: M.G., Data Collection or Processing: S.K., Analysis or Interpretation: İ.S., Literature Search: C.Y., Writing: C.Y.

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

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

REFERENCES

1. Crawford F, Welch K, Andras A, Chappell FM. Ankle brachial index for the diagnosis of lower limb peripheral arterial disease. Cochrane Database Syst Rev 2016;9:CD010680.
2. TKDCD Periferik arter ve ven hastalıkları tedavi kılavuzu-2008;s:2.
3. Norgren L, Hiatt WR, Dormandy JA, Nehler MR, Harris KA, Fowkes FG. Inter-society consensus for the management of peripheral arterial disease (TASC II). J Vasc Surg 2007;45:5-67.
4. Paulus N, Jacobs M, Greiner A. Primary and secondary amputation in critical limb ischemia patients: different aspects. Acta Chir Belg 2012;112:251-4.
5. Lee JT. Could the endo-first strategy really be better? Arch Surg 2012;147:846.
6. Welten GM, Schouten O, Chonchol M, Hoeks SE, Bax JJ, Van Domburg RT, et al. Prognosis of patients with peripheral arterial disease. J Cardiovasc Surg (Torino) 2009;50:109-21.
7. Norgren L, Hiatt WR, Dormandy JA, Nehler MR, Haris KA, Fowkes FG. Inter-society consensus for the management of peripheral arterial disease (TASC II). Eur J Vasc Endovasc Surg 2007;33: P1-75.
8. Aboyans V, Ricco JB, Bartelink MEL, Björck M, Brodmann M, Cohnert T, et al. 2017 ESC Guidelines on the Diagnosis and Treatment of Peripheral Arterial Diseases, in collaboration with the European Society for Vascular Surgery (ESVS): Document covering atherosclerotic disease of extracranial carotid and vertebral, mesenteric, renal, upper and lower extremity arteriesEndorsed by: the European Stroke Organization (ESO)The Task Force for the Diagnosis and Treatment of Peripheral Arterial Diseases of the European Society of Cardiology (ESC) and of the European Society for Vascular Surgery (ESVS). Eur Heart J 2018;39:763-816.
9. Abello N, Kretz B, Picquet J, Magnan PE, Hassen-Khodja R, Chevalier J, et al. Long-term results of stenting of the aortic bifurcation. Ann Vasc Surg 2012;26:521-6.
10. Van Overhagen H, Spiliopoulos S, Tsetis D. Below the-knee interventions. Cardiovasc Intervent Radiol 2013;36:302-1.

ORIGINAL ARTICLE

DOI: 10.4274/eamr.galenos.2019.94809
Eur Arch Med Res 2019; 35 (4):233-40

Comparison of the Effects of Smoking and Smokeless Tobacco "Maras Powder" Use on Pulmonary Function, Electrocardiogram and Other Parameters

İlim Irmak[1], Hüseyin Arpağ[2], Nurhan Atilla[2], Umut Sabri Kasapoğlu[3], Pınar Atagün Güney[4], Maşuk Taylan[5]

[1]Göksun State Hospital, Clinic of Chest Diseases, Kahramanmaraş, Turkey
[2]Kahramanmaraş Sütçü İmam University Faculty of Medicine, Department of Pulmonary Medicine, Kahramanmaraş, Turkey
[3]Marmara University İstanbul Pendik Training and Research Hospital, Unit of Pulmonology and Intensive Care, İstanbul, Turkey
[4]İstanbul Süreyyapaşa Chest Diseases and Thoracic Surgery Training and Research Hospital, Clinic of Chest Diseases, İstanbul, Turkey
[5]Dicle University Faculty of Medicine, Department of Pulmonary Medicine, Diyarbakır, Turkey

Abstract

Objective: Through the years, tobacco has been used in many ways. While the most common way of consumption is through smoking cigarettes, smokeless use by chewing or nasal snuffing are also quite common. Smokeless tobacco, also named "Maras powder", is generally used as a substitute to reduce or quit smoking. The effects of smokeless tobacco use on the immune system, respiratory system and cardiovascular system have been extensively researched. In our study, we aimed to investigate the effects of Maras powder on the respiratory, electrocardiogram (ECG) findings and biochemical methods.

Methods: One hundred and forty-nine cases were included and the cases were classified into the following four groups: only using Maras powder; using Maras powder and smoking; only smoking and control group neither smoking nor using Maras powder. Physical examination findings, ECG findings, results of pulmonary function tests, results of biochemical analysis including complete blood count and lipid profile of all participants were recorded on admission.

Results: The risk of mouth sores was 7.9 times higher in the Maras powder group due to direct contact to the oral mucosa. There is a relationship between the daily use frequency of smokeless tobacco and the development of oral wounds, but the total period of use or the duration in mouth was not related to this situation. The ECG findings of both the smoking and Maras powder consuming group was found to be significantly higher than the control group.

Conclusion: The smokeless tobacco use, which is considered as an alternative way of quitting smoking, does not have adverse effects on respiratory functions. However, it is an important risk factor for many life-threatening health conditions such as ECG abnormalities and occurrence of oral lesions. Social awareness must be created for smokeless tobacco use in order to fight this habitual threat to public health.

Keywords: Tobacco smokeless, Maras powder, tobacco

INTRODUCTION

Over the years, tobacco has been used in many ways. While the most common form of consumption is through smoking cigarettes, smokeless use by chewing or nasal snuffing are also quite common (1,2).

The use of "smokeless tobacco" is popular in Eastern Anatolia and South-Eastern Anatolia regions of Turkey, especially within and around the cities of Kahramanmaraş and Gaziantep. Two studies conducted in Turkey reported the smokeless tobacco use rate as 4.0% and 16.8%, respectively (3,4). Smokeless tobacco, also named "Maras powder," has generally been used as a substitute to reduce or quit smoking. Maras powder is made from leaves of a plant called Nicotiana rustica Linn. The leaves of this tobacco plant are dried and powdered, followed by mixing with ashes of vine, oak, or walnut sticks at a rate of 1/2 or 1/3 and mildly moisturized with some water. The final product refined through this process is used orally. The refined mixture is wrapped in cigarette paper or directly applied between lower lip or cheek mucosa and jaw. It is kept in the mouth for 5 to 10 minutes or sometimes for 1 or 2 hours until it is disposed. This process

Address for Correspondence: İlim Irmak, Göksun State Hospital, Clinic of Chest Diseases, Kahramanmaraş, Turkey
Phone: +90 505 680 18 03 E-mail: ilimirmak@hotmail.com ORCID ID: orcid.org/0000-0001-5230-1575

Cite this article as: Irmak İ, Arpağ H, Atilla N, Kasapoğlu US, Atagün Güney P, Taylan M. Comparison of the Effects of Smoking and Smokeless tobacco "Maras Powder" Use on Pulmonary Function, Electrocardiogram and Other Parameters. Eur Arch Med Res Res 2019; 35 (4): 233-40

Received: 19.06.2017
Accepted: 23.01.2019

is repeated several times according to the addiction level of the individual. Interestingly, the addict can even sleep with the tobacco in his/her mouth to curb the cravings of nicotine (5-7). The effects of smokeless tobacco use on the immune system, respiratory system and cardiovascular system have been extensively researched. Studies suggest that Maras powder has no effect on respiratory function, since it is not inhaled (8). On the other hand, the use of Maras powder was found to be associated with the development of atherosclerosis due to its ability to reduce nitric oxide production and increase oxidative stress (9,10). Thus, it has been concluded that use of Maras powder has negative effects on the cardiovascular system (11). Additionally, it has been suggested that Maras powder affects chronic inflammatory modifications at organ and systemic level due to its nicotine content and tobacco-specific nitrosamine (12).

As shown in our study, local people use Maras powder in order to prevent the harmful effects of smoking on respiratory functions. Therefore, in addition to the detrimental effects of Maras powder on respiratory functions, electrocardiogram (ECG) abnormalities, oral lesions and changes in routine biochemical blood counts have been also demonstrated by our results. In addition, our study was conducted in the region where the Maras powder is used heavily. More patients were included in this study than in previous studies. With this aspect, this study will contribute to the literature of Maras powder use in Turkey.

METHODS

This retrospectively designed study included patients referred to the Department of Pulmonary Medicine at Dr. Sureyya Adanalı Göksun State Hospital and Kahramanmaraş Sütçü İmam University Faculty of Medicine between June 2013 and August 2014. Exclusion criteria were the presence of accompanying systemic diseases such as Chronic Obstructive Pulmonary disease (COPD), malignancies, hypertension, heart failure, ischemic heart disease, diabetes mellitus, liver and kidney failure, and/or current medical treatment. One hundred and forty-nine men were included and were classified into the following four groups: only using Maras powder (n=38); using Maras powder and smoking (n=41); only smoking (n=33); and control group of neither smoking nor using Maras powder (n=37). Frequency of use, duration of use and method of use (direct contact of powder with oral mucosa or using wrapped in cigarette paper) were noted for the participants using Maras powder. Duration of smoking, packs per year and current smoking status were noted with the participants' coal or biomass exposure and additional medical conditions. Systolic and diastolic blood pressure, physical examination findings (sores in mouth, gum abnormalities, abnormal respiratory sounds), ECG findings (presence of arrhythmias), results of pulmonary function tests (FEV1, FVC, FEV1/FVC measurements), results of routine biochemical analysis including complete blood count and lipid profile of all participants were recorded upon admission (Tables 1, 2). The study was approved by the Local Ethics Committee and was in accordance with the Declaration of Helsinki (24.11.2014/181).

Statistical Analysis

SPSS version 18.0 was used for statistical analysis. A p value of less than 0.05, with confidence interval of 95%, was considered statistically significant. Kolmogorov-Smirnov test was used to determine the consistency of numeric variables with normal distribution. Parametric tests were used in the analysis of data consistent with normal distribution. One-way ANOVA test was used to compare the numerical variables between the groups. Multivariate analysis was processed to determine if the statistically significant data at ANOVA test were originating from group variables or other factors such as age and gender. Chi-square analysis was used to compare abnormal ECG findings between the case groups. Binary logistic regression test was used to identify the data associated with mouth sores. ROC analysis was used to determine cut-off values, specificity and sensitivity values and statistically significance of numeric variables of factors that could be associated with development of sores such as duration of Maras powder, use (in years), daily amount, duration held in mouth, and amount of smoking (as pack per year).

RESULTS

One hundred forty nine cases were included and 91.3% of them were male. The mean age of the participants was 43.7±16.3 years. The rate of Maras powder users and smokers were 53.0% and 49.7%, respectively. Maras powder users (91.1%) and smokers (54.1%) were currently using these products. Among the Maras powder users, 84.8% were using it wrapped in paper (indirect contact) and the frequency of daily use was 13.8±11.4 days, mean duration of use was 13.4±12.1 years, and mean duration held in mouth was 17.0±14.9 minutes. Mean duration of use was 18.8±15.6 packs per year for the smokers. Dust or smoke exposure and coal or biomass exposure cases were 40.3% and 79.2%, respectively (Table 1). Eight point one percent of the cases had mouth lesions and 14.8% had abnormal ECG findings (arrhythmia, etc.). The demographic distribution of complete blood count and biochemical analysis of the cases are shown in Table 2. The binary logistic regression test, which

was used to identify causative parameters for mouth lesions, found the method and number of daily use of Maras powder as statistically significant variables (p=0.026 and p=0.035, respectively). The risk for mouth sores was 7.9 times higher in the Maras powder group due to direct contact with the oral mucosa. For each additional daily session of Maras powder use, risk for mouth sores increased by 1.055 times (Table 3). Among the numerical parameters that could be associated with mouth sores, ROC analysis revealed statistically insignificant findings for the duration of Maras powder use in years and duration held in mouth (p=0.566 and p=0.243, respectively). However, the amount of daily use was found to be statistically significant (p=0.035, area under curve=0.692). The cut-off value for the amount of daily use was 17.5. The sensitivity for mouth sores was 66.7% and specificity was 74.6% (Figure 1). When the groups were compared with chi-square analysis for abnormal ECG findings, the control group had no ECG abnormality. In contrast, 15.8% of only Maras powder users, 18.2% of only smokers, and 24.4% of both Maras powder users and smokers had statistically significant increases in ECG abnormalities (p=0.021) (Figure 2). Diastolic blood pressure, hemoglobin (Hb), leukocytes and cholesterol levels were found to be statistically and significantly different as determined by One-way ANOVA test, which compared physical examination findings, results of pulmonary function test, complete blood count, lipid profile, and numerical parameters of the other biochemical variables (Table 3). Tukey's

Table 1. Descriptives of case groups

		Control		Maras powder		Tobacco		Maras powder + tobacco		Total	
		(n=37)		(n=38)		(n=33)		(n=41)		(n=149)	
		n	%	n	%	n	%	n	%	n	%
Gender	Female	9	24.3	2	5.3	2	6.1	0	0.0	13	8.7
	Male	28	75.7	36	94.7	31	93.9	41	100.0	136	91.3
Comorbidity	No	37	100.0	38	100.0	33	100.0	41	100.0	149	100.0
	Yes	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Dust exposition	No	24	64.9	18	47.4	25	75.8	22	53.7	89	59.7
	Yes	13	35.1	20	52.6	8	24.2	19	46.3	60	40.3
Biomass exposition	No	12	32.4	4	10.5	14	42.4	1	2.4	31	20.8
	Yes	25	67.6	34	89.5	19	57.6	40	97.6	118	79.2
Tobacco smoking status	Non smoker	37	100.0	38	100.0	0	0.0	0	0.0	75	50.3
	Smoker	0	0.0	0	0.0	33	100.0	41	100.0	74	49.7
Still tobacco smoker	No	0	0.0	0	0.0	5	15.2	29	70.7	34	45.9
	Yes	0	0.0	0	0.0	28	84.8	12	29.3	40	54.1
Maras powder status	No	37	100.0	0	0.0	33	100.0	0	0.0	70	47.0
	Yes	0	0.0	38	100.0	0	0.0	41	100.0	79	53.0
Still using Maras powder	No	0	0.0	4	10.5	0	0.0	3	7.3	7	8.9
	Yes	0	0.0	34	89.5	0	0.0	38	92.7	72	91.1
Direct mouth contact	No	0	0.0	0	0.0	0	0.0	38	92.7	38	92.7
	Yes	0	0.0	0	0.0	0	0.0	3	7.3	3	7.3
Mouth lesion	No	37	100.0	27	71.1	33	100.0	39	95.1	136	91.3
	Yes	0	0.0	11	28.9	0	0.0	2	4.9	13	8.7
Arrhythmia	No	37	100.0	32	84.2	27	81.8	31	75.6	127	85.2
	Yes	0	0.0	6	15.8	6	18.2	10	24.9	22	14.8
Operation history	No	34	91.9	31	81.6	27	81.8	27	65.9	119	79.9
	Yes	3	8.1	7	18.4	6	18.2	14	34.1	30	20.1
Complication during general anesthesia	No	3	100.0	5	71.4	5	83.3	8	57.1	21	70.0
	Yes	0	0.0	1	14.3	1	16.7	2	14.3	4	13.3
	Unknown	0	0.0	1	14.3	0	0.0	4	28.6	5	16.7

Table 2. Comparation of vital findings. pulmonary functions and laboratory results between case groups (Independent t and ANOVA test result)															
Case groups															
	Control (n=37)		**Maras powder (n=38)**		**Tobacco (n=33)**		**Maras powder + tobacco (n=41)**		**p values**						
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**p values**	**p1**	**p2**	**p3**	**p4**	**p5**	**p6**
Age	40.6	14.3	45.6	21.0	40.3	12.9	47.5	15.3	0.140						
Tobacco smoking duration (year)					19.2	12.1	16.2	10.2	0.253						
Tobacco smoking amount (packet/ year)					19.1	13.3	18.8	17.4	0.934						
Maras powder amount (number/ day)			14.6	13.0			13.2	10.0	0.612						
Maras powder using duration (year)			14.9	12.9			12.1	11.4	0.301						
Retention time in mouth (min)			16.2	11.0			17.9	17.9	0.617						
Heart rate	88.1	16.4	85.9	16.9	86.5	17.1	83.0	14.7	0.567						
Systolic BP	116.5	15.8	118.2	11.8	126.4	18.8	117.9	20.3	0.730						
Diastolic BP	73.0	9.4	74.5	8.6	79.4	12.0	77.1	8.1	0.030	0.904	0.028	0.233	0.136	0.620	0.725
SPO^2	97.2	1.5	97.2	1.1	97.2	1.2	96.9	1.7	0.790						
FVC (mL)	3.459.2	1.092.1	3.507.1	893.8	3.703.3	813.0	3.344.5	904.7	0.450						
FVC (%)	81.5	15.9			82.4	12.6	77.7	16.4	0.383						
FEV1 (mL)	3.201.6	946.8	3.277.7	821.7	3.359.1	801.3	3.007.1	846.2	0.160						
FEV1 (%)	91.4	15.7	93.3	14.5	90.2	14.3	85.5	16.5	0.346						
FEV1/FVC	93.6	7.2	93.7	6.3	90.6	7.4	89.9	8.4	0.056						
FEF 25-75 (mL)	4.346.8	1.599.5			4.050.9	1.409.4	3.797.4	1.485.4	0.290						
FEF 25-75 (%)	100.5	27.7			94.0	26.4	94.4	29.6	0.540						
PEF (mL)	4.076.2	1.073.6			4.361.5	837.3	3.719.2	954.6	0.022		0.220	0.130			0.004
PEF (%)	80.4	14.2			84.6	20.7	71.9	16.4	0.008		0.310	0.020			0.005
WBC	7.367.3	1.750.2	7.318.4	1.921.5	7.450.6	2.308.3	7.249.5	1.930.1	0.018	0.440	0.570	0.567	0.032	1.000	0.034
PLT	255.702.7	54.074.0	242.342.1	67.008.4	231.727.3	48.957.4	226.780.5	46.842.6	0.110						
HB	13.9	1.3	14.0	1.6	14.9	1.2	14.5	1.0	<0.001	1.000	0.010	0.179	0.011	0.248	0.499
NE (%)	59.1	8.7	62.0	9.2	56.8	7.3	61.1	9.6	0.070						
EO (%)	2.6	2.2	2.9	4.0	2.2	1.2	3.2	4.9	0.610						
MO (%)	8.1	1.8	7.9	2.8	8.9	4.2	7.2	3.5	0.150						
LY (%)	28.7	8.6	25.7	8.5	30.1	6.1	26.9	7.4	0.090						
BA (%)	0.6	0.7	0.4	0.6	0.5	0.3	0.4	0.8	0.494						
Albumin	4.3	0.4	4.1	0.3	4.3	0.4	4.2	0.4	0.050						
Glucose	100.4	15.2	104.0	21.0	101.2	18.3	107.7	52.7	0.740						
AST	21.8	10.4	22.3	7.6	19.7	6.5	19.8	6.2	0.370						
ALT	20.0	10.0	18.6	6.3	21.8	13.5	20.5	13.0	0.670						
Amylase	64.0	17.2	74.2	22.3	72.0	21.2	73.7	26.1	0.170						
Cholesterol	156.6	34.0	163.6	34.5	178.9	35.6	172.7	37.5	0.040	0.830	0.040	0.195	0.268	0.668	0.874
HDL	43.1	9.4	40.3	8.9	44.9	13.9	41.0	11.5	0.280						
LDL	97.7	25.3	102.7	28.4	116.0	27.9	113.7	27.6	0.012	0.850	0.029	0.052	0.170	0.280	0.980

VLDL	26.4	18.0	27.8	14.3	32.2	18.9	33.8	15.1	0.160						
TG	130.5	90.4	134.8	71.7	169.8	94.4	157.0	75.3	0.150						
LDH	189.4	30.2	201.8	40.7	178.3	23.4	201.1	42.6	0.018	0.430	0.560	0.460	0.032	0.990	0.034
CK	183.9	182.1	182.2	182.2	144.8	85.6	111.0	50.1	0.060						

SD: Standart deviation, SPO[2]: Pulse oxygen saturation, FVC: Forced vital capacity, FEV1: Forced expirator volume (1. second), PEF: Peak expiratory flow, WBC: White blood cell, LDL: Low-density lipoprotein, VLDL: Very low-density lipoprotein, LDH: Lactate dehydrogenase, TG: Thyroglobulin, HDL: High-density lipoprotein, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, CK: Creatine kinase, HB: Hemoglobin, PLT: Platelets, p: (ANOVA or T test)

significiant value, p[1]: Control-tobacco, p[2]: control-maras powder, p[3]: Control-tobacco plus maras powder, p[4]: Tobacco-maras powder, p[5]: Tobacco-tobacco plus maras powder, p6: Maras powder-tobacco plus maras powder sig. values

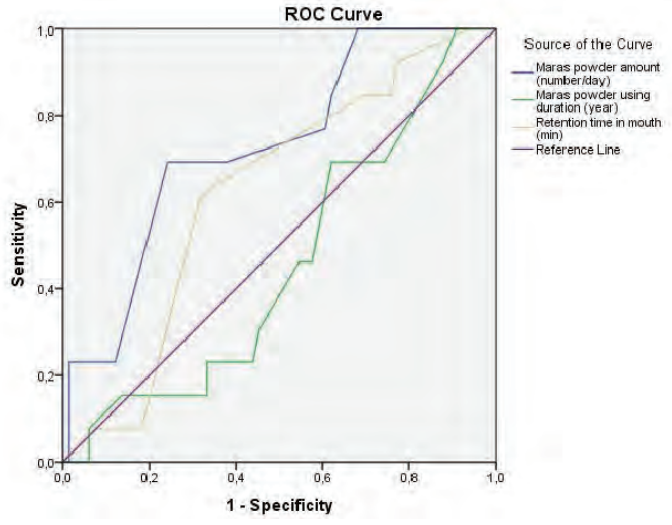


Figure 1. Sensitivity and spesifity of amount of usage, using duration and retention time in mouth for maras powder (ROC curve)

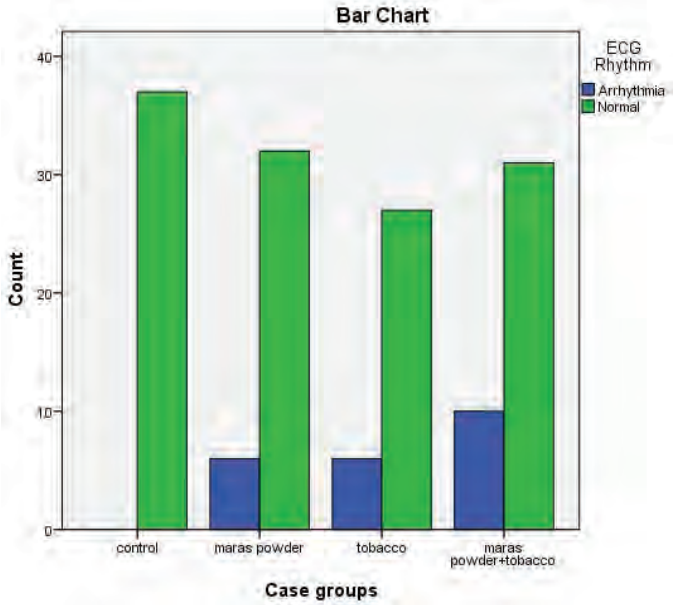


Figure 2. Bar chart of electrocardiogram comparisons for patient groups

ECG: Electrocardiogram

test in ANOVA was processed to determine which subgroups had statistically significant differences. Multivariate analysis was processed to determine whether this statistically significant

Table 3. Factors determining mouth lesion (binary logistic test)

	B	S.E	Wald	df	p	OR		CI
Direct mouth contact (direct contact group)	2.072	0.765	7.332	1	0.007	7.942	0	
Maras powder amount (number/day)	0.054	0.026	4.431	1	0.035	1.055	0.99	1.42
Constant	-3.044	0.639	22.686	1	0	0.048		

CI: Confidence interval, OR: Odds ratio

Variables entered at step 1: Direct mouth contact. Maras powder amount (number/day). Retention time in mouth (minimum). Maras powder using duration (year)

difference originated from the group variables or other factors such as age and gender. We found that none of these parameters actually differed between group variables (p=0.716). Instead, they were determined by age and gender (p<0.001 and p=0.009, respectively). The same analysis revealed the age factor to be associated with diastolic blood pressure (p=0.02), Hb (p<0.001) and cholesterol level (p=0.001). Also, the age factor was associated with Hb level (p<0.001).

DISCUSSION

Tobacco use is a global problem similar to drug addiction and alcohol abuse. While the developed countries have reduced smoking rates as a government policy, smoking still remains an important health problem for most developing countries. Turkey has come a long way in the fight against tobacco use through the restrictions on tobacco consumption, purchasing of products and the creation of social awareness about the issue. As a tobacco variety, cigarette alternative products such as smokeless tobacco should be carefully evaluated for in order to increase public awareness. Unfortunately, the targeted level of smokeless tobacco use (especially in the Eastern and Southeastern regions of Turkey) has not been reached yet (notice no: 2013/4 published by Kahramanmaraş Governorship, Tobacco Control Council). The

common belief of "it is less harmful than smoking", cheaper prices, consideration of smokeless tobacco as an alternative method to stop smoking, and its ease of use in "non-smoking" areas are some of the underlying reasons why smokeless tobacco use is rapidly increasing (13-16). Numerous studies have researched the effects of smokeless tobacco on carcinogenesis, oral health, the respiratory system, and the cardiovascular system as well as the immunological, biochemical and hematological parameters. The nicotine content of Nicotiana rustica L. has been reported to be 6-10 times higher than the nicotine content of Nicotiana tabacum used for cigarettes (17). One study revealed that blood nicotine levels were 15 times higher when tobacco was consumed orally, compared to smoking (18). A limiting factor in the evaluation of the results of our study was that we did not measure nicotine levels of participants.

Nicotine and other chemicals in tobacco are responsible for the harmful effects on multiple organs, including the respiratory system. The effects of smokeless tobacco on these same systems have been documented to be equal or greater (19,20). Smoking has been considered as harmful on the cardiovascular system through nicotine, and increased blood nicotine levels of smokeless tobacco consumers can increase the risk of cardiovascular diseases. Several studies have reported that using these products can increase the risk factors for fatal myocardial infarctions (21-24). Güven et al. (11) conducted a study on Maras powder consumers and found that serum lipid levels were high and that diastolic function parameters were impaired compared to the control group. They stated that Maras powder was as harmful to the cardiovascular system as smoking cigarettes. Similarly, Allen et al. (25) investigated the incidence of cardiovascular diseases in smokeless tobacco consumers (26). They observed that blood pressure, heart rate and functions, and lipoprotein levels were different from the control group. These values were increased or decreased among smokeless tobacco users, while they remained stable within the control group. In our study, we detected statistically and significantly increased ECG findings in participants regardless of their method of consuming tobacco. While there was no significant difference between only Maras powder group and only smoking group, the ECG findings of both smoking and Maras powder consuming group was found to be statistically and significantly higher than the control group. When age and gender of the participants were considered, other cardiac parameters such as blood pressure, heart rate and lipid levels were not different between groups. This indicates that tobacco use is a risk factor for cardiovascular diseases alone, but using Maras powder along with smoking increases that risk. Smokers are exposed to more than 3,000 substances, including alkaloids, as well as many toxic and carcinogenic substances through tobacco consumption. For smokers, exposure to carcinogenic substances may also happen by burning the cigarette and inhaling the smoke of it. Smoke from the cigarette causes chronic inflammation in the airways and is the underlying etiology of various diseases such as chronic bronchitis, COPD, oral and oropharyngeal cancers and lung cancers. Recently, it has been reported that free radicals increase in patients with COPD, which may be responsible for the disease (27,28). Several studies have proved that the use of smokeless tobacco causes systemic effects by the association among usage of smokeless tobacco, free radicals, and endothelia (19,21). Köksal et al. (8) investigated the effects of Maras powder on the airways and cardiovascular system. According to the results of the study, Maras powder had no effect on airways because it was not inhaled, although it did affect the cardiovascular system equivalent to smoking cigarettes. In another similar study, Büyükbese et al. (6) investigated the effects of Maras powder on pulmonary function and they observed that pulmonary function was negatively affected. In our study, we could not detect any difference in pulmonary function parameters between the smokers and the control group. This may be associated with the fact that we conducted our study in the rural regions of Turkey, which exposed patients to biomass in varying degrees. There was no statistically significant difference in pulmonary function parameters between the cases of Maras powder use and smoking cigarette use. This was associated with the fact that Maras powder did not circulate through the respiratory system like smoking cigarettes. Maras powder contains many harmful substances such as nicotine and this can affect hematological and biochemical parameters. In a research conducted by Ukoha et al. (29) in Wistar rats treated with smokeless tobacco, hematological and homeostatic effects of smokeless tobacco in sublethal doses were shown to increase leukocyte levels and decrease erythrocyte and platelets compared to the control rat group. They suggested that smokeless tobacco use in high doses and in chronic processes might be a risk factor for abnormal homeostatic and hematological conditions. Another clinical study by Kılınç et al. (12) found high leukocyte levels and low monocyte and thrombocyte levels in participants using Maras powder. It has been shown that Maras powder can adversely affect biochemical and hematologic parameters negatively. Many studies have found a positive correlation between Maras powder use, Hb, leukocyte, lipid profiles, and C-reactive protein values. However, there are reports in the literature that suggest no statistical correlation (30). When comparing the participants

of our study, leukocyte levels were found to be higher in the group using Maras powder compared to the smokers group. The leukocyte levels were also higher in the group using both Maras powder and smoking compared to the only smoking group. Further analyses suggested that increased Hb and lipid profiles were associated with age and gender. There was no difference between the groups in terms of other biochemical and hematological parameters. The obtained data support the opinion that Maras powder has systemic adverse effects and it may increase peripheral leukocyte levels. It is a well-known fact that differences in the pack-per-year consumption, the kind of tobacco, and depth and duration of inspiration of the smoke are important contributors to the adverse effects of smoking cigarettes. Frequency, using method, duration held in the mouth, oral flora, and amount of saliva are among other factors on the emergence of adverse effects of tobacco (31-34). There are studies available emphasizing that smokeless tobacco may cause disturbances in or around mouth. Also, it can cause oral-oropharyngeal cancers, leukoplakia, bleeding gums, and gum abnormalities (35-37). Considering leukoplakia, there are studies indicating that smokeless tobacco has a 3.0% lower rate of progressing into dysplasia when compared to normal cigarette smoking. Therefore, progression into cancer is less and slower (38,39). But, adverse effects of smokeless tobacco have varying degrees of risk depending on various conditions such as consumption route and frequency. In an analysis of case-control studies, risk for oral and respiratory cancers was found to be statistically and significantly higher in participants using dry snuff, lower in moist snuff and chewing tobacco (40). In our study, we found that effective parameters on developing oral lesions were the amount of daily use and method of use. We concluded that direct contact of Maras powder with mucosa increased the risk for oral lesions up to 8 times compared to using it wrapped in paper. Also, a positive correlation between daily use frequency and oral lesions was found. According to this, we can conclude that use of smokeless tobacco may contribute to deterioration of mucosal integrity, leukoplakia, and dysplasia. The progression of oral-oropharyngeal cancer development by consumption with direct contact to mucosa and number of daily use sessions is also a concern. This is consistent with results of many studies within the literature (35-37). But the fact that we did not obtain samples from lesions or mucosa of the participants to process histopathologically was a limiting factor of our study.

CONCLUSION

In conclusion, the smokeless tobacco use, which is considered as an alternative way of quitting smoking, does not have adverse effects on respiratory functions. However, it is an important risk factor for many life-threatening health conditions such as cardiac diseases, impairment of several blood parameters, oral lesions, and gum abnormalities that contribute to malignancies. Also, daily use frequency and method of use (direct contact to mucosa) for these tobacco products should be considered due to their harmful effects. Social awareness should be created for smokeless tobacco use, similar to smoking, in order to fight this habitual threat to public health. Additionally, more comprehensive studies are necessary to raise awareness about the effects of smokeless tobacco as a serious health problem.

Ethics

Ethics Committee Approval: The study was approved Department of Pulmonary Medicine at Dr. Sureyya Adanalı Göksun State Hospital and Kahramanmaraş Sütçü İmam University Faculty of Medicine by the Local Ethical Committee and was in accordance to the Declaration of Helsinki (24.11.2014/181).

Informed Consent: There is not informed consent because of nature of the retrospective study.

Peer-review: External and internal peer-reviewed.

Authorship Contributions

Concept: İ.I., H.A., Design: İ.I., H.A., Data Collection or Processing: U.S.K., P.A.G., Analysis or Interpretation: İ.I., N.A., Literature Search: İ.I., M.T., Writing: İ.I.

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

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

REFERENCES

1. Ashley DL, Burns D, Djordjevic M, Dybing E, Gray N, Hammond SK, et al; WHO Study Group on Tobacco Product Regulation. The scientific basis of tobacco product regulation. World Health Organ Tech Rep Ser 2008;1-277, 1 p following 277.
2. Örsel O. Dünyada kullanılan tütün çesitleri. www.toraks.org.tr/sub/sigarasiz/DunyadaKullanilanTutunUrunleriosman orsel.pdf. Son erisim 07.12.2009.
3. Kafas A: Analysis of factors affecting cigarete smoking and Maras powder use among adults in the urban area of Kahramanmaras. Master's Thesis, Institute of Science, Department of Agricultural Economics, Kahramanmaras Sutcu Imam University, Turkey, 2011, https://tez.yok.gov.tr/UlusalTezMerkezi/SearchTez
4. Sucaklı MH, Kahraman H, Çelik M, Keten HS. An Evaluation of Knowledge, Attitudes and Behavior regarding Smoking and Smokeless Tobacco (Maras Powder) Use among High School Children. Gaziantep Med J 2015;21:225-32.
5. Erenmemisoğlu A, Tekol Y, Kartal M, Kurucu S. The use of smokeless tobacco in our country "Maras Powder". Turk J Med Sci 1992;16:567-76.

6. Büyükbese MA, Köksal N, Güven A, Çetinkaya A. Effects of smokeless tobacco "Maras Powder" use on respiratory function. Tohoku J Exp Med 2004;204:173-8.
7. Ozkul Y, Donmez H, Erenmemisoglu A, Demirtas H, Imamoglu N. Induction of micronuclei by smokeless tobacco on buccal mucosa cells of habitual users. Mutagenesis 1997;12:285-7.
8. Köksal N, Güven A, Çetinkaya A. Büyükbese MA. Dumansız Tütün "Maras Otu" Kullanımının Solunum Fonksiyonları Üzerine Olan Etkileri. Akciger Arsivi 2004;5:174-8.
9. Kiowski W, Linder L, Stoschitzky K, Pfisterer M, Burckhardt D, Burkart F, et al. Diminished vascular response to inhibition of endothelium-derived nitric oxide and enhanced vasoconstriction to exogenously administered endothelin-1 in clinically healthy smokers. Circulation 1994;90:27-34.
10. Powell JT, Higman DJ. Smoking, nitric oxide and the endothelium. Br J Surg 1994;81:785-7.
11. Güven A, Köksal N, Büyükbese MA, Çetinkaya A, Sökmen G, Aksu E, et al. Effects of Using a Different Kind of Smokeless Tobacco on Cardiac Parameters: "Maras powder". Anadolu Kardiyoloji Dergisi 2003;3:230-5.
12. Kılınç M, Okur E, Yıldırım I, Inanç F, Kurutas EB. The Investigation of the Effect of Maras Powder (Smokeless Tobacco) on Hematological Parameters. Turk J Haematol 2004;21:131-6.
13. Goo MJ. Smokeless Tobacco. The Health Conseoquences of Snuff and Chewing Tobacco. Nurse Practitioner 1992;17:24-36.
14. Rodu B. An Alternatine Approach to Smoking Control. Am J Med Sci 1994;308:32-4.
15. Castiglia PT. Smokeless Tobacco. J Pediatr Health Care 1994;8:274-6.
16. Cok I, Oztürk R. Smokeless Tobacco Usage in the World and Turkey. Journal of Faculty of Pharmacy of Gazi University 1996;13:105-10.
17. Saıtoh F, Noma M, Kawashıma N. The Alcaloid Contents of Sixty Nicotiana Species. Phytochemistry 1985;24:477-480.
18. Benowitz NL. Pharmacological aspects of cigarette smoking and nicotine addiction. N Engl J Med 1998;319:1318-30.
19. Mitchell BE, Sobel HL, Alexander MH. The adverse health effects of tobacco and tobacco related products. Prim Care 1999;26:463-98
20. Bartal M. Health effects of tobacco use and exposure. Monaldi Arch Chest Dis 2001;56:545-54.
21. Traber MG, van der Vliet A, Reznick AZ, Cross CE. Tobacco-related diseases. Is there a role for antioxidant micronutrient supplementation? Clin Chest Med 2000;21:173-87.
22. Russell MA, Jarvis MJ, Devitt G, Feyerabend C. Nicotine intake by snuff users. Br Med J (Clin Res Ed) 1981;283:814-7.
23. Lee PN. Circulatory disease and smokeless tobacco in Western populations: a review of the evidence. Int J Epidemiol 2007;36:789-804.
24. Boffetta P, Straif K. Use of smokeless tobacco and risk of myocardial infarction and stroke: systematic review with metaanalysis. BMJ 2009;18:b3060.
25. Allen SS, Hatsukami D, Jensen J, Grillo M, Bliss R. Effects of Treatment on Cardiovascular Risk Among Smokeless Tobacco Users. Preventive Medicine 2002;6:242-5.
26. Tabak L. Is smokeless tobacco less harmful than smoking? Anadolu Kardiyol Derg 2003;3:236-7.
27. Kharitonov SA, Barnes PJ. Biomarkers of some pulmonary diseases in exhaled breath. Biomarkers 2002;7:1-32.
28. Couillard A, Koechlin C, Cristol JP, Varray A, Prefaut C. Evidence of local exercise-induced systemic oxidative stress in chronic obstructive pulmonary disease patients. Eur Respir J 2002;20:1123-9.
29. Ukoha UU, Dimkpa U, Maduka SO. "The effect of sub-lethal doses of smokeless tobacco (snuff) on certain hematological and hemostatic parameters in Wistar rats." Journal of Experimental and Integrative Medicine 2012;225-30.
30. Asplund K, Nasic S, Janlert U, Stegmayr B. Smokeless tobacco as a possible risk factor for stroke in men: a nested case-control study. Stroke 2003;34:1754-9.
31. Hatsukami DK, Lemmonds C, Tomar SL. Smokeless Tobacco Use: Harm Reduction or Induction Approach. Preventive Medicine 2004;38:309-17.
32. Rodu B, Jansson C. Smokeless Tobacco and Oral Cancer: A Review of the Risks and Determinants. Critical Reviewvs in Oral Biology and Medicine 2004;21:156-7.
33. Boyle P, Macfarlane GJ, Maisonneuve P, Zheng T, Scully C, Tedesco B. Epidemiology of mouth cancer in 1989: a review. J R Soc Med 1990;83:724-30.
34. Scully C. Oncogenes, tumor suppressors and viruses in oral squamous cell carcinoma. J Oral Pathol Med 1993;22:337-47.
35. Fant RV, Henningfield JE, Nelson RA, Pickworth WB. Pharmacokinetics and Pharmacodynamics of Moist Snuff in Humans. Tob Control 1999;8:387-92.
36. Walsh PM, Epstein JB. The Oral Effects of Smokeless Tobacco. J Can Dent Assoc 2000;66:22-5.
37. Hatsukami DK, Lemmonds C, Tomar SL. Smokeless Tobacco Use: Harm Reduction or Induction Approach. Prev Med 2004;38:309-17.
38. Greer RO, Poulson TC, Boone ME, Lindenmuth JE, Crosby L. Smokeless tobacco associated oral changes in juvenile, adult and geriatic patients: Clinical and histomorphologic features. Gerodontics 1986;2:87-98.
39. Axell T, Mornstad H, Sundstrom B. The relation of the clinical picture to the histopathology of snuff dipper's lesions in a Swedish population. J Oral Pathol 1976;5:229-36.
40. Rodu B, Cole P. Smokeless tobacco use and cancer of the upper respiratory tract. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2002;93:511-5.

ORIGINAL ARTICLE

DOI: 10.4274/eamr.galenos.2019.02418
Eur Arch Med Res 2019; 35 (4):241-4

The Diagnostic Power of the Gallium-68 Prostate-specific Membrane Antigen Positron Emission Tomography-Computed Tomography in Biochemical Recurrence After Primary Curative Treatment in Patients with Prostate Cancer: A Single-center Experience

Sevda Sağlampınar Karyağar[1], Selvi Tabak Dinçer[2]

[1]İstanbul Okmeydanı Training and Research Hospital, Clinic of Nuclear Medicine, İstanbul, Turkey
[2]İstanbul Okmeydanı Training and Research Hospital, Clinic of Radiation Oncology, İstanbul, Turkey

Abstract

Objective: Our study aimed to investigate the efficacy of Gallium-68 (^{68}Ga) prostate-specific membrane antigen (PSMA) positron emission tomography-computed tomography (PET-CT) in terms of focus detection in the presence of biochemical recurrence (BCR) after primary curative treatment in patients with prostate cancer (PCa).

Methods: This study included 34 PCa patients who underwent ^{68}Ga-PSMA PET-CT for BCR following radical prostatectomy (RP) or primary curative radiotherapy (RT), between August 2017-December 2018 at Okmeydanı Training and Research Hospital, Clinic of Nuclear Medicine.

Results: Thirteen patients (38.2%) had RP and 21 (61.8%) had RT. PSMA-positive lesion was detected in 21 (61.7%) of 34 patients. PSMA positive lesion was present in six of 13 patients (46.1%) in the RP group and 15 of the 21 patients (71.4%) in the RT group. There was a PSMA-positive lesion in five out of 13 patients with serum prostate-specific antigen (PSA) values of 0.01-1 ng/mL, in five of eight patients with serum PSA values of 1-2 ng/mL, in four of five patients with serum PSA values of 2-5 ng/mL, and in seven of eight patients with serum PSA values of >5 ng/mL. PSA levels of PSMA-positive patients were found to be significantly higher than those of PSMA-negative, whereas PSA-positive and negative patients did not differ significantly in terms of PSA doubling time, time to BCR, and Gleason score.

Conclusion: ^{68}Ga-PSMA PET-CT is an effective method in the diagnosis of BCR after primary curative treatment in PCa patients.

Keywords: Gallium-68 prostate-specific membrane antigen positron emission tomography-computed tomography, biochemical recurrence, curative radiotherapy, radical prostatectomy

INTRODUCTION

Prostate cancer (PCa) is the most common cancer in men and is responsible for 1/3 of cancer-related deaths. In PCa patients, biochemical recurrence (BCR) occurs in 25% of patients after radical prostatectomy (RP) or primary curative radiotherapy (RT) (1,2). Prostate-specific membrane antigen (PSMA) is a cell surface protein that is 100-1.000 fold increased expression in PCa cells compared to benign prostate tissues (3,4). Gallium-68 (^{68}Ga)-labeled PSMA ligands bind to the extracellular part of PSMA and internalize to PCa cells (3,4). PSMA is a preferable and suitable target for imaging with these features. ^{68}Ga-PSMA positron emission tomography-computed tomography (PET-CT) imaging has been increasingly used in recent years in PCa patients for staging purposes and the purpose of focus detection in the presence of BCR (5-8). The aim of this study was to determine the efficacy of ^{68}Ga-PSMA PET-CT for the detection of recurrence focus in the presence of BCR after RP or primary curative RT treatments in PCa patients, and to investigate the correlation between PSMA positive focus detection and serum prostate-specific antigen (PSA) value, PSA doubling time (PSAdt), time to BCR, Gleason score.

METHODS

This study included 34 PCa patients who underwent ^{68}Ga-PSMA PET-CT for BCR following RP or primary curative RT between August 2017-December 2018 at Okmeydanı Training and

Address for Correspondence: Sevda Sağlampınar Karyağar, İstanbul Okmeydanı Training and Research Hospital, Clinic of Nuclear Medicine, İstanbul, Turkey
Phone: +90 505 479 31 64 E-mail: ssaglampinar@gmail.com ORCID ID: orcid.org/0000-0002-6356-8280

Cite this article as: Sağlampınar Karyağar S, Tabak Dinçer S. The Diagnostic Power of the Gallium-68 Prostate-specific Membrane Antigen Positron Emission Tomography-Computed Tomography in Biochemical Recurrence After Primary Curative Treatment in Patients with Prostate Cancer: A Single-center Experience. Eur Arch Med Res 2019; 35 (4): 241-4

Received: 03.05.2019
Accepted: 26.05.2019

Research Hospital, Clinic of Nuclear Medicine. The following were defined as BCR criterion: >0.2 ng/mL PSA value at post-RP follow-up, PSA levels that were increased 2 ng/mL compared to the rare value in the patient at post-RT follow-up, or, increasing PSA value in a patient who had achieved rare value in three consecutive PSA measurements. In patients with BCR, the diagnostic power of ^{68}Ga-PSMA PET-CT imaging in the detection of recurrence focus and the relationship between imaging findings and serum PSA value, PSAdt, time to BCR, and Gleason score were investigated. Whole-body ^{68}Ga-PSMA PET-CT imaging was performed with PET-CT scanner (Siemens Biograph 6, Chicago, IL, USA) consisting of full-ring HI-REZ lutetium oxyorthosilicate PET and 6-section CT scan at 60th minutes following the intravenous injection of 2 MBq/kg ^{68}Ga PSMA I&T (Scintomics GRP, Germany) obtained from Germanium-68/^{68}Ga generator (iThemba LABS, South Africa). Images were evaluated visually by two nuclear medical experts who knew just the patients' primary diagnosis. ^{68}Ga-PSMA I&T uptake, which is located outside the physiological activity regions and increased compared to background activity, was considered positive for recurrence. The standardized uptake value maximum (SUV_{max}) value of all ^{68}Ga-PSMA I&T uptake foci was measured, but any SUV_{max} threshold value was not used as the criterion of positivity. Ethics committee approval was obtained from Okmeydanı Training and Research Hospital Ethics Committee with the 1181 decision number and on 03.05.2019 date, for this clinical study, which was designed retrospectively.

Statistical Analysis

For statistical analysis, IBM SPSS Statistics 22 (IBM SPSS, Turkey) program was used. In the evaluation of the study data, the conformity of the parameters to the normal distribution was evaluated by Shapiro-Wilks test, and it was found that the parameters did not show normal distribution. In addition to descriptive statistical methods (mean, standard deviation, frequency), Mann-Whitney U test was used for the comparison of quantitative data between two groups. Significance was evaluated as $p<0.05$.

RESULTS

Thirty-four male patients were included in the study. The mean age was 70.76±6.94 years (range, 56-84). Thirteen patients (38.2%) had RP, and 21 (61.8%) had RT as primary treatment. While ^{68}Ga-PSMA PET-CT was applied with the diagnosis of BCR in patients, the mean serum PSA level was 6.33±11.06 ng/mL (median 1.23; range=0.01-40.19) and the mean PSA_{dt} was 9.97±6.77 (median 8; range=1-31) months. The mean time to BCR was 71.03±52.05 months (median 52; range=9-179). Gleason score was 3+3 in nine patients, 3+4 in nine patients, 4+3 in three patients, 4+4 in eight patients, and 4+5 in five patients. The PSMA-positive lesion was detected in 21 (61.7%) of the 34 patients: In six of the 13 patients (46.1%) in the RP group and 15 of the 21 patients (71.4%) in the RT group. The pathologies detected with ^{68}Ga-PSMA PET-CT were local recurrence in 12 patients, local recurrence plus pelvic metastatic lymph node in one patient, local recurrence plus bone metastasis in two patients, pelvic metastatic lymph node alone in two patients and bone metastasis alone in four patients. The mean SUV_{max} value was 18.37 (range=3.6-56.43) in local recurrent lesions, 6.58 (range=3.38-13.51) in metastatic lymph nodes, and 10.52 (range=3.69-43.14) in bone metastases (Figure 1). There was a PSMA-positive lesion in five out of 13 patients with serum PSA values of 0.01-1 ng/mL, in five of eight patients with serum PSA values of 1-2 ng/mL, in four of five patients with serum PSA values of 2-5 ng/mL, and in seven of eight patients with serum PSA values of >5 ng/mL. PSA levels were found to be significantly higher in patients who had a positive lesion in ^{68}Ga-PSMA PET-CT compared to patients with no lesion ($p=0.008$). Between the patients with and without a positive focus in ^{68}Ga-PSMA PET-CT, no statistically significant difference was found in terms of PSA_{dt}, time to BCR, and Gleason score ($p>0.05$) (Table 1). ^{68}Ga-PSMA PET-CT imaging was found to provide a potential change in the treatment plan through detection of the metastatic lesion without local recurrence, in addition to the detection of local recurrence in nine patients.

DISCUSSION

In PCa patients, RP or primary curative RT is the primary

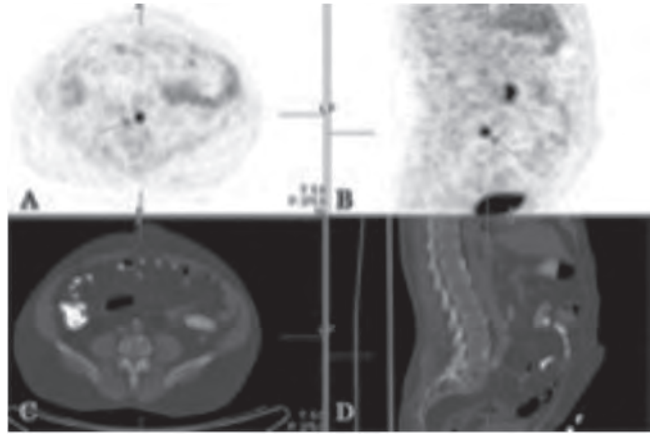


Figure 1. A 62-year-old man with prostate cancer, Gleason score 4+4, who underwent radio theraphy June 2017. Gallium-68 prostate-specific membrane antigen positron emission tomography-computed tomography (^{68}Ga-PSMA PET/CT) was performed for a persistently raised prostate-specific antigen level (0.47 ng/mL). (A) Axial, (B) Sagittal ^{68}Ga PSMA PET and (C) Axial, (D) Sagittal CT images showed ^{68}Ga PSMA-positive sclerotic lesion at the lumbar 4th vertebra corpus (black arrow)

Table 1. Relationship between Gallium-68 prostate-specific membrane antigen positron emission tomography-computed tomography findings and serum prostate-specific antigen, time to biochemical recurrence, and prostate-specific antigen doubling time

	PSMA positive	PSMA negative	Totaly	p
Serum PSA	8.41±12.5 (2.39)	2.97±7.47 (0.89)	6.33±11.06 (1.23)	0.008*
BCR time	80.67±54.22 (58)	55.46±46.1 (29)	71.03±52.05 (52)	0.070
PSA_{dt}	10.2±7.34 (8)	9.62±6.05 (8)	9.97±6.77 (8)	0.986

PSA_{dt}: Prostate-specific antigen doubling time, PSMA: Prostate-specific membrane antigen, BCR: Biochemical recurrence

treatment option in the presence of localized disease. BCR is seen in 20-30% of patients after RP, and 60% after curative RT in 10-15 years follow-up (5,9). Although adjuvant RT is the first and most important treatment option in patients with BCR, the efficacy of this treatment is related to the detection of the recurrent disease as early as possible and is limited to the prostate region (6). In a meta-analysis of Tan et al. (10) which included 5113 patients with BCR who underwent ^{68}Ga-PSMA PET-CT to detect the recurrence focus, ^{68}Ga-PSMA PET-CT scan was reported to have a 70% focus detection rate, and, for the serum PSA values of <0.5 ng/mL, 0.5-0.9 ng/mL, 1-1.9 ng/mL and ≥2 ng/mL, these rates were reported as 44.9%, 61.3%, 78.2%, and 93.9%, respectively. In our study, at least one PSMA-positive lesion was detected in 21 of the 34 patients (61.7%) as the BCR focus, and this rate was 46.1% in the RP group and 71.4% in the RT group. The pathologies detected with ^{68}Ga-PSMA PET-CT were local recurrence in 12 patients, local recurrence plus pelvic metastatic lymph node in one patient, local recurrence plus bone metastasis in two patients, pelvic metastatic lymph node alone in two patients and bone metastasis alone in four patients. In the study of Vinsensia et al. (11) as BCR focus, 30.4% local recurrence and pelvic metastatic lymph nodes, 42.1% bone metastasis, 13.7% retroperitoneal metastatic lymph nodes and 13.7% distant metastasis were detected. Accurate and early detection of local recurrence and possible metastatic focus in the presence of BCR is essential for appropriate treatment planning (12,13). In our study, there was a PSMA-positive lesion in five out of 13 patients with serum PSA values of 0.01-1 ng/L in five of eight patients with serum PSA values of 1-2 ng/mL, in four of five patients with serum PSA values of 2-5 ng/mL, and in seven of eight patients with serum PSA values of >5 ng/mL. In a prospective study that included 314 cases, Caroli et al. (14) reported that PSMA-positive focus detection rate was found to be 94.8% among patients with BCR who had a serum PSA value of ≥2 ng/mL. In a study conducted with patients with serum PSA values <5 ng/mL, Kabasakal et al. (15) detected a PSMA positive focus in 58% of patients by ^{68}Ga-PSMA PET-CT imaging, and, this rate was reported to be 31% in patients with serum PSA <0.2 ng/mL and 54% in patients with 0.2-2 ng/mL. In patients with BCR, the major effective factor for detecting the focus with imaging methods is serum PSA, and as the PSA increases, the focus detection rate increases (10,16). In a study by Sanli et al. (17) on the ^{68}Ga-PSMA PET-CT imaging performed due to BCR, at least one focus was detected in 83.4% of the patients. Furthermore, there was a significant difference between the PSA positive and negative patients in terms of serum PSA levels, but no significant relationship was found in terms of the Gleason score. In our study, we showed that PSA levels were significantly higher in patients who had PSMA-positive lesion in ^{68}Ga-PSMA PET-CT compared to patients without a PSMA-positive lesion, but there was no statistically significant difference in terms of Gleason score, time to BCR and PSAdt. In a meta-analysis examining 37 investigations conducted by Eissa et al. (18) the rate of focus detection was found to be 47-96.6% with ^{68}Ga-PSMA PET-CT imaging in patients with BCR. Moreover, this rate was 11.1% to 75% in patients who had <0.5 ng/mL serum PSA value. In this study, it was also reported that there was a significant relationship between high serum PSA values and PSMA positive focus detection, and ^{68}Ga-PSMA PET-CT imaging provided a change in treatment in 28.6-87.1% of the patients (18). In a study of 70 cases, conducted by Ceci et al. (5) a significant relationship was found between positive focus detection in ^{68}Ga-PSMA PET-CT imaging and the PSA_{dt}. Also, It was reported that PSMA positive focus detection rate was 85% among patients with PSA_{dt} <6.5 months and serum PSA value <2 ng/mL (5). However, in the study of Afaq et al. (16) no significant relationship was found between PSAdt and PSMA positive focus detection. The treatment plan change provided by ^{68}Ga-PSMA PET-CT imaging in BCR patients has been reported in the literature in 39-76%. Significant treatment changes have been in the form of RT area-dose change for pelvic lymph node metastasis in patients scheduled rescue RT and the transition to systemic therapy in patients who were detected distant metastases (9,19-21). In our study, ^{68}Ga-PSMA PET-CT imaging was found to provide a potential change in the treatment plan through detection of the metastatic lesion without local recurrence, in addition to the detection of local recurrence in nine patients.

CONCLUSION

^{68}Ga-PSMA PET-CT imaging is an effective method for detecting BCR after primary curative treatment in PCa patients and provides optimal treatment planning. In our study, there was

a significant relationship between high PSA level and PSMA positive focus detection rate, while there was no significant difference between PSA positive and negative patients in terms of PSAdt, time to BCR, and Gleason score.

Ethics

Ethics Committee Approval: Ethics committee approved.

(İstanbul Okmeydanı Training and Research Hospital, desicion number 1181/date 03.05.2019).

Informed Consent: Obtained from all patients.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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

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

REFERENCES

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin 2011;61:69-90.
2. Hamed MAG, Basha MAA, Ahmed H, Obaya AA, Afifi AHM, Abdelbary EH. 68Ga-PSMA PET/CT in parients with rising prostatic specific antigen after definitive treatment of prostate cancer: Detection efficacy and diagnostic accuracy. Acad Radiol 2019;26:450-60.
3. Afshar-Oromieh A, Haberkorn U, Eder M, Eisenhut M, Zechmann CM. [68 Ga] Gallium-labelled PSMA ligand as superior PET tracer for the diagnosis of prostate cancer: comparison with 18F-FECH. Eur J Nucl Med Mol Imaging 2012;39:1085-6.
4. Meredith G, Wong D, Yaxley J, Coughlin G, Thompson L, Kua B, et al. The use of 68Ga-PSMA PET CT in men with biochemical recurrence after definitive treatment of acinar prostate cancer. BJU İnt 2016;118:49-55.
5. Ceci F, Uprimny C, Nilica B, Geraldo L, Kendler D, Kroiss A, et al. 68Ga-PSMA PET/CT for restaging recurrent prostate cancer: which factors are associated with PET/CT detection rate?. Eur J Nucl Med Mol Imaging 2015;42:1284-94.
6. Eiber M, Maurer T, Souvatzoglou M, Beer AJ, Ruffani A, Haller B, et al. Evaluation of Hybrid 68Ga-PSMA ligand PET/BT in 248 Patients with Biochemical recurrence after radical Prostatectomy. J Nucl Med 2015;56:668-74.
7. Afsahar-Oromieh A, Avtzi E, Giesel FL, Holland-Letz T, Linhart HG, Eder M, et al. The diagnostic value of PET/CT imaging with the 68Ga-labelled PSMA ligand HBED-CC in the diagnosis of recurrent prostate cancer. Eur J Nucl Med Mol Imaging 2015;42:197-209.
8. Meyrick DP, Asokenderan M, Skelly LA, Lenzo NP, Henderson A. The role of 68Ga-PSMA-I&T PET/CT in the pretreatment staging of primary prostate cancer. Nucl Med Commun 2017;38:956-63.
9. Bluemel C, Linke F, Herrmann K, Simunovic I, Eiber M, Kestler C, et al. Impact of 68Ga-PSMA PET/CT on salvage radiotherapy planning in patients with prostate cancer and persisting PSA values or biochemical relapse after prostatectomy. EJNMMI Res 2016;6:78.
10. Tan N, Bavadian N, Calais J, Oyoyo U, Kim J, Turkbey IB, et al. Imaging of PSMA-targeted Radiotracers for the detection of Prostate Cancer Biochemical Recurrence After Definitive Theraphy: A Systematic Review and Meta-aalysis. J Urol 2019;202:231-240.
11. Vinsensia M, ChyokePl, Hadaschik B, Holland-Letz T, Moltz J, Kopka K, et al. 68Ga-PSMA PET/CT and volumetric Morphology of PET-Positive Lymph Nodes Stratified by Tumor Differentiation of Prostate cancer. J Nucl Med 2017;58:1949-55.
12. Uprimny C, Kroiss AS, Fritz J, Decristoforo C, Kendler D, von Guggenberg E, et al. Early PET imaging with [68] Ga-PSMA-11 increases the detection rate of local recurrence in prostate cancer patients with biochemical recurrence. Eur J Nucl Med Mol Imaging 2017;44:1647-55.
13. Pfister D, Bolla M, Briganti A, Carrol P, Cozzarini C, Joniau S, et al. Early salvage radiotherapy following radical prostatectomy. Eur Urol 2014;651034-43.
14. Caroli P, Sandler I, Matteucci F, De Giorgi U, Uccelli L, Celli M, et al. 68Ga-PSMA PET/CT in patients with recurrent prostate cancer after radical treatment: prospective results in 314 patients. Eur J Nucl Med Mol Imaging 2018;45:2035-44.
15. Kabasakal L, Demirci E, Nematyazar J, Akyel R, Razavi B, Ocak M, et al. The role of PSMA PET/CT imaging in restaging of prostate cancer patients with low prostate-specific antigen levels. Nuc Med Commun 2017;38:149-55.
16. Afaq A, Bomanji J. Prostate-specific membrane antigen positron emission tomography in the management of recurrent prostate cancer. Br Med Bull 2018;128:37-48.
17. Sanli Y, Kuyumcu S, Sanlı O, Buyukkaya F, İribas A, Alcin G, et al. Relationships between serum PSA levels, Gleason scores and results of 68Ga-PSMAPET/CT in patients with recurrent prostate cancer. Ann Nuc Med 2017;31:709-17.
18. Eissa A, Elsherbiny A, Coelho RF, Rassweiler J, Davis JW, Porpiglia F, et al. The role of 68Ga-PSMA PET/CT scan in biochemical recurrence after primary treatment for prostate cancer: a systematic review of the literatüre. Minerva Urol Nefrol 2018;70:462-78.
19. Afaq A, Alahmed S, Chen SH, Lengana T, Haroon A, Payne H, et al. Impact of 68Ga prostate-specific membrane antigen PET/CT on prostate cancer management. J Nucl Med 2018;59:89-92.
20. Albisinni S, Artigas C, Aoun F, Biaou I, Grosman J, Gil T, et al. Clinical impact of 68Ga-prostate-specific membrane antigen (PSMA) positron emission tomography/computed tomography (PET/ CT) in patients with prostate cancer with rising prostate specific antigen after treatment with curative intent: preliminary analysis of a multidisciplinary approach. BJU Int 2017;120:197-203.
21. vanLeeuwen PJ, Stricker P, Hruby G, Kneebone A, Ting F, Thompson B, et al. 68Ga-PSMA has a high detection rate of prostate cancer recurrence outside the prostatic fossa in patients being considered for salvage radiation treatment. BJU Int 2016;117:732-9.

ORIGINAL ARTICLE

DOI: 10.4274/eamr.galenos.2019.30301
Eur Arch Med Res 2019; 35 (4):245-8

Is Idiopathic Benign Paroxysmal Positional Vertigo Associated with Serum 25-Hydroxy Vitamin D Deficiency?

Belgin Tutar, Güler Berkiten, Fatih Akgün, Semih Karaketir, Onur Üstün, Yavuz Uyar, Ömür Biltekin Tuna

İstanbul Okmeydanı Training and Research Hospital, Clinic of Ear Nose Throat, İstanbul, Turkey

Abstract

Objective: Benign paroxysmal positional vertigo (BPPV) is a common cause of peripheral vertigo in the general population. We investigated the role of 25-hydroxy (25-OH) vitamin D deficiency in BPPV by comparing 25-OH vitamin D levels in healthy controls and in patients with BPPV.

Methods: 25-OH vitamin D levels of 125 patients with idiopathic BPPV who were diagnosed at our clinic between January 2018 and September 2018 and 101 healthy controls without vertigo were compared statistically.

Results: In this study, vitamin D deficiency was detected in patients diagnosed with BPPV, but there was no statistically significant difference with the control group.

Conclusion: The prevalence of the vitamin D deficiency is very high in our population. Despite the major studies in the literature, vitamin D deficiency was not related to BPPV as a result of this research.

Keywords: Benign paroxysmal positional vertigo, 25-hydroxy vitamin D, peripheral vertigo

INTRODUCTION

Benign paroxysmal positional vertigo (BPPV) is the most common cause of peripheral vertigo in the general population (1). BPPV is characterized by vertigo that is triggered by head movements and lasts for seconds, and accompanied by a feeling of imbalance and nausea. In the pathophysiology of BPPV, there are two theories called cupulolithiasis and canalithiasis. There are three semicircular canals located perpendicular to each other in the inner ear and sensing the angular movements of the head: posterior, lateral (horizontal), superior (anterior) canal crura are associated with the utricle, known as autolytic organ. Calcium (Ca) carbonate crystals are found in the otoconial layer above the maculae found in the utricle. The otoconia separated from the utricle macula can pass into semicircular canals. Vertigo and nystagmus can occur when these otoconia stimulates cupula. The "canalithiasis" theory suggests that the free movement of these otoconia in the canal plays a role in the pathophysiology of the disease. It was first described by Hall, Ruby and McClure in 1979 and was first proven in vivo by Parnes and McClure in 1992. "Cupulolithiasis", defined by Schuknecht in 1969, refers to the adherence of the otoconia to the cupula (2). Otoconia contains Ca carbonate in the form of Ca crystals and an organic core consisting mainly of glycoproteins. Ca metabolism also plays a primary role in the synthesis/absorption of otoconia and is therefore theoretically thought to be an etiological factor at the onset of BPPV (3). The aim of our study was to compare 25-hydroxy (25-OH) vitamin D levels in patients with idiopathic BPPV and healthy controls, and to investigate the role of 25-OH vitamin D in the development of BPPV.

METHODS

One hundred and twenty-five (100 female and 25 male, mean age=52±14 years) patients, who were admitted to the vertigo outpatient clinic between June 2018 and September 2018 and were diagnosed with idiopathic BPPV, were included in this retrospective case-control study. The patients had no history of Meniere's disease, vestibular migraine, labyrinth hypofunction, head trauma or other vestibular diseases. The control group

Address for Correspondence: Belgin Tutar, İstanbul Okmeydanı Training and Research Hospital, Clinic of Ear Nose Throat, İstanbul, Turkey
Phone: +90 505 261 05 02 E-mail: belgintutar@gmail.com ORCID ID: orcid.org/0000-0001-7783-0908

Received: 28.11.2018
Accepted: 19.06.2019

Cite this article as: Tutar B, Berkiten G, Akgün F, Karaketir S, Üstün O, Uyar Y, Biltekin Tuna Ö. Is Idiopathic Benign Paroxysmal Positional Vertigo Associated with Serum 25-Hydroxy Vitamin D Deficiency?. Eur Arch Med Res 2019; 35 (4): 245-8

consisted of 101 (74 female and 27 male, mean age=48±13 years) healthy volunteers. The patients had no history of neurological symptoms or vestibular disease. Patients with neurotologic symptoms and complaints of dizziness and imbalance were excluded from the study. All participants did not receive Ca or vitamin D treatment within the last year.

Vestibular evaluation was performed using computerized (videonystagmography: ICS Charter EP, GN Otometrics, USA). BPPV was diagnosed by Dix-Hallpike and Pagnini-McClure maneuvers. There was posterior canal involvement in 80 patients (64%), horizontal canal involvement in 38 patients (30.4%) and anterior canal involvement in seven patients (5.6%). Epley maneuver was used in patients with posterior canal involvement and Barbecue maneuver was applied to those with horizontal canal involvement. In the anterior canal involvement, "reverse Epley maneuver" was performed. Canalithiasis was detected in 72% (90 patients) of these patients and cupulolithiasis was responsible for the pathophysiology in 28% (35 patients) (Table 1). Blood was collected from patients with BBPV and healthy volunteers and 25-OH vitamin D levels were measured. 25-OH vitamin D levels were classified as normal (≥30 ng/mL), insufficient (>20 to <30 ng/mL) and deficiency (≤20 ng/mL). The approval of the Ethics Committee was obtained (dated: 6.11.2018, numbered: 48670771-514.10). Informed consent was obtained from the patients.

Statistical Analysis

Statistical analysis was performed using SPSS version 23.0. Descriptive data are presented using mean and standard deviation for normally distributed variables, and median, minimum and maximum values for non-normally distributed variables (and frequency tables for ordinal variables). Chi-square was used to compare categorical variables. The suitability of the measured variables to normal distribution was examined by visual (histogram) and analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk tests). Pairwise comparisons were performed using Student's t-test for normally distributed parameters and Mann-Whitney U and Kruskal-Wallis tests for non-normally distributed parameters. P<0.05 was evaluated as statistically significant.

Results

The mean serum 25-OH vitamin D levels were 16.36 ng/mL (3.52-53.91) in the BPPV group and 17.09 ng/mL (4.46-53.51) in the control group. Vitamin D levels were low in both groups. In the BPPV group, 81 patients (65.3%) had serum 25-OH vitamin D deficiency, 33 patients (26.6%) had insufficient and 10 patients (8.1%) had normal levels. In the control group, 74 patients (73.3%) had serum 25-OH vitamin D deficiency, 16 patients (15.8%) had insufficient and 11 patients (10.9%) had normal levels (Table 2). There was no statistically significant difference between the BBPV

Table 1. Rate of semicircular canal involvement and 25-hydroxy vitamin D levels

		n	% Median (minimum-maximum)	Vitamin D levels	H	p[a]
Affected canal	Superior	7	5.6	14.52 (9.60-36.04)	0.645	0.724
	Posterior	38	30.4	18.00 (3.52-53.91)		
	Lateral	80	64	16.18 (6.12-49.78)		

[a]Kruskal Wallis-H test

Table 2. Comparison of 25-hydroxy vitamin D levels between benign paroxysmal positional vertigo and control group

n		BPPV (n=125)		Control group (n=101)		Significance	
		%	n	%			
Gender	Male	25	20.0	27	26.7	x^2=1.429	p=0.232
	Female	100	80.0	74	73.3		
Age*		52±14		48±13		t=1.903	p=0.058
Vitamin D groups	Deficient (≤20 ng/mL)	81	65.3	74	73.3	x^2=3.952	p=0.139
	Insufficient (>20 to <30 ng/mL)	33	26.6	16	15.8		
	Normal (≥30 ng/mL)	10	8.1	11	10.9		
Vitamin D groups	Deficient (<30 ng/mL)	114	91.9	90	89.1	x^2=0.526	p=0.468
	Normal (≥30 ng/mL)	10	8.1	11	10.9		
Vitamin D value**		16.36 (3.52-53.91)		17.09 (4.46-53.51)			

BPPV: Benign paroxysmal positional vertigo, *Mean ± standard deviation values are given, **Since vitamin D values do not show normal distribution, median (minimum-maximum) values are given, Pearson chi-square test, Independent samples t-test, Mann-Whitney U test

group and control group in terms of 25-OH vitamin D deficiency (p=0.139). There was no difference between 25-OH vitamin D levels in BPPV patients regarding affected canals (p=0.724).

DISCUSSION

BPPV is the most common cause of peripheral vertigo at any age. The mechanism of BPPV is explained by the passage of the otoconia separated from the utricle into semicircular canals. There is no consensus on the factors that cause BPPV. Since the etiologic factors are unclear, most cases are considered idiopathic. Predisposing factors are senility, female gender, hormonal factors and viral causes (4). Inner ear consists of cochlea and labyrinth system. The bony labyrinth consists of three semicircular canals: superior (anterior), posterior and horizontal (lateral). There is a fluid called perilymph in the bony labyrinth. Membranous labyrinth consists of utricle, saccule and membranous semicircular canals, and it contains endolymph. Membranous semicircular canals are located perpendicular to each other. The dilated parts are called ampulla. There are special cells in this region called "crista ampullaris" that is the sensory organ of balance. Within the wall of the utricle, there are cells called the macula of utricle, which lie horizontally and receive the sense of balance, and supporting cells. These cells have Ca^{+2} particles called otoconia. Otoconia crystals have central and peripheral portions. The core is predominantly organic with a lower Ca^{+2} level and the periphery is largely inorganic with a higher Ca^{+2} level (5). Endolymphatic Ca^{+2} concentration is critical for normal auditory and balance system (6-8). According to theoretical considerations, a link between otolytic disorders and vitamin D deficiency is highly probable. Endolymphatic (cochlea 23 µM and vestibule 280 µM) Ca concentration is much lower than perilymph. Yamauchi et al. (9) first demonstrated the expression of a complete Ca^{+2} absorptive system in cochlear and vestibular tissues in mice. Regulation by vitamin D allows this system to be regulated at the transcript level. Brookes (8) thinks that low endolymphatic Ca^{+2} concentrations at pathological level are the cause of hearing loss due to vitamin D deficiency and hypoparathyroidism. Karataş et al. (3) conducted a study in our country and compared the prevalence of osteoporosis and vitamin D deficiency in 78 BPPV patients and in 78 controls. Osteoporosis and vitamin D deficiency rates in BPPV patients were very high. The mean serum 25-OH vitamin D levels in BPPV patients and controls were 23.0±14.4 ng/mL and 17.0±12.3 ng/mL, respectively. The prevalence of vitamin D deficiency in these two groups was 28% (22 individuals) and 40% (31 individuals), respectively. However, there was no significant difference in the prevalence of osteoporosis and vitamin D deficiency between BPPV group and controls. Since the prevalence of osteoporosis and vitamin D deficiency is quite high in the general population, they thought that osteoporosis and vitamin D deficiency are not risk factors for BPPV. In our study, mean serum 25-OH vitamin D levels were 16.36 ng/mL (3.52-53.91) in the BPPV group and 17.09 ng/mL (4.46-53.51) in the control group. Vitamin D levels were low in both groups. There was no statistical difference between the groups. Lee et al. (4) evaluated the relationship between Ca^{+2} and vitamin D status, and the presence of BPPV formation and bone biochemical markers in 132 osteoporotic patients diagnosed with idiopathic BPPV. They divided patients into three groups according to bone mineral density (BMD). The incidence of vitamin D deficiency was 11.8% (4/34) in the normal BMD group, 15% (6/40) in the osteopenia group, and 43.1% (25/58) in the osteoporosis group. They found a positive correlation between 25-OH vitamin D and BMD results in BPPV patients. They reported that the prevalence of BPPV in osteoporotic patients is associated with vitamin D deficiency and systemically high bone turnover rates, and may impair local Ca^{+2} homeostasis in the inner ear. Han et al. (10) examined the BMD and serum 25-OH vitamin D levels of 80 postmenopausal women with BPPV and compared them with healthy volunteers. Decreased BMD was significantly higher in women with BPPV than in healthy controls (71.8% vs. 51.2%). Mean serum 25-OH vitamin D levels were also significantly lower in women with BPPV than in healthy controls (19.1±5.2 vs. 22.5±5.8, p<0.001). They thought that low 25-OH vitamin D might be a risk factor for BPPV in the postmenopausal period. Maslovara et al. (11) compared serum vitamin D vitamin levels in BPPV patients with and without recurrence, and found no significant difference. Most of the patients found low serum vitamin D level and recommended vitamin D for these patients. Vitamin D levels were significantly lower in patients with clinical canalithiasis than cupulolithiasis. In our study, 72% of cases had canalithiasis pathology and 28% had cupulolithiasis. There was no difference in vitamin D deficiency.

Talaat et al. (12) divided 80 patients (52 females, 28 males, mean age=47.6±9.1 years, range=31-71 years) with BPPV into two groups: 36 patients with primary and 44 patients with recurrent BPPV. The control group included 100 healthy volunteers with similar age and gender distribution. BMD and serum 25-OH vitamin D were measured. Vitamin D levels were significantly lower in the recurrence group (p<0.05). Studies that correlate BPPV and both vitamin D deficiency and low BMD suggest that these disorders should be investigated and treated in patients with recurrence. Büki et al. (13) measured 25-OH vitamin D levels in 18 BPPV patients and found it to be low. Four of these patients were recurrent cases with 4-6 attacks per year. Patients with idiopathic

positional vertigo had a low mean serum 25-OH vitamin D (23 ng/mL) levels similar to that of the general Austrian population at high rates. Patients with recurrences were given vitamin D and their serum levels were corrected. They were followed-up for 8 months and vertigo attacks did not recur after vitamin D supplementation. They suggest further epidemiological research to determine whether BPPV patients with low vitamin D levels may benefit from supplementation and the effect of correcting vitamin D deficiency on vertigo recurrence. Jeong et al. (14) measured 25-OH vitamin D serum levels in 100 patients (63 female and 37 male, mean age=61.8±11.6 years) with idiopathic BPPV and compared the data with 192 controls (101 female and 91 male, mean age=60.3±11.3 years). Serum 25-OH vitamin D level was lower in BPPV patients (14.4±8.4 vs. 19.1±6.8 ng/mL, p=0.001). Moreover, patients with BPPV showed a higher prevalence of lower serum 25-OH vitamin D (≤20 ng/mL, 80.0 vs. 60.1%, p<0.001) than controls. It was shown that there was a relationship between idiopathic BPPV and decreased serum 25-OH vitamin D. Decreased serum 25-OH vitamin D may be a risk factor for BPPV.

CONCLUSION

The relationship between idiopathic BPPV and vitamin D deficiency is controversial in the literature. As in our country, vitamin D deficiency is common in populations with short and variable sun exposure. We found low levels of vitamin D in both study and control groups. In this respect, further studies are needed to investigate the relationship between BPPV and vitamin D deficiency.

Ethics

Ethics Committee Approval: Ethics committee approval was received for this study from the local Ethics Committee of Okmeydanı Training and Research Hospital (dated: 6.11.2018, numbered: 48670771-514.10).

Informed Consent: Informed consent was obtained from the patients.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: B.T., Y.U., Design: B.T., Y.U., Data Collection or Processing: G.B., Ö.B.T., Analysis or Interpretation: S.K., F.A., O.Ü., Writing: B.T.

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

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

REFERENCES

1. von Breven M, Radtke A, Lezius F, Feldmann M, Ziese T, Lempert T, et al.Epidemiology of bening paroxysmal positional vertigo: a populationbased study. J Neurol Neurosurg Psychitry 2007;78:710-5.
2. Parnes LS, Agrawal SK, Atlas J. Diagnosis and management of benign paroxysmal positional vertigo (BPPV). CMAJ 2003;169:681-93.
3. Karataş A, Acar Yüceant G, Yüce T, Hacı C, Cebi IT, Salviz M. Association of Benign Paroxysmal Positional Vertigo with Osteoporosis and Vitamin D Deficiency: A Case Controlled Study. J Int Adv Otol 2017;13:259-65.
4. Lee SB, Lee CH, Kim YJ, Kim HM. Biochemical markers of bone turnover in benign paroxysmal positional vertigo. PLoS One 2017;12:e0176011.
5. Jang YS, Kang MK. Relationship between bone mineral density and clinical features in women with idiopathic benign paroxysmal positional vertigo. Otol Neurotol 2009;30:95-100.
6. Tanaka Y, Asanuma A, Yanagisawa K. Potentials of outer hair cells and their membrane properties in cationic environments. Hear Res 1980;2:431-8.
7. Ohmori H. Mechano-electrical transduction currents in isolated vestibular hair cells of the chick. J Physiol 1985;359:189-217.
8. Brookes GB. Vitamin D deficiency--a new cause of cochlear deafness. J Laryngol Otol 1983;97:405-20.
9. Yamauchi D, Raveendran NN, Pondugula SR, Kampalli SB, Sanneman JD, Harbidge DG, et al. Vitamin D upregulates expression of ECaC1 mRNA in semicircular canal. Biochem Biophys Res Commun 2005;33:1353-7.
10. Han W, Fan Z, Zhou M, Guo X, Yan W, Lu X, et al. Low 25-hydroxyvitamin D levels in postmenopausal female patients with benign paroxysmal positional vertigo. Acta Otolaryngol 2018;138:443-6.
11. Maslovara S, Butkovic Soldo S, Sestak A, Milinkovic K, Rogic-Namacinski J, Soldo A. 25 (OH) D3 levels, incidence and recurrence of different clinical forms of benig paroxysmal positional vertigo. Braz J Otorhinolaryngol. 2018;84:453-9.
12. Talaat HS, Abuhadied G, Talaat AS, Abdelaal MS. Low bone mineral density and vitamin D deficiency in patients with benign positional paroxysmal vertigo. Eur Arch Otorhinolaryngol 2015;272:2249-53.
13. Büki B, Ecker M, Jünger H, Lundberg YW. Vitamin D deficiency and benign paroxysmal positioning vertigo. Med Hypotheses 2013;80:201-4.
14. Jeong SH, Kim JS, Shin JW, Kim S, Lee H, Lee AY, et al. Decreased serum vitamin D in idiopathic benign paroxysmal positional vertigo. J Neurol 2013;260:832-8.

CASE REPORT

DOI: 10.4274/eamr.galenos.2018.57778
Eur Arch Med Res 2019; 35 (4):249-52

Primary Cutaneous Anaplastic Large Cell Lymphoma: A Case Report

Sinem Ayşe Örnek[1], Şule Güngör[1], Ömer Özdemir[1], Tuğrul Elverdi[2], Özben Yalçın[3], Şeyma Sinem Özbey[3], Emek Kocatürk[1]

[1]İstanbul Okmeydanı Training and Research Hospital, Clinic of Dermatology, İstanbul, Turkey
[2]İstanbul Okmeydanı Training and Research Hospital, Clinic of Hematology, İstanbul, Turkey
[3]İstanbul Okmeydanı Training and Research Hospital, Clinic of Pathology, İstanbul, Turkey

Abstract

Primary cutaneous anaplastic large cell lymphoma (PCALCL) is a rare cutaneous CD30 + T cell lymphoproliferative disorder. PCALCL usually presents as solitary and localized nodules and tumors, or may be multiple and ulcerated. Extracutaneous involvement may occur rarely. Systemic anaplastic large cell lymphoma with secondary cutaneous involvement has a similar clinical appearance to PCALCL, but differs in treatment and prognosis. Thus, systemic evaluation of patients considered as PCALCL is critical. An 82-year-old male patient presented with three progressive lesions on the scalp. The final diagnosis was PCALCL with extracutaneous involvement based upon the involvement of regional lymph nodes, liver and spleen, lack of systemic B symptoms and the results of histopathological and immunophenotypical studies. This case is presented to emphasize the necessity of systemic evaluation in patients considered as PCALCL and to remind how to differentiate PCALCL from systemic lymphomas.

Keywords: Primary cutaneous anaplastic large cell lymphoma, cutaneous lymphoma, CD30 positivity

INTRODUCTION

Primary cutaneous anaplastic large cell lymphoma (PCALCL) is a rare CD30 + T cell lymphoproliferative disease of the skin (1). PCALCL is clinically seen as one or more ulcerable nodules and tumors (2). Rarely, extracutaneous involvement may occur (3). Cutaneous involvement secondary to systemic anaplastic large cell lymphoma is clinically similar to PCALCL, but treatment and prognosis vary (4). Therefore, it is important to investigate systemic disease in patients with PCALCL. The diagnosis of PCALCL is usually made by clinical findings, histopathological and immunophenotypical examination and the absence of systemic disease (5).

CASE REPORT

An 82-year-old male patient presented to our clinic with three lesions on the scalp that grew and became a mass. The history revealed that the first lesion had occurred one year ago and had occasional bleeding; the other two had developed in the last 2 months and had no symptoms. Dermatological examination revealed a 3x3x1.5 cm sized, hard, brown-red-colored nodular lesion with hemorrhagic crust and yellow squama on the right frontoparietal region. Two 1.5x1.5x0.5 cm sized, brown-red-colored, nodular lesions were observed on bilateral posterior parietal regions (Figures 1, 2). He did not have weight loss, night sweats, decreased appetite, and fever. Histopathological examination revealed a large infiltrate of atypical lymphocytes in the dermis. Immunohistochemically, cells were positive for CD30, CD4, CD3, CD45 RO, MUM1, and negative for CD20, anaplastic lymphoma kinase (ALK), epithelial membrane antigen (EMA), Melan A, S100, BCL6, glial fibrillary acidic protein and Epstein-Barr virus. The KI 67 proliferation index was 90% (Figures 3-9). Laboratory tests and thoracic computed tomography (CT) evaluating systemic involvement were normal. Abdomen CT examination revealed a 10 mm hypodense nodule in the left and right lobes of the liver and 1 mm in the medial spleen.

Address for Correspondence: Sinem Ayşe Örnek, İstanbul Okmeydanı Training and Research Hospital, Clinic of Dermatology, İstanbul, Turkey
Phone: +90 537 510 40 30 E-mail: drsinemornek@yahoo.com ORCID ID: orcid.org/0000-0002-1536-6035

Cite this article as: Örnek SA, Güngör Ş, Özdemir Ö, Elverdi T, Yalçın Ö, Özbey ŞS, Kocatürk E. Primary Cutaneous Anaplastic Large Cell Lymphoma: A Case Report. Eur Arch Med Res 2019; 35 (4): 249-52

Received: 05.01.2018
Accepted: 19.09.2018

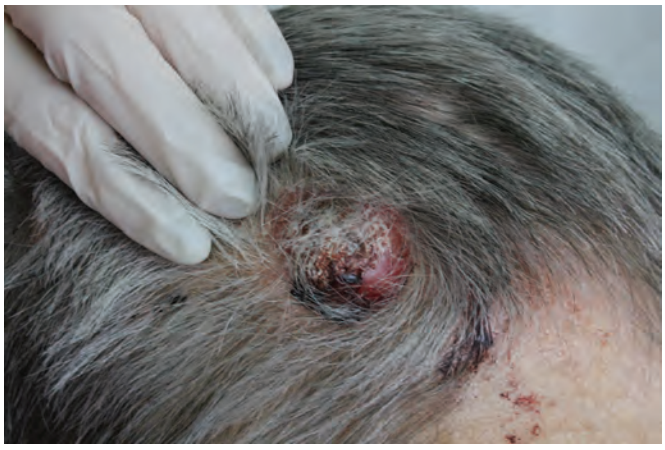


Figure 1. A 3x3x1.5 cm sized, hard, brown-red nodular lesion with hemorrhagic crust and yellow squama on the right frontoparietal region

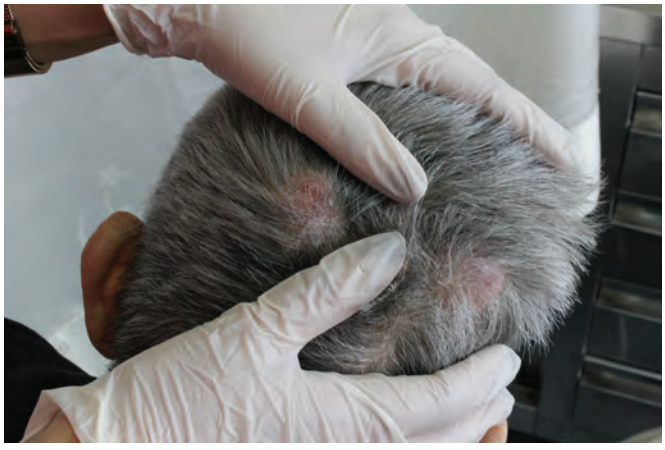


Figure 2. Two 1.5x1.5x0.5 cm sized, brown-red nodular lesions with a slight squama on the posterior of the bilateral parietal region

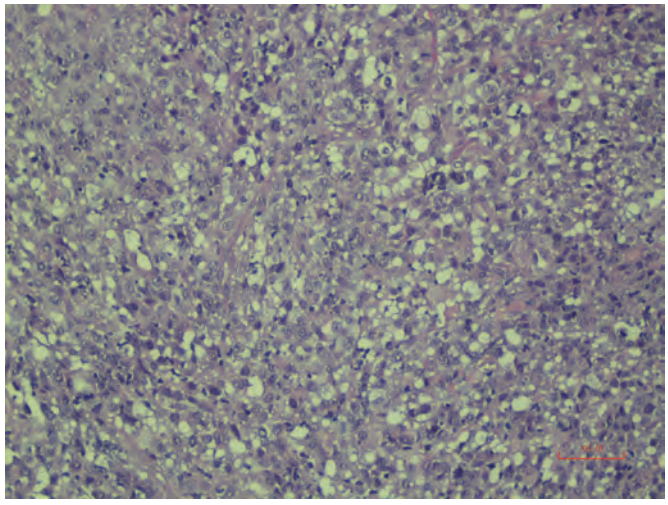


Figure 3. Infiltrate of large atypical lymphocytes in the dermis (hematoxylin and eosin, x100)

Positron emission tomography examination showed increased F-18 fluorodeoxyglucose uptake in localized sites that matched the skin-subcutaneous lesions on the scalp, left posterior cervical lymph nodes in the neck, and in the liver and spleen that matched the hypodense nodules seen on CT. As the lesion was immunohistochemically negative for ALK and EMA, the patient was diagnosed as PCALCL with extracutaneous involvement

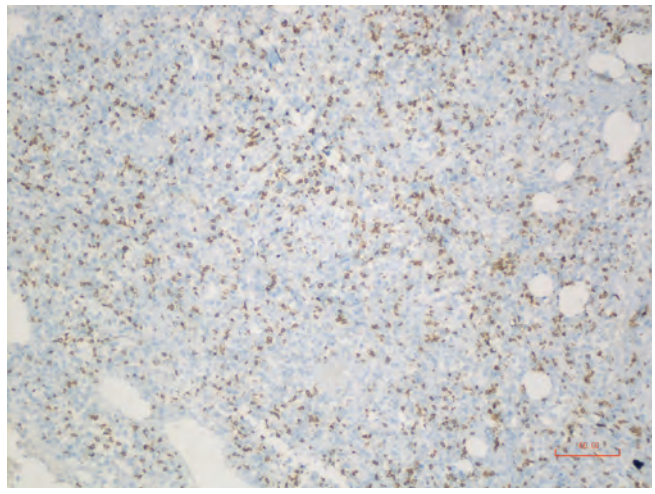


Figure 4. Positive staining with CD3

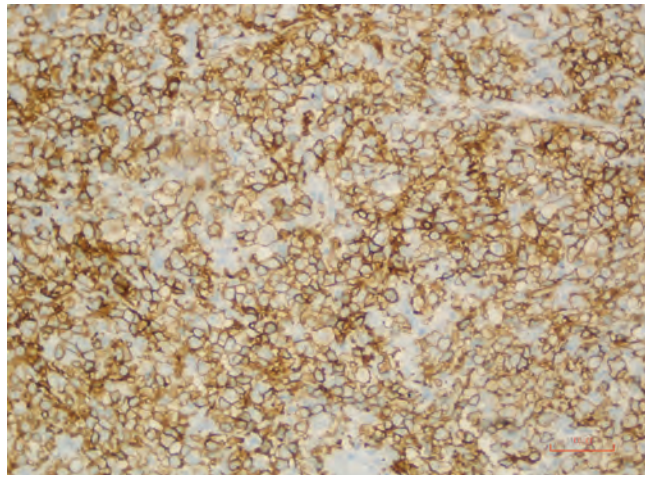


Figure 5. Positive staining with CD30

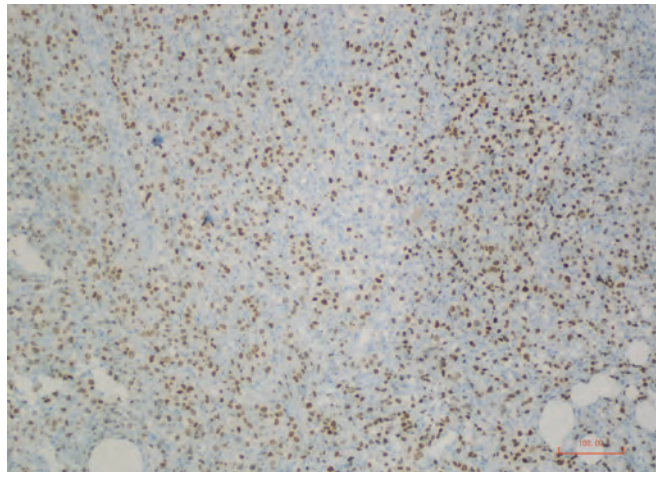


Figure 6. Positive staining with MUM1

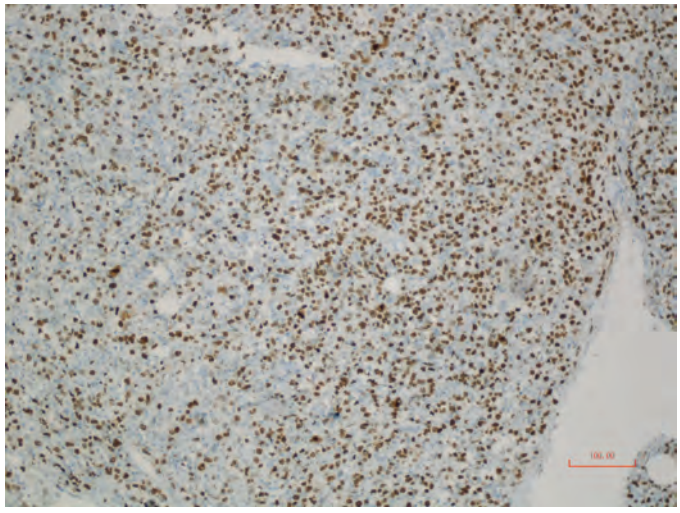


Figure 7. 90% Ki67 proliferation index

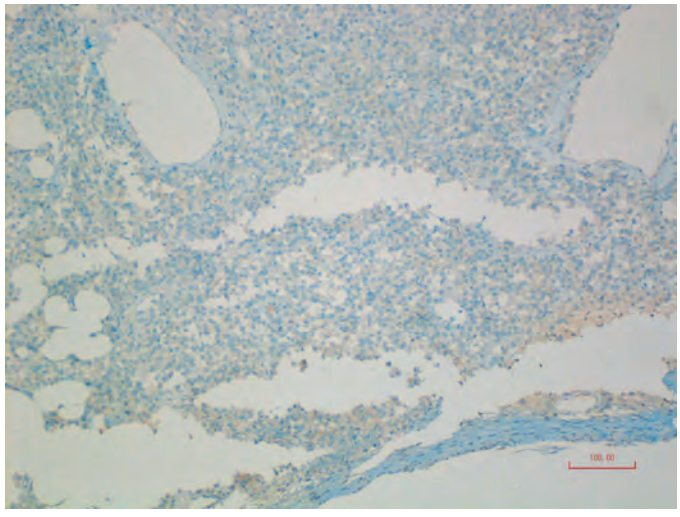


Figure 8. Negative staining with anaplastic lymphoma kinase

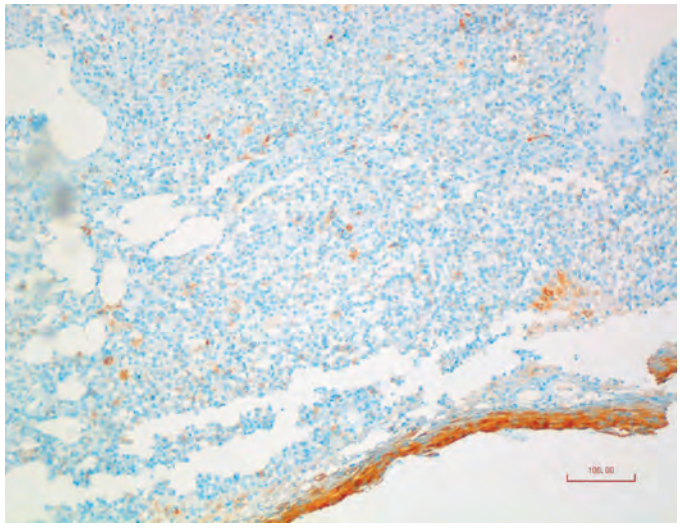


Figure 9. Negative staining with epithelial membrane antigen

DISCUSSION

Primary cutaneous lymphomas are a heterogeneous group of diseases consisting of extranodal non-Hodgkin's lymphomas that present in the skin. Unlike nodal non-Hodgkin lymphomas, 75% of primary cutaneous lymphomas are of T lymphocyte origin and the majority are mycosis fungoides and Sezary syndrome. This is followed by lymphoid papulosis and that present in the skin as CD30 + T cell lymphomas (6). PCALCL accounts for 10% of all cutaneous T cell lymphomas. It is usually seen after the 6th decade and is common in men. The most common site of involvement is the head and extremities. Clinically, it is seen as one or more nodules and tumors that can ulcerate. The etiology is unknown. Unlike systemic ALCL, it has a good prognosis and spontaneous regression can be seen (7). Rarely, extracutaneous dissemination may occur and involves regional lymph nodes and viscera (6). The diagnosis of PCALCL is made by clinical findings, histopathological and immunophenotypical examination and imaging methods (5). In histopathological examination, CD4+ T lymphocytes with CD30 + anaplastic morphology form non-epidermotropic diffuse infiltrate in the dermis. Mitosis rate is high. Ulcerated lesions may be accompanied by inflammatory infiltrates of reactive lymphocytes, histiocytes, eosinophils and neutrophils (6). Immunohistochemical studies are important in distinguishing between primary and secondary lesions that are clinically and histopathologically similar. Unlike systemic CD30 + lymphomas, PCALCL is not stained with EMA and ALK immunostaining (8). Imaging techniques are necessary both for staging the disease and for distinguishing between primary and secondary. In order to diagnose PCALCL, it should be shown that there is no systemic involvement (7). In our case, there were regional lymph nodes, liver and spleen involvement as well as skin involvement at the time of diagnosis. It could not be determined that the involvement of lymph nodes and viscera were before or after the cutaneous lesions, since there was no previous examination. However, the patient was diagnosed as PCALCL with extracutaneous involvement due to lack of B symptoms (fever, night sweats, weight loss) which are suggestive of systemic lymphoma and immunohistochemical studies showing negative ALK and EMA staining. Surgical excision and radiotherapy are the treatment options for localized PCALCL cases. In case of extracutaneous involvement, systemic multiple chemotherapy is applied (9). Recent studies have reported cases of complete remission with brentuximab vedotin, an anti-CD30 monoclonal antibody (10). We did not have the opportunity to initiate any treatment because our patient died one month after the diagnosis.

PCALCL has a better prognosis than systemic lymphomas. Ten-year survival has been reported to be 90% (8). However, the presence of widespread skin lesions, muscle and deep fascia involvement, the occurrence of lesions in the leg and extracutaneous involvement reduces survival (11-13). In the study of Benner and Willemze (3), 14.8% of 135 patients with PCALCL showed extracutaneous invasion. It was reported that 70% of the patients were alive and in remission, 6% were alive and under treatment, 8.9% died due to lymphoma and 14% died due to another cause. In the study of Hapgood et al. (14) 15% of 47 PCALCL cases showed extracutaneous spread. It was reported that 57% of the patients were alive and in remission, 13% were alive and under treatment, 9% died due to lymphoma and 21% died due to another cause. In another study by Benner and Willemze (15), 5-year survival rates of patients with and without lymph node and visceral organ involvement were reported as 51% and 80%, respectively. In conclusion, since treatment and prognosis differ by origin, staging is important in patients with PCALCL and systemic and primary cutaneous lymphoma should be differentiated by clinical findings, imaging methods, histopathological and immunophenotypical examination.

Ethics

Informed Consent: Writing concept of the patient was received.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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

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

REFERENCES

1. Swerdlow SH, Campo E, Harris NL, Jaffe ES, Stein H, Thiele J,Vardiman J. WHO classification of tumours of haematopoietic and lymphoid tissue. 4th ed. Lyon: IARC Press; 2008
2. Bekkenk MW, Geelen FA, van Voorst Vader PC, Heule F, Geerts ML van Vloten WA, et al. Primary and secondary cutaneous CD30 (+) lymphoproliferative disorders: a report from the Dutch Cutaneous Lymphoma Group on the long-term follow-up data of 219 patients and guidelines for diagnosis and treatment. Blood 2000;95:3653-61.
3. Benner MF and Willemze R. Applicability and prognostic value of the New TNM Classification System in 135 patients with Primary Cutaneous Anaplastic Large Cell Lymphoma. Arch Dermatol 2009;145:1399-404.
4. Willemze R, Hodak E, Zinzani PL, Specht L, Ladetto M. ESMO Guidelines Working Group. Primary cutaneous lymphomas: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. Ann Oncol 2013;24:149-54.
5. Brown RA, Fernandez-Pol S, Kim J. Primary cutaneous anaplastic large cell lymphoma. J Cutan Pathol 2017;44:570-7.
6. Willemze R, Jaffe ES, Burg G, Cerroni L, Berti E, Swerdlow SH, et al. WHO-EORTC classification for cutaneous lymphomas. Blood 2005;105:3768-85.
7. Rao SD, Ravi R, Govindarajan M. Primary cutaneous CD 30+ anaplastic large cell lymphoma. Indian J Surg 2010;72:283-5.
8. Kempf W, Willemze R, Jaffe ES, Burg G, Kadin ME. CD30+ T-cell lymphoproliferative disorders. In: LeBoit PE, Burg G, Weedon D, Sarasin A, eds. WHO/IARC classification of tumours. Pathology and genetics of skin tumours. Lyon: IARC Press; 2006;179-81.
9. Liu HL, Hoppe RT, Kohler S, Harvell JD, Reddy S, Kim YH. CD30+ cutaneous lymphoproliferative disorders: the Stanford experience in lymphomatoid papulosis and primary cutaneous anaplastic large cell lymphoma. J Am Acad Dermatol 2003;49:1049-58.
10. Desai A, Telang GH, Olszewski AJ. Remission of primary cutaneous anaplastic large cell lymphoma after a brief course of brentuximab vedotin. Ann Hematol 2013;92:567-8.
11. Sugaya M, Fujita H, Izutsu K, Oshima K, Takazawa Y, Ohmatsu H, et al. Primary cutaneous anaplastic large cell lymphoma with leg involvement: a case report and review of 11 cases. J Dermatol 2011;38:1009-12.
12. Woo DK, Jones CR, Vanoli-Storz MN, Kohler S, Reddy S, Advani R, et al. Prognostic factors in primary cutaneous anaplastic large cell lymphoma: characterization of clinical subset with worse outcome. Arch Dermatol 2009;145:667-74.
13. Lee WJ, Moon IJ, Lee SH, Won CH, Chang SE, Choi JH, et al. Cutaneous anaplastic large-cell lymphoma (ALCL): A comparative clinical feature and survival outcome analysis of 52 cases according to primary tumor site. J Am Acad Dermatol 2016;74:1135-43.
14. Hapgood G, Pickles T, Sehn LH, Villa D, Klasa R, Scott DW, et al. Outcome of primary cutaneous anaplastic large cell lymphoma: a 20-year British Columbia Cancer Agency experience. Br J Haematol 2017;176:234-40.
15. Benner MF and Willemze R. Bone marrow examination has limited value in the staging of patients with an anaplastic large cell lymphoma first presenting in the skin. Retrospective analysis of 107 patients. Br J Dermatol 2008;159:1148-51.

CASE REPORT

DOI: 10.4274/eamr.galenos.2018.23600
Eur Arch Med Res 2019; 35 (4):253-6

Cleidocranial Dysplasia: A New Mutation

Nimet Pınar Yılmazbaş[1], Diğdem Bezen[1], Biray Ertürk[2]

[1]İstanbul Okmeydanı Training and Research Hospital, Clinic of Pediatrics, İstanbul, Turkey
[2]İstanbul Okmeydanı Training and Research Hospital, Clinic of Medical Genetics, İstanbul, Turkey

Abstract

Cleidocranial dysplasia is a rare disease with mostly autosomal dominant inheritance. *De novo* mutations are rare and the disease is characterized by generalized dysplasia of bone tissue. Delay in closure of cranial sutures and fontanels, short stature, dental anomalies, hypoplasic or aplasic clavicles and some other bone anomalies are seen clinically. A 9.5 month old infant who presented with anterior and posterior fontanelle enlargement and separated sagittal suture is presented in this paper.

Keywords: Cleidocranial dysplasia, enlarged fontanels, relative macrocephaly

INTRODUCTION

Cleidocranial dysplasia (CCD) is an autosomal dominant disorder with rare *de novo* mutations and with an incidence of 1/1000000. It is generally seen as a result of mutations in the *RUNX2* gene in chromosome 6p21 that regulates normal osteoblastic differentiation and appropriate bone formation (1,2). In such bone dysplasia, flat bones such as head bones, clavicle, and teeth are affected. Short stature, frontal and parietal bossing, seperated cranial sutures, late closure of fontanels, hypoplasia or aplasia of clavicles, maxilla hypoplasia, hypoplasia of iliac wings and brachydactyly can be seen. Although the eruption time of primary teeth is normal, early tooth loss may accompany in adulthood (1,3). In affected cases, the clavicles may not be present partially or completely. Therefore, increased opposition at the shoulders is a specific finding for this disease (4). The diagnosis is confirmed by clinical findings, radiological examinations and gene sequence analysis. Cases should be followed for orthopedic complications, dental anomalies, upper respiratory tract obstruction, sinus and ear infections, hearing loss, osteoporosis and osteopenia. There is no specific treatment for CCD. Bone deformities can be operated after 5 years of age and orthodontic intervention may be required due to tooth loss (5).

CASE REPORT

Written consent was obtained from the mother of the patient to present the case.

A 9.5 month old girl was admitted to the outpatient clinic with enlarged anterior and posterior fontanels and separated sagittal suture. The patient was the first child of a 24 year old mother and a 36 year old father from a non-consanguineous marriage. Birth weight was 3150 g [25-50% percentile, standard deviation score (SDS): -0.51], birth height was 49 cm (25-50% percentile, SDS: -0.12%) and birth head circumference was 35 cm (50th percentile, SDS: 0.18). Her family history revealed short stature, early tooth loss, and similar facial dimorphism in her mother, uncle, two male cousins and her grandmother, but no cases were diagnosed. On physical examination, body weight was 8000 g (25-50% p, SDS: -0.74), height was 69 cm (10-25% p, SDS: -0.55), head circumference was 45.5 cm (50-75% p, SDS: 1.05), weight by height was 99% (SDS: -0.07), anterior fontanel was 4x5 cm and posterior fontanel was 2x2 cm, metopic-coronal and sagittal sutures were separated, and there were flattened nose and frontal bossing. The patient had two primary teeth and the other system examinations were normal (Figure 1). In terms of neuromotor development, the patient achieved head control at 3 months, supported sitting at 5 months and unsupported

Address for Correspondence: Nimet Pınar Yılmazbaş, İstanbul Okmeydanı Training and Research Hospital, Clinic of Pediatrics, İstanbul, Turkey
Phone: +90 535 321 74 33 E-mail: drpinary@yahoo.com ORCID ID: orcid.org/0000-0002-1283-1712

Received: 11.06.2018
Accepted: 05.10.2018

Cite this article as: Yılmazbaş NP, Bezen D, Ertürk B. Cleidocranial Dysplasia: A New Mutation. Eur Arch Med Res 2019; 35 (4): 253-6

sitting at 8 months. Our case erupted her first primary tooth at the age of 6 months and started to spell at 8 months. Laboratory tests were within normal limits as follows: thyroid stimulating hormone (TSH): 2.75 microIU/mL (0.73-8.35 microIU/mL), free T4: 1.26 ng/dL (0.92-1.99), calcium (Ca): 10.64 mg/dL (9-11 mg/dL), phosphorus (P): 6.2 mg/dL (4.5-6.7 mg/dL), parathyroid hormone (PTH): 21.52 pg/mL (3.6-32 pg/mL), and 25 (OH) vitamin D: 32.9/g/L (20-50/g/L). The alkaline phosphatase level of our case was 97 U/L and this value was close to the lower limit according to age (n: 70-1107 U/L). On the direct X-ray, sagittal suture and posterior fontanel were open, anterior fontanel was large for age, clavicle development was normal and bone age was consistent with 6 months (Figure 2). On cranial ultrasound examination, the ventricular horn index was within normal limits, lateral ventricular contours were smooth, the 3rd and 4th ventricles were normal in size, localization and configuration, and cerebral-cerebellar hemispheres, basal ganglia, intra- and periventricular sites were in normal sonographic appearance. On physical examination of the mother of our case, height was 148 cm, weight was 64 kilogram, and head circumference was 61 cm. She had frontal bossing with 0.5 cm open anterior fontanelle and accompanying unilateral hip dysplasia. The mother had increased opposition of shoulders and was able to connect both shoulders to the midline. Based on these findings, a diagnosis of bone dysplasia was considered in the mother. Cranial ultrasonography was reported to be normal when we looked at the causes of large fontanelle, and no lesion causing intracranial pressure increase was detected and this diagnosis was ruled out. Ca, P, PTH and 25 (OH) vitamin D levels were normal, so we ruled out the diagnosis of rickets. In our case, normal TSH and free T4 levels allowed us to rule out hypothyroidism that may cause large fontanelle. Hypophosphatasia, a rare hereditary disease associated with bone and dental mineral deficiency accompanied by low or normal autosomal recessive alkaline phosphatase, normal or high serum calcium and phosphate levels, is a disease that causes neuromotor growth retardation in the early period, and the neuromotor development of our case was normal according to the month and the tone was normal, so we ruled out this diagnosis (6,7).

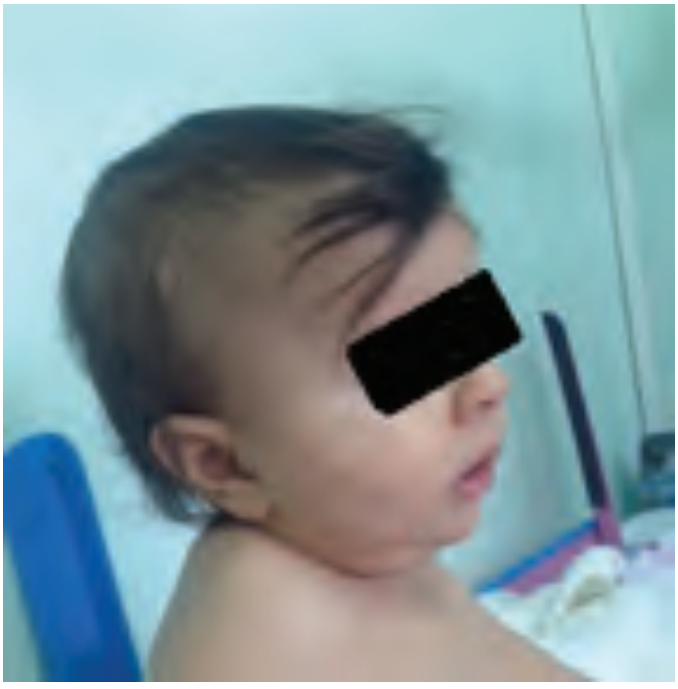


Figure 1. Side view of brachiocephalic head of case

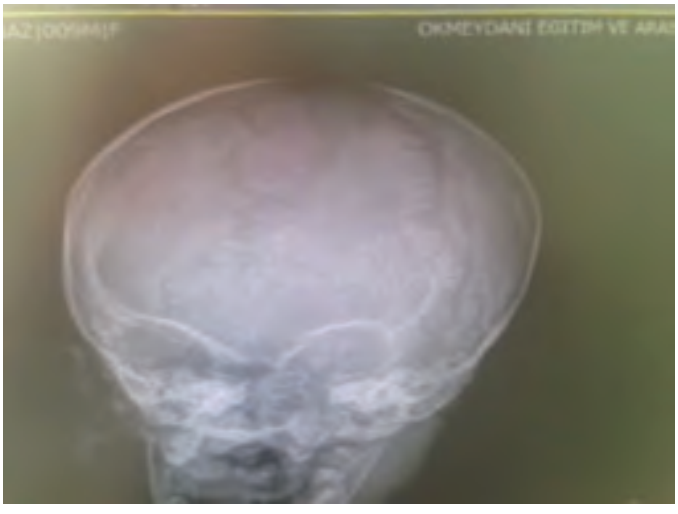


Figure 2. View of enlarged fontanels and sutures on anteroposterior posterior radiograph of the case

The present findings and family history of our case were suggestive of bone dysplasias. The increased opposition of shoulders in mother was specific for the diagnosis of CCD and *RUNX2* gene mutation was sent from case and mother for definitive diagnosis. As a result of the sequence analysis, heterozygous c.1281delC (p.Gly428Alafs*56) mutation was detected in exon 8 in our case and mother, which was not previously defined. After the genetic diagnosis was confirmed, our case was consulted and followed-up with orthopedics and neurosurgery clinics. In addition, genetic counseling was provided to all affected cases in the family and thus orthodontic and orthopedic problems were intervened.

DISCUSSION

CCD is a genetic disease that occurs as a result of a mutation in the *RUNX2* gene, which is involved in osteoblastic differentiation and function, with an incidence of 1/1000000. Flat bone anomalies such as head bones and clavicle, and tooth

anomalies are present in each case. Short stature, frontal and parietal bossing, open cranial sutures, late closure of fontanels, hypoplasia or aplasia of clavicles, maxilla hypoplasia, hypoplasia of iliac wings, brachydactyly and early dental loss are the accompanying clinical conditions. Our case was admitted to our hospital because of an enlarged anterior and posterior fontanelle and he was admitted to another tertiary training and research center before his admission to our hospital; however, he could not be diagnosed as his laboratory findings and neuromotor development were normal. Hypothyroidism and rickets can cause enlarged fontanels in an infant of this age, but normal biochemical tests ruled out these diagnoses. In our case with normal cranial ultrasonography findings, diseases that could cause increased intracranial pressure were also ruled out. The serum alkaline phosphatase level of our case was at the lower limit and suggested the diagnosis of hypophosphatasia. While infantile hypophosphatasia is a disease with fontanel patency, bone mineralization disorder and low alkaline phosphatase in early infancy, it also causes neuromotor developmental retardation in the early period; however, normal neuromotor development, lack of hypotonicity and alkaline phosphatase level being not as low as in hypophosphatasia ruled out the diagnosis in our case. The presence of similar cases in the family of our case indicated a genetic disease. The presence of unilateral developmental dysplasia of the hip, open anterior fontanelle, increased opposition of the shoulders and accompanying tooth loss in the mother made us think of the diagnosis of CCD. Diagnosis was confirmed by gene analysis and a new mutation was detected. Since the majority of craniofacial anomalies are prominent in adolescence in this disease, the number of cases diagnosed during infancy is low (8-10). The mean age at diagnosis was reported to be 18.3 years in the study by Golan et al. (11) including 24 CCD cases. Our case is an early-diagnosed case and early diagnosis is important for endocrinological, orthopedic and orthodontic follow-ups and early interventions when necessary. In previous series, shortness of clavicle and increased opposition in CCD were reported to accompany 88% of cases (11). In the mother of our case, there was increased opposition in shoulder movements, but the clavicle size was normal on the bone radiographs of our case and there was no increased opposition movement. As indicated in previous publications, this shows us that CCD can cause different clinical presentations among the same family members (12).

When the causes of fontanel patency are listed, bone dysplasia is a rare disease and diagnosis is therefore delayed. However, simple laboratory tests and radiological examinations can easily exclude other possible diagnoses. If the findings of other members of the family are carefully recorded, bone dysplasia should be considered and the diagnosis should be easier. With this case, we wanted to draw attention to the early findings of CCD and to emphasize the importance of early diagnosis.

Many patients are examined in healthy pediatric outpatient clinics due to the relative macrocephaly and enlarged fontanelle. Bone dysplasias should be considered when evaluating these patients, but these cases are not always diagnosed because they are rare. In this case report, we aimed to raise awareness of the rarely seen CCD and to emphasize the importance of early diagnosis.

Ethics

Informed Consent: Was taken.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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

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

REFERENCES

1. Mundlos S. Cleidocranial dysplasia: clinical and molecular genetics. J Med Genet 1999;36:177-82.
2. Ott CE, Leschik G, Trotier F, Brueton L, Brunner HG, Brussel W, et al. Deletions of the RUNX2 gene are present in about 10% of individuals with cleidocranial dysplasia. Hum Mutat 2010;31:E1587-E93.
3. Turek's Orthopaedics: Principles And Their Application. Lippincott Williams and Wilkins 2005.pp.251-2.
4. John J. Paul and Juhl's essentials of radiologic imaging, 7th ed. Lippincott-Raven 1998.
5. Pediatric Orthopedics in Practice. Springer 2007.p.478.
6. Soto E, Richani K, Gonçalves LF, Devers P, Espinoza J, Lee W, et al. Three-dimensional ultrasound in the prenatal diagnosis of cleiodocranial dysplasiz associated with B-cell immundeficiency. Ultrasound Obstet Gynecol 2006;27:574-9.
7. Sağlam H, Erdöl Ş. Hipofosfatazya. Turkiye Klinikleri J Med Genet-Special Topics 2016;1:87-92.
8. Suba Z, Balaton G, Gyulai-Gaal S, Balaton P, Barabas J, Tarjan I. Cleidocranisl dysplasia: diagnostic criteria and combined treatment. J Craniofac Surg 2005;16:11226.
9. Ringe KI, Schirg E, Galanski M. Cleidocranial dysplazia (CCD) causing respiratory distress syndrome in a newborn infant. A case report. J Radiol Case Rep 2010;4:9-12.

10. Stewart PA, Wallerstein R, Moran E, Lee MJ. Early prenatal ultrasound diagnosis of cleidocranial dysplasia. Ultrasound Obstet Gynecol 2000;15:154-6.
11. Golan I, Baumert U, Hrala BP, Schaumburger J, Wiech O, Grifka J, et al. Symptoms and signs in cleidocranial dysplasia (CCD) Z Orthop Ihre Grenzgeb 2003;141:336-40.
12. Cavalli P, Santorelli FM, Bontardelli M, Tessa A, Bosi A, Poggiani C. Prenatal exclusion of cleidocranial dysplasia Prenat Diagn 2003;23:945-6.

CASE REPORT

DOI: 10.4274/eamr.galenos.2019.18189
Eur Arch Med Res 2019; 35 (4):257-9

Kounis Syndrome as a Result of Anaphylactic Reaction to Gold Dust: A Case Report

Öner Bozan, Asım Kalkan, Şeref Emre Atiş, Bora Çekmen, Adnan Türk

İstanbul Okmeydanı Training and Research Hospital, Clinic of Emergency Medicine, İstanbul, Turkey

Abstract

Kounis syndrome (KS) is described as the provocation of an acute coronary syndrome through activation of mast cells resulting in allergy, hypersensitivity, anaphylaxis, or anaphylactoid reaction. The case is here presented of a patient who developed KS with retrosternal chest pain and ST-segment elevation myocardial infarction 30 minutes after the ingestion of gold dust. A 40-year-old male without a history of allergy or atopy was admitted to the emergency room with complaints of red, itchy skin, chest pain, and shortness of breath 30 minutes after exposure to gold dust. Arterial blood pressure was 129/72 mmHg, pulse rate was 94 bpm, and oxygen saturation was 98%. On physical examination, urticarial lesions were observed on the anterior chest wall without uvula edema and pulmonary bronchospasm. Electrocardiogram (ECG) showed ST elevation in the V1-V6 and aVL leads. High sensitivity Troponin I level was measured as 12.1 ng/L (reference=0-19.8 ng/L), and the Troponin I levels increased to 229.7 ng/L two hours later. KS was considered because of the findings of chest pain, urticaria on the body, and lack of cardiac disorder. KS should be kept in mind in cases that present with allergic reactions together with chest pain following exposure to agents to which the immune system may be allergic. Cardiac enzymes can either be normal or elevated in these patients. ECG usually has ST, and the T wave changes.

Keywords: Kounis syndrome, anaphylactic reaction, gold dust

INTRODUCTION

Kounis syndrome (KS) is described as the provocation of an acute coronary syndrome (ACS) by the activation of mast cells resulting in allergy, hypersensitivity, anaphylaxis, or an anaphylactoid reaction. Drugs, food, environmental factors (insect bite, bee sting, pollens, latex contact), and intracoronary stent placement can be there as on which trigger the allergic reaction (1). In this paper, the development of KS with retrosternal chest pain and ST segment elevation myocardial infarction after 30 minutes following the ingestion of gold dust exposure is discussed.

CASE REPORT

A 40-year-old male without a history of allergy or atopy was admitted to the emergency room with complaints of red, itchy skin, chest pain, and shortness of breath 30 minutes after exposure to gold dust. Arterial blood pressure was 129/72 mmHg, pulse rate 94 was bmp, and oxygen saturation was 98%. On physical examination, urticarial lesions were found on the patient's anterior chest wall without uvula edema and pulmonary bronchospasm (Figure 1). His electrocardiogram (ECG) showed ST elevation in V1-V6 leads (Figure 2). High sensitivity Troponin I level was measured as 12.1 ng/L (reference=0-19.8 ng/L), and the Troponin I levels increased to 229.7 ng/L two hours later. KS was considered because of the findings of chest pain, urticaria on the body, and lack of cardiac disorder. The patient was consulted with cardiology. Echocardiography showed segmental cardiac wall abnormalities with 50% ejection fraction. Coronary angiography revealed no sign of atherosclerosis. The patient was diagnosed to have a KS type 1 variant, secondary to gold dust ingestion.

DISCUSSION

Allergic events can cause not only angina episodes but also acute myocardial infarction (2,3). KS was first described in 1991 and has a clinical spectrum ranging from chest pain with an acute or chronic allergic reaction to acute myocardial infarction (1).

Address for Correspondence: Öner Bozan, İstanbul Okmeydanı Training and Research Hospital, Clinic of Emergency Medicine, İstanbul, Turkey
Phone: +90 505 512 15 00 E-mail: onerbozan@gmail.com ORCID ID: orcid.org/0000-0002-4195-2601

Received: 06.03.2019
Accepted: 31.05.2019

Cite this article as: Bozan Ö, Kalkan A, Atiş ŞE, Çekmen B, Türk A. Kounis Syndrome as a Result of Anaphylactic Reaction to Gold Dust: A Case Report. Eur Arch Med Res 2019; 35 (4): 257-9

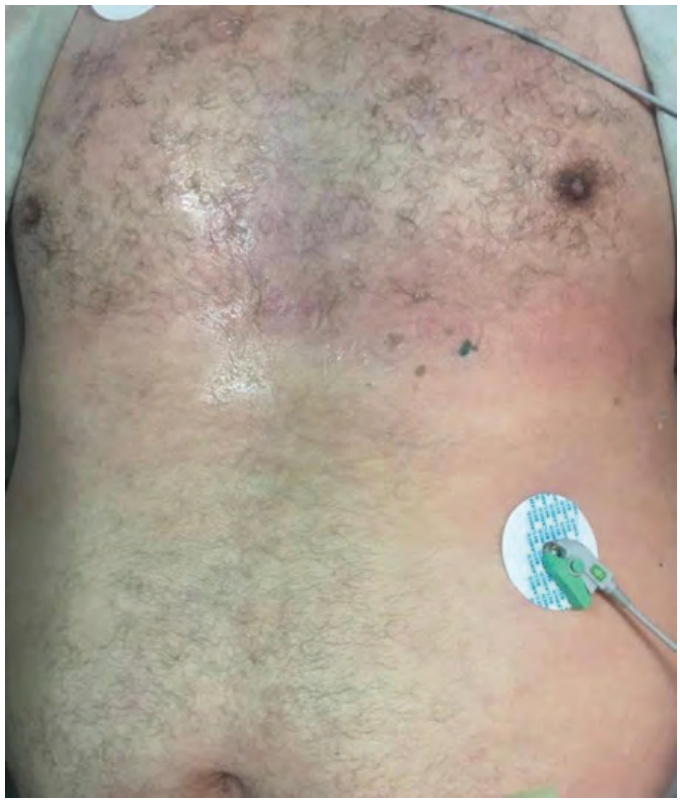


Figure 1. Patient's rush on anterior thorax and abdomen

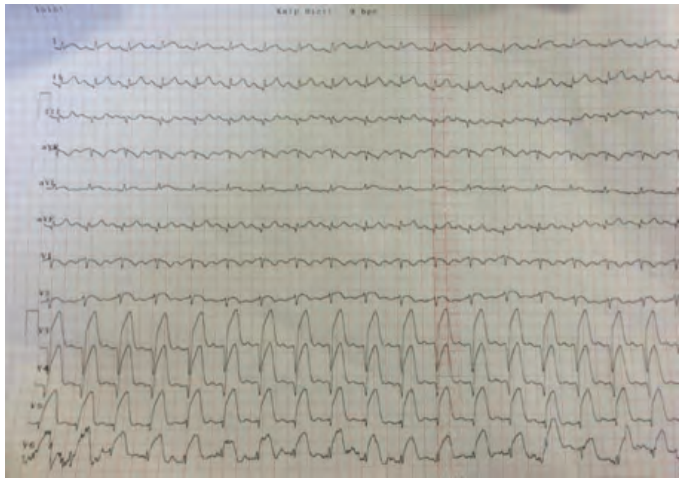


Figure 2. Patient's initial electrocardiogram when admitted to hospital

Vasospasm of the coronary arteries has been suggested to be the main pathophysiologic mechanism (4). In 1998, Braunwald (5) reported that allergic reactions could induce vasospastic angina with mediators such as histamine and leukotrienes acting on coronary vascular smooth muscle. Two types of KS have been described (6). In type 1 variant, patients have normal coronary arteries. An acute allergic event induces coronary artery spasm, resulting in chest pain and ischemic electrocardiographic changes. Cardiac enzymes can either be normal or elevated, which reflects progression to an acute myocardial infarction (2). The mechanism responsible for this type would be endothelial dysfunction or microvascular angina (7). In type 2, patients have underlying coronary artery disease, and chest pain occurs during an acute allergic reaction (8). An acute allergic episode can induce plaque erosion or rupture manifesting as an acute myocardial infarction (2,9). We know that several antibiotics have been associated with allergic reactions and KS. An ACS with ST elevation after exposure to amoxicillin was reported by Vivas et al. (3). In the present case, the allergic reactions resulting in chest pain were seen 30 minutes after exposure to gold dust. According to our knowledge, this is the first case with gold dust induced by KS.

The primary treatment of KS is the management of ACS and regression of the allergic reaction. The regression of symptoms of the allergic reaction with steroids and antihistamines may be enough to resolve coronary vasospasm (10).

CONCLUSION

KS should be kept in mind in cases that present with allergic reactions along with chest pain following exposure to agents that may be allergic to the immune system. Cardiac enzymes can either be normal or elevated in these patients. ECG would have ST and T wave changes, which would be normal.

Ethics

Informed Consent: Was obtained.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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

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

REFERENCES

1. Kounis NG, Zavras GM. Histamine-induced coronary artery spasm: the concept of allergic angina. Br J Clin Pract 1991;45:121-8.
2. Lopez PR, Peiris AN. Kounis syndrome. South Med J 2010;103:1148-55.
3. Vivas D, Rubira JC, Ortiz AF, Macaya C. Coronary spasm and hypersensitivity to amoxicillin: Kounis or not Kounis syndrome? Int J Cardiol 2008;128:279-81.

4. Gázquez V, Dalmau G, Gaig P, Gómez C, Navarro S, Mercé J. Kounis syndrome: report of 5 cases. J Investig Allergol Clin Immunol 2010;20:162-5.

5. Braunwald E. Unstable angina: an etiologic approach to management. Circulation 1998;98:2219-22.

6. Biteker M. A new classification of Kounis syndrome. Int J Cardiol 2010;145:553.

7. Tavil Y, Turfan M, Türkoğlu S, Abaci A. Kounis syndrome secondary to amoxicillin/clavulanic acid use. Int J Cardiol 2008;124:e4-e7.

8. Kounis GN, Soufras GD, Kouni SA, Kounis NG. Hypersensitivity myocarditis and hypersensitivity coronary syndrome (Kounis syndrome). Am J Emerg Med 2009;27:506-8.

9. Kounis NG. Kounis syndrome (allergic angina and allergic myocardial infarction): a natural paradigm? Int J Cardiol 2006;110:7-14.

10. Tok D, Ozcan F, Sentürk B, Gölbaşı Z. Parenteral penisilin kullanımını takiben gelişen akut koroner sendrom olgusu: Kounis sendromu. Arch Turk Soc Cardiol 2012;40:615-9.

2019 Referee Index

2019 Author Index

2019 Author Index

2019 Subject Index

2019 Subject Index