ISSN : 2149-9322
E-ISSN : 2149-9330

TURKISH JOURNAL OF OBSTETRICS AND GYNECOLOGY

March 2019 Volume: 16 Issue: 1 www.tjoddergisi.org

Clinical Investigations

TURKISH SOCIETY OF OBSTETRICS AND GYNECOLOGY

galenos yayınevi

TURKISH JOURNAL OF OBSTETRICS AND GYNECOLOGY

TURKISH JOURNAL OF OBSTETRICS AND GYNECOLOGY

TURKISH JOURNAL OF OBSTETRICS AND GYNECOLOGY

Contact
Çetin Emeç Bulvarı Hürriyet Caddesi Harbiye Mahallesi 1/13 Öveçler, Ankara, Turkey
Phone: +90 312 481 06 06 Fax: +90 312 481 28 28 E-mail: editor@tjod.org

Galenos Publishing House
Owner and Publisher
Erkan Mor

Publication Coordinator
Burak Sever

Web Coordinators
Soner Yıldırım
Turgay Akpınar

Graphics Department
Ayda Alaca
Çiğdem Birinci
Gülşah Özgül

Project Coordinators
Eda Kolukısa
Hatice Balta
Lütfiye Ayhan İrtem
Sedanur Sert
Zeynep Altındağ

Project Assistants
Gamze Aksoy
Nurcan Acarçağ

Finance Coordinator
Sevinç Çakmak

Research&Development
Kerim Sancar Ölmez
Mert Köse

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 at: Üniform Basım San. ve Turizm Ltd. Şti.
Matbaacılar Sanayi Sitesi 1. Cad. No: 114 34204 Bağcılar, İstanbul, Turkey
Phone: +90 (212) 429 10 00 **Certificate Number:** 42419
Printing Date: March 2019
ISSN: 2149-9322 **E-ISSN:** 2149-9330
International scientific journal published quarterly.

TURKISH JOURNAL OF OBSTETRICS AND GYNECOLOGY

AIMS AND SCOPE

Turkish Journal of Obstetrics and Gynecology (formerly called Türk Jinekoloji ve Obstetrik Derneği Dergisi) is the official peer-reviewed journal of the Turkish Society of Obstetrics and Gynecology and is published quarterly on March, June, September and December.

It is an independent peer-reviewed international journal published in English language since 2014 September. Manuscripts are reviewed in accordance with "double-blind peer review" process for both referees and authors.

The target audience of Turkish Journal of Obstetrics and Gynecology includes gynecologists, obstetricians, urogynecologists, reproductive medicine specialists, gynecological oncologists and primary care physicians interested in gynecology practice. It publishes original work on all aspects of obstetrics and gynecology. The aim of Turkish Journal of Obstetrics and Gynecology is to publish high quality original research articles. In addition to research articles, reviews, editorials, letters to the editor and case presentations are also published.

The General Guidelines for manuscript preparation specified below are based on "Recommendations for the Conduct, Reporting, Editing, & Publication of Scholarly Work in Medical Journals (ICMJE Recommendations)" by the International Committee of Medical Journal Editors (2016, archived at http://www.icmje.org/).

- Turkish Journal of Obstetrics and Gynecology is indexed in **PubMed Central (PMC), Web of Science-Emerging Sources Citation Index (ESCI), EBSCO, DOAJ, Index Copernicus, Scopus, CINAHL, Google Scholar, Tübitak/Ulakbim Turkish Medical Database, Turk Medline and Turkiye Citation Index.**

Open Access Policy

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

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

Permission

Financial expenses of the journal are covered by Turkish Society of Obstetrics and Gynecology.

Subscription Information

Turkish Journal of Obstetrics and Gynecology is distributed free of charge to all physicians, specialists in obstetrics and gynecology field. The access to tables of contents, abstracts and full texts of all articles published since 2004 are free to all readers via the journal's webpage "http://www.tjoddergisi.org". Visit the journal's home pages for details of the aims and scope and instruction to authors.

Manuscripts can only be submitted electronically through the Journal Agent website (http://journalagent.com/tjo/) after creating an account. This system allows online submission and review.

Instructions for Authors

Instructions for authors page of the journal is available in the journal content and at www.tjoddergisi.org

Disclaimer

The statements and opinions expressed contained in the articles of the Turkish Journal of Obstetrics and Gynecology are solely those of the individual authors and contributors not of the Turkish Society of Obstetrics and Gynecology or Galenos Yayınevi.

Advertising

Enquiries concerning advertisements should be addressed to Editorial Office or Publisher:

Editorial Office

Editor-in-Chief: Eray Çalışkan, M.D.

Address : Çetin Emeç Bulvarı Hürriyet Caddesi Harbiye Mahallesi 1/13 Öveçler, Ankara - Turkey

Phone : +90 (312) 481 06 06

Fax : +90 (312) 481 28 28

E-mail : info@tjod.org

Publisher

Galenos Yayınevi Tic. Ltd. Şti.

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

TURKISH JOURNAL OF OBSTETRICS AND GYNECOLOGY

INSTRUCTIONS FOR AUTHORS

The "Turkish Journal of Obstetrics and Gynecology" is the official publication of the Turkish Society of Obstetricians and Gynecologists. The journal is published quarterly (March, June, September and December) in English and publishes original peer-reviewed articles, reviews, case reports and commentaries in the fields of gynecology, gynecologic oncology, endocrinology and reproductive medicine and obstetrics. The journal gives publication priority to original research articles over case reports. Reviews are considered for publication only if they are prepared by authors who have at least three published manuscripts in international peer-reviewed journals on the topic of the review and these studies should be cited in the review. Otherwise only invited reviews will be considered for peer-review from qualified experts in the area.

The "Turkish Journal of Obstetrics and Gynecology" is a peer-reviewed journal and adheres to the highest ethical and editorial standards. The editors also adhere to the Committee on Publications Ethics (COPE) recommendations (http://publicationethics.org).

The journal should be abbreviated as Turk J Obstet Gynecol when referenced.

Turkish Journal of Obstetrics and Gynecology does not charge any article submission or processing charges.

Turkish Journal of Obstetrics and Gynecology is indexed in **PubMed Central (PMC), Web of Science-Emerging Sources Citation Index (ESCI), EBSCO, DOAJ, Index Copernicus, Scopus, CINAHL, Google Scholar, Tübitak/Ulakbim Turkish Medical Database, Turk Medline and Turkiye Citation Index.**

Submission of Manuscripts

Turkish Journal of Obstetrics and Gynecology has specific instructions and guidelines for submitting articles. Those instructions and guidelines are readily available on the submission service site. Submit all manuscripts through the journal's web page at www.tjoddergisi.org. New users should first create an account. Once a user is logged onto the site, submissions should be made via the Author Centre. Download the Instructions to Authors for detailed notes on how to prepare your manuscript.

The ORCID (Open Researcher and Contributor ID) number of the correspondence author should be provided while sending the manuscript. A free registration can be done at http://orcid.org.

Manuscripts submitted via any other medium will not be evaluated. During the submission please make sure to provide all requested information to prevent any possible delays in the evaluation process. Only those submitted articles are not currently being considered by another journal, or have not been previously published, will be considered for publication in Turkish Journal of Obstetrics and Gynecology. The submitted articles are firstly evaluated over by the non-baised editors. The articles that meet the originality and other requirements of the journal are peer-reviewed by the national or international referees. Acceptance for publication is based on significance, novelty, and quality of the article.

Authors who have any queries regarding the submission process can contact the journal's editorial office:

Çetin Emeç Bulvarı Harbiye Mahallesi Hürriyet Caddesi 1/3 Öveçler/ Ankara.

Phone number: +90 (312) 481 06 06

E-mail: editor@tjod.org

Editorial Policies

All manuscripts will be evaluated for their scientific contribution, originality and content by the editorial board. Only those submitted articles are not currently being considered by another journal, or have not been previously published, will be considered for publication in Turkish Journal of Obstetrics and Gynecology. Authors are responsible for the accuracy of the data presented in their manuscript. The journal retains the right to make appropriate changes on the grammar and language of the manuscript when needed. When suitable the manuscript will be send to the corresponding author for revision. The manuscript, if accepted for publication, will become the property of the journal and copyright will be taken out in the name of the journal.

All manuscripts submitted to the journal for publication are checked by Crossref Smilarity Check powered by iThenticate software for plagiarism. If plagiarism is detected, relevant institutions may be notified. In this case, the authors might be asked to disclose their raw data to relevant institutions.

Peer-review

Turkish Journal of Obstetrics and Gynecology is an independent international journal based on double-blind peer-review principles. The manuscript is assigned to the Editor-in-Chief, who reviews the manuscript and makes an initial decision based on manuscript quality and editorial priorities. These manuscripts then sent for external peer-review, the Editor in Chief assigns Associate Editor. The Associate Editor sends the manuscript to the 3 internal and external reviewers. The reviewers must review the manuscript in 21 days. Associate Editor recommends decision based on the reviewers' recommendations and sends the manuscript to the Editor-in-Chief. The Editor-in-Chief makes a final decision based on editorial priorities, manuscript quality and reviewer recommendations. If there are any conflicting recommendation of reviewers, Editor-in-Chief can assign a new reviewer. The scientific board guiding the selection of the papers to be published in the journal consists of elected experts of the journal and if necessary, selected from national and international experts in the relevant field of research. All manuscripts are reviewed by the editor, section associate editors and at least three internal and external expert referees. All research articles undergo review by statistics editor as well.

Full text of all articles can be downloaded at the web site of the journal: www.tjoddergisi.org

Authorship

The role of authorship in Turkish Journal of Obstetrics and Gynecology is reserved for those individuals who meet the criteria recommended by the International Committee of Medical Journal Editors (ICMJE; http://www.icmje.org). Describe each authors' contribution by using ICMJE's criteria: substantial contributions to the conception or design; the acquisition, analysis, or interpretation of data; drafting the work or revising it critically for important intellectual content; final approval of the version to be published; agreement to be accountable for all aspects of the study in ensuring that questions related to the accuracy or integrity of any part of the work are

INSTRUCTIONS FOR AUTHORS

appropriately investigated and resolved. The statement about the authors' contributions should be placed in the cover letter. All persons who contributed to the work, but not sufficiently to be authors, must be acknowledged.

Cover Letter

Cover letter to the editors addressing the following points:

· The authors' intent to submit solely to Turkish Journal of Obstetrics and Gynecology.

· Verification that the manuscript is not under consideration elsewhere, and indication from the authors that it will not be submitted elsewhere until a final decision is made by the editors of Turkish Journal of Obstetrics and Gynecology.

· The declaration of transparency from the corresponding author.

· Clinical trial registration, if applicable.

· Institutional review board (IRB) approval or exemption.

· Informed consent.

· Any explanations related to reporting guidelines.

· The statement about the authors' contributions.

Preparation of Manuscripts

The "Turkish Journal of Obstetrics and Gynecology" follows the Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals" (International Committee of Medical Journal Editors - http://www.icmje.org/). Upon submission of the manuscript, authors are to indicate the type of trial/research and provide the checklist of the following guidelines when appropriate:

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

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

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

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

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

CARE guidelines are designed to increase the accuracy, transparency, and usefulness of case reports. (Gagnier JJ, Kienle G, Altman DG, Moher D, Sox H, Riley D; the CARE Group. The CARE Guidelines: Consensus-based Clinical Case Reporting Guideline Development.) (http://www.care-statement.org/)

Human and Animal Studies

Manuscripts submitted for publication must contain a statement to the effect that all human studies have been reviewed by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards described in an appropriate version of the 1964 Declaration of Helsinki, as revised in 2013 (http://www.wma.net/en/30publications/10policies/b3/). It should also be stated clearly in the text that all persons gave their informed consent prior to their inclusion in the study. Details that might disclose the identity of the subjects under study should be omitted. In case of usage of any image media that potentially can expose patients' identity requires obtaining permission for publication from the patients or their parents/guardians. Experimental animal studies should be presented with the disclosure of the appropriateness to the institutional/national/international ethical guides on care and use of laboratory animals.

Reports of animal experiments must state that the "Principles of laboratory animal care" (NIH publication No. 86-23, revised 1985) were followed, as well as specific national laws where applicable.

The editors reserve the right to reject manuscripts that do not comply with the above mentioned requirements. The author will be held responsible for false statements or for failure to fulfill the above mentioned requirements.

Authors must provide statement on the absence of conflict of interests between authors and provide authorship contributions and declare if any financial/material support.

Copyright

The author(s) transfer(s) the copyright to his/their article to the Turkish Journal of Obstetrics and Gynecology effective if and when the article is accepted for publication. The copyright covers the exclusive and unlimited rights to reproduce and distribute the article in any form of reproduction (printing, electronic media or any other form); it also covers translation rights for all languages and countries. For U.S. authors the copyright is transferred to the extent transferable.

After receiving and accept decision for publication, submissions must be accompanied by the "Copyright Transfer Statement". The form is available for download on the journal's manuscript submission and evaluation site. The copyright transfer form should be signed by all contributing authors and a scanned version of the wet signed document should be submitted.

Manuscript Structure

All manuscripts must be submitted as Microsoft Word (.doc or .docx) files. All manuscript pages (including references, tables, and figure legends) must be double-spaced. Use a standard, 12-point typeface such as Times New Roman. Top, bottom, and side margins should be set at 1 inch. Authors must include the following in the manuscript file:

TURKISH JOURNAL OF OBSTETRICS AND GYNECOLOGY

INSTRUCTIONS FOR AUTHORS

Title Page

A separate title page should list;

-The manuscript title, which should contain no more than a total of 100 characters (counting letters and spaces) and should not be declarative; do not use abbreviations or commercial names in the title.

- A short title of no more than 50 characters, including spaces, for use as a running foot.

- All author name(s), institutional, corporate, or commercial affiliations, and up to two major degree(s).

- Corresponding author's name, address, telephone (including the mobile phone number), fax numbers and e-mail address (the corresponding author will be responsible for all correspondence and other matters relating to the manuscript).

Precis

The precis is a one-sentence synopsis of no more than 30 words that describes the basic findings of the article. Precis sample can be seen below:

'Using a 45 point questionnaire, we have evaluated the trend of Robotic surgery training in the gynecologic surgery fellowship programs across the nation'.

Abstract

All manuscripts should be accompanied by an abstract. All information in the abstract should be consistent with the information in the text, tables, or figures. Avoid use of commercial names in the abstract. Original research reports should have a structured abstract of no more than 250 words, using the following headings:

· Objective: Main question, objective, or hypothesis (single phrase starting with, for example, "To evaluate..." or "To estimate." [never start with "To determine."]).

· Materials and Methods: Study design, participants, outcome measures, and in the case of a negative study, statistical power.

· Results: Measurements expressed in absolute numbers and percentages, and when appropriate indicate relative risks or odds ratios with confidence intervals and level of statistical significance; any results contained in the abstract should also be presented in the body of the manuscript, tables, or figures.

· Conclusion: Directly supported by data, along with clinical implications.

Authors from Turkey or Turkish speaking countries are expected to submit a Turkish abstract including subheadings such as "Amaç, Gereç ve Yöntemler, Bulgular, Sonuç". The abstract of Authors whose native language is not Turkish will be provided free of charge translation services into Turkish language.

A structured abstract is not required with review articles and case reports.

Keywords

Below the abstract provide 3 to 5 keywords. Abbreviations should not be used as keywords. Keywords should be picked from the Medical Subject Headings (MeSH) list (www.nlm.nih.gov/mesh/MBrowser.html).

Turkish abstracts should have keywords "Anahtar Kelimeler" picked from www.atifdizini.com under "Türkiye Bilim Terimleri" link.

Several types of articles can be submitted for publication in Turkish Journal of Obstetrics and Gynecology: Original research, case reports, systematic reviews, current commentaries, procedures and instruments, and letters. Stated word counts and page limits were shown in Table 1. Copyright transfer forms, the cover letter, and figures do not contribute to the page limits.

Table 1. Manuscript length at a glance

Article type	Abstract Length	Manuscript Word Count*	Maximum Number of Authors	Maximum Number of References$^{\Phi}$
Original Research	250 words	5,500 words (~22 pages)$^{\Psi}$	NA	30
Case report	150 words	2,000 words (~8 pages)	4	8
Systematic review	300 words	6,250 words (~25 pages)	4	60
Current commentary	250 words	3,000 words (~12 pages)	4	12
Procedure and Instruments	200 words	2,000 words (~8 pages)	4	10
Letters	NA	350 words	4	5

*Manuscript length includes all pages in a manuscript (ie, title page, abstract, text, references, tables, boxes, figure legends, and appendixes). $^{\Phi}$Suggested limit. $^{\Psi}$The Introduction should not exceed 250 words. ~approximately; NA, not applicable.

Original researches should have the following sections;

Introduction

State concisely the purpose and rationale for the study and cite only the most pertinent references as background. Avoid a detailed literature review in this section.

Materials and Methods

Describe the research methodology (the patients, experimental animals, material and controls, the methods and procedures utilized, and the statistical method(s) employed) in sufficient detail so that others could duplicate the work. Identify methods of statistical analysis and when appropriate, state the basis (including alpha and beta error estimates) for their selection. Cite any statistical software programs used in the text. Express p values to no more than two decimal places. Indicate your study's power to detect statistical difference.

Address "IRB" issues and participants informed consent as stated above, the complete name of the IRB should be provided in the manuscript. State the generic names of the drugs with the name and country of the manufactures.

Results

Present the detailed findings supported with statistical methods. Figures and tables should supplement, not duplicate the text; presentation of data in either one or the other will suffice. Authors should report

INSTRUCTIONS FOR AUTHORS

outcome data as both absolute and relative effects since information presented this way is much more useful for clinicians. Actual numbers and percentages should be given in addition to odds ratios or relative risk. When appropriate, number needed to treat for benefits (NNTb) or harm (NNTh) should be supplied. Emphasize only your important observations; do not compare your observations with those of others. Such comparisons and comments are reserved for the discussion section.

Discussion

Begin with a description of what your study found in relation to the purpose or objectives as stated in the Introduction. State the importance and significance of your findings to clinicians and actual patient care but do not repeat the details given in the Results section. Limit your opinions to those strictly indicated by the facts in your report. Compare your finding with previous studies with explanations in cases where they differ, although a complete review of the literature is not necessary.

Study Limitations

Provide information on the limitations of the study. No new data are to be presented in this section. A final summary is not necessary, as this information should be provided in the abstract and the first paragraph of the Discussion. Although topics that require future research can be mentioned, it is unnecessary to state, "Further research is needed."

Conclusion

The conclusion of the study should be highlighted. The study's new and important findings should be highlighted and interpreted.

Conflict of Interest

Authors must indicate whether or not they have a financial relationship with the organization that sponsored the research.

The main text of case reports should be structured with the following subheadings:

Introduction, Case Report, Discussion and References.

References

References are numbered (Arabic numerals) consecutively in the order in which they appear in the text (note that references should not appear in the abstract) and listed double-spaced at the end of the manuscript. The preferred method for identifying citations in the text is using within parentheses. Use the form of the "Uniform Requirements for Manuscripts" (http://www.icmje.org/about-icmje/faqs/icmje-recommendations/). If number of authors exceeds seven, list first 6 authors followed by et al.

Use references found published in peer-reviewed publications that are generally accessible. Unpublished data, personal communications, statistical programs, papers presented at meetings and symposia, abstracts, letters, and manuscripts submitted for publication cannot be listed in the references. Papers accepted by peer-reviewed publications but not yet published ("in press") are not acceptable as references.

Journal titles should conform to the abbreviations used in "Cumulated Index Medicus".

Examples

Journals; Zeyneloglu HB, Onalan G. Remedies for recurrent implantation failure. Semin Reprod Med 2014;32:297-305.

Book chapter; Ayhan A, Yenen MC, Dede M, Dursun P, Gultekin M. How to Manage Pre-Invasive Cervical Diseases? An Overview. In: Ayhan A, Gultekin M, Dursun P, editors. Textbook of Gyneaecological Oncology. Ankara, Turkey: Gunes Publishing; 2010. p. 28-32.

Book; Arici A, Seli E. Non-invasive Management of Gynecologic Disorders. In: Arici A, Seli E (eds). London: Informa Healthcare; 2008.

Tables and Figures

Tables should be included in the main document after the reference list. Color figures or gray-scale images must be at minimum 300 DPI resolutions. Figures should be submitted in "*.tiff", "*.jpg" or "*.pdf" format and should not be embedded in the main document. Tables and figures consecutively in the order they are referred to within the main text. Each table must have a title indicating the purpose or content of the table. Do not use internal horizontal and vertical rules. Place explanatory matter in footnotes, not in the heading. Explain all abbreviations used in each table in footnotes. Each figure must have an accompanying descriptive legend defining abbreviations or symbols found in the figure. If photographs of people are used, the subjects must be unidentifiable and the subjects must have provided written permission to use the photograph. There is no charge for color illustrations.

Units of Measurement and Abbreviations

Units of measurement should be in Systéme International (SI) units. Abbreviations should be avoided in the title. Use only standard abbreviations. If abbreviations are used in the text, they should be defined in the text when first used.

Revisions

Revisions will be sent to the corresponding author. Revisions must be returned as quickly as possible in order not to delay publication. Deadline for the return of revisions is 30 days. The editorial board retains the right to decline manuscripts from review if authors' response delays beyond 30 days. All reviewers' comments should be addressed a revision note containing the author's responses to the reviewers' comments should be submitted with the revised manuscript. An annotated copy of the main document should be submitted with revisions. The Editors have the right to withdraw or retract the paper from the scientific literature in case of proven allegations of misconduct.

Accepted Articles

Accepted articles are provided with a DOI number and published as ahead of print articles before they are included in their scheduled issue.

Journal and Society Web sites:

www.tjod.org (Turkish Society of Obstetrics and Gynecology)

www.tjoddergisi.org (Turkish Journal of Obstetrics and Gynecology)

TURKISH JOURNAL OF OBSTETRICS AND GYNECOLOGY

CONTENTS

Clinical Investigations

TURKISH JOURNAL OF OBSTETRICS AND GYNECOLOGY

CONTENTS

LETTER FROM THE PRESIDENT

Dear Collagues,

Firstly, I would like to thank everyone, especially the the editorial board that enables us to reach more collagues and to maintain our progress.

It is the time of the year that all members of the obstetrics and gynecology community come together and unite in the most prosperous obstetrics and gynecology congress in terms of the scientific and the social programme. We are excited for our upcoming congress, the TSOG 2019 Congress, which will be held between April 24-28, 2019 in Antalya, with your participations.

As we have done in the previous years, we hereby aim to share and discuss the recent developments in our field. Therefore,we are hosting such precious national and international speakers who will share their knowledge and experiences with their collagues.

We will be glad to reunite with our collagues in an environment built upon peace, freedom and sympathy. We look forward to meet all our collagues in our congress.

Best regards,

Ateş Karateke, Prof. M.D.,
President of TSOG

TURKISH JOURNAL OF OBSTETRICS AND GYNECOLOGY

EDITORIAL

Dear Collegues,

A new issue of Turkish Journal of Obstetrics and Gynecology is now available as an electronic journal. The hard copy era is over now and from now on all the manuscripts and editorials will be available online as it was since the last 5 years. With the new publishing strategy we also want to support the environment by decreasing the paper use.

With the new year our editorial board has undergone revision. As editorial work is considerably time consuming it is hard to do it for a long time. Revision included editors, section editors exchange places with editorial and advisory board. We would like also to wellcome the new joining editorial board members who have H-index over 10 which is compatible with our prior commitments.

We would like to thank all the editorial borad for their time donated for advancement of our journal and science. An editorial board meeting will be held in the National Congress in 2019. I would like to invite all our collegues interested in scientific publishing.

We seek your support with high quality research papers.

Sincerely

Eray Calışkan
Editor in Chief

Clinical Investigation / Araştırma

DOI: 10.4274/tjod.galenos.2018.33410

Turk J Obstet Gynecol 2019;16:1-6

A comparison of emergency and therapeutic modified Shirodkar cerclage: an analysis of 38 consecutive cases

Acil ve terapötik modifiye Shirodkar serklajın karşılaştırılması: 38 olgunun analizi

Alper Başbuğ[1], Ozan Doğan[2]

[1]*Düzce University Faculty of Medicine Hospital, Clinic of Obstetrics and Gynecology, Düzce, Turkey*

[2]*University of Health Sciences, İstanbul Şisli Hamidiye Etfal Training and Research Hospital, Clinic of Obstetrics and Gynecology, İstanbul, Turkey*

Abstract

Objective: To compare the maternal and neonatal outcomes of patients with emergency versus therapeutic cerclage.
Materials and Methods: The study included 38 female patients who underwent cervical cerclage using the modified Shirodkar method in the Obstetrics and Gynecology Clinics of Düzce University Medical Faculty Hospital and Düzce Atatürk State Hospital.
Results: The operating time for the emergency cerclage group was significantly longer than that of the therapeutic group (30.40 minutes vs 19.85 minutes, p=0.001). Following the cerclage procedure, the cervical length was longer in the therapeutic cerclage group [29.90 millimeters (mm) vs. 22.45 mm, p=0.001]. The cerclage to birth interval was also longer in the therapeutic group (91 vs. 138 days).
Conclusion: In comparison with therapeutic cerclage, the total duration of pregnancy after emergency cerclage is shorter, and newborns have a greater need for intensive care. Both methods, however, protect against advanced prematurity, which causes neonatal loss.
Keywords: Premature birth, cervical incompetence, cervical cerclage

Öz

Amaç: Acil ve terapötik serklajlı olguların maternal ve neonatal sonuçlarını karşılaştırmaktır.
Gereç ve Yöntemler: Düzce Üniversitesi Tıp Fakültesi Hastanesi Kadın Hastalıkları ve Doğum Kliniği ve Düzce Atatürk Devlet Hastanesi'nde modifiye Shirodkar yöntemi ile servikal serklaj uygulanan 38 kadın hasta çalışmaya alındı.
Bulgular: Acil serklaj grubunun çalışma süresi, terapötik gruba göre anlamlı olarak daha uzundu (30,40 dakikaya 19,85 dakika, p=0,001). Serklaj işleminden sonra, servikal uzunluk terapötik serklaj grubunda daha uzundu (29,90 mm'ye 22,45 mm, p=0,001). Doğum aralığı, terapötik grupta daha uzundu (91 güne 138 gün).
Sonuç: Terapötik serklaj ile kıyaslandığında, acil serklajdan sonraki toplam gebelik süresi kısadır ve yenidoğanların yoğun bakım ihtiyacı daha yüksektir. Bununla birlikte, her iki yöntem de, neonatal kayba neden olan ileri prematüre karşı koruma sağlar.
Anahtar Kelimeler: Prematüre doğum, servikal yetmezlik, servikal serklaj

Introduction

Preterm labor and the resulting preterm births remain a major problem for physicians, and despite all the advances made, it remains the leading health problem in the field of obstetrics in both developed and developing countries[1,2]. There is a negative correlation between gestational age at birth and mortality and morbidity, primarily due to respiratory distress syndrome, intraventricular bleeding, necrotizing enterocolitis, and sepsis[3,4]. Several factors are responsible for the etiology of preterm labor[5]. One of these is cervical incompetence, which is seen in two percent of all pregnancies, and which leads to miscarriage in the second trimester and early third trimester[6,7].

PRECIS: Both cerclage methods administered by experienced surgeons in appropriate patients could be effective.

Address for Correspondence/Yazışma Adresi: Ozan Doğan, MD,
University of Health Sciences, İstanbul Şisli Hamidiye Etfal Training and Research Hospital, Clinic of Obstetrics and Gynecology, İstanbul, Turkey
Phone: +90 505 506 07 20 **E-mail:** ozandogan02@hotmail.com **ORCID ID:** orcid.org/0000-0002-0016-8749
Received/Geliş Tarihi: 11.08.2018 **Accepted/Kabul Tarihi:** 06.11.2018

During the last 50 years, cervical cerclage has been frequently used to prolong the period until birth in pregnancies with cervical incompetence. In patients with cervical dilatation with or without prolapse of the amniotic membranes into the vagina, the procedure is conducted as an emergency cerclage. If cervical shortening is seen on serial ultrasonography measurements, the procedure can be conducted as a therapeutic cerclage[8,9]. The cervical cerclage procedure can be performed transvaginally with the McDonald and Shirodkar methods or transabdominally[10-12]. The efficacy of these methods in therapeutic and emergency cerclage is controversial, but the McDonald type of cerclage is more widely used because the application is easier to perform[13,14]. The aim of this study was to evaluate the maternal and neonatal outcomes when modified Shirodkar cervical cerclage was performed as an emergency or a therapeutic procedure in a three-year period in two clinics.

Materials and Methods

This retrospective case-control study included 38 pregnant women who were diagnosed as having cervical incompetence and treated with cervical cerclage using the modified Shirodkar method. The patients were treated in the Obstetrics and Gynecology Clinics of Düzce University Medical Faculty Hospital (a tertiary hospital) and Düzce Atatürk State Hospital (a secondary hospital) between June 2015 and May 2018.

Approval for the study was granted by the Institutional Ethics Committee, and all procedures were performed in accordance with the 1964 Helsinki Declaration. Informed consent was obtained from all study participants. This study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.

The therapeutic cerclage procedure was administered to patients with a cervical length of less than 25 mm without cervical dilatation in the second trimester sonographic cervical examination. The emergency cerclage procedure was performed on those with cervical dilatation determined during physical examinations and/or prolapse of the fetal membranes into the vagina.

Patients with symptoms of clinical and biochemical chorioamnionitis [tenderness of the cervix and/or the uterus, a temperature greater than 38° Celsius, a white blood cell count greater than 15.000 per cubic millimeter (mm^3), and a C-reactive protein level greater than 2.0 milligrams per deciliter (mg/dL)], as well as those who had preterm premature rupture of membranes, a fetus with anomalies, a history of cone biopsy and loop electrosurgical excision procedures, mechanical dilation of the cervix during pregnancy termination, and uterine malformations were excluded from the study. A 12-hour waiting period was imposed before performing cerclage in order to exclude patients with preterm deliveries.

Modified Shirodkar cerclage procedure

All procedures were conducted under regional or general anesthesia with the patient in the dorsal lithotomy position. A non-absorbable monofilament suture of 0.5 centimeters (cm) in thickness and 50 cm in length was used as the suture material, with a double needle for application (Braun, Aesculap, Tuttlingen, Germany). Before the therapeutic procedure, the cervix was visualized using a Sims retractor, and vaginal lavage was applied with a diluted iodine solution. Then, holding the anterior lip of the cervix with an Allis clamp, the physician performed an anterior colpotomy by making a 3 cm transverse incision into the anterior wall of the vagina after pulling it downward. The bladder was avoided. From the twelve o'clock position at the level of the cardinal ligament, 3-4 cm from the cervical os, the two-way needle with the suture material was passed to the level of four o'clock and eight o'clock. Then, removing both needles at the six o'clock position, the physician knotted the suture at the posterior of the cervix. The incision in the vaginal anterior wall was closed with a non-absorbable 3/0 multifilament suture. In the emergency cerclage procedure, all steps were applied in the same way, and the amniotic membrane that was prolapsed into the vagina was gently replaced into the uterus using wet sponges.

All operations were performed by A.B. and O.D., who are both experienced in these procedures.

The conventional Shirodkar suture was modified to decrease the operation time and to minimize the potential harmful effects of the anesthetic on the fetus, as well as to avoid the occurrence of hemorrhaging during the posterior vaginal wall dissection. Moreover, the modification simplifies the removal of the cerclage suture upon the commencement of labor.

After the procedure, all patients were observed for the following two days in the obstetric unit.

Prophylaxis of 1 gram (g) cefazolin sodium (Sefazol, M. Mevzat, Turkey) was applied before all the cerclage procedures. To inhibit uterine contractions, 100 mg of indomethacine (Endol, Deva, Turkey) was administered rectally. For the relief of postoperative pain, 1 g of acetaminophen (Parol, Atabay, Turkey) was administered. Additionally, in the emergency cerclage group, all patients were given 1 g of cefazolin sodium intravenously twice a day and 500 mg of metronidazole intravenously twice a day for five days. All patients in both groups received 200 mg of natural progesterone vaginally for as long as their pregnancies continued or until the 37th gestational week. Tocolysis with oral nifedipine was implemented on an individual basis.

The presence of uterine contractions that did not respond to tocolysis and the rupture of the membranes were regarded as indications for preterm removal of the cerclages. Otherwise, the cerclages were electively removed at 37 gestational weeks. The primary goal was to evaluate the relationship between maternal and perinatal outcomes and the cerclage procedure

type. The secondary goal was assessing the relationship between cervical dilatation, residual cervical length and vaginal contact with the amniotic membranes, and births before the 32nd gestational week.

Statistical Analysis

Analysis of the data obtained in the study was made using the SPSS (Ver. 22.0) software. Quantitative data are presented in the tables as mean ± standard deviation and median (minimum-maximum) values. Categorical data are stated as number (n) and percentage (%). In the comparison between independent groups, Student's t-test and the Mann-Whitney U test were used, and in the comparison of categorical variables, Pearson's chi-square test and Fisher's exact test were used. Univariate binary logistic regression analysis was used to determine relationships between risk factors that were independently associated with delivery at less than 32 gestational weeks. No power calculation was performed for this study because all eligible patients were included in the study. The data were examined at 95% confidence intervals (CI). A p value of <0.05 was accepted as being statistically significant.

Results

The study included a total of 38 patients, comprising 22 emergency cases and 16 therapeutic cases. No statistically significant difference was determined between the groups with respect to demographic characteristics, premature birth history, the manner of pregnancy (natural or *in vitro* fertilization) or multiple pregnancies (Table 1).

The operating time was determined to be statistically significantly longer in the emergency cerclage group than in the therapeutic group (30.40 minutes vs 19.85 minutes, p=0.001). The cervical length after the cerclage procedure was determined to be statistically significantly shorter in the emergency group (20.24±3.97 mm) compared with the therapeutic group (29.90±4.65 mm) (p=0.001). During the procedure, membrane rupture was observed in two patients (9.1%) in the emergency group, and no membrane rupture associated with the procedure was seen in any of the therapeutic cerclage group. Following the procedure, chorioamnionitis was seen in one patient (4.5%) in the emergency cerclage group and in no patients in the therapeutic group. The cerclage procedure was applied at an earlier gestational week in the therapeutic group than in the emergency group, but the difference was not statistically significant (19 weeks vs 21 weeks, p=0.59). The time period from the procedure to birth was longer, and the gestational week was later in the therapeutic group than in the emergency group (128 days vs 80 days, p=0.001; 37 weeks vs 32 weeks, p=0.001).

Birth occurred at full-term (≥37 weeks) in 10 patients (62.5%) in the therapeutic cerclage group and in three patients (13.6%) in the emergency group. The difference between the groups was determined to be statistically significant (p=0.002). The rates of vaginal delivery and caesarean section were similar in both groups (p=0.22, p=0.51, respectively).

There was a greater need for tocolytic therapy in the emergency cerclage group following the procedure (p=0.001). There was a greater need for neonatal intensive care in the emergency group (p=0.001). The neonatal mortality rate was not observed in the therapeutic cerclage group, and it was 9.1% (n=2) in the emergency cerclage group, with no statistically significant difference determined between the groups (Table 2).

The cervical length after cerclage that was shorter than 20 mm, the vaginal contact of amniotic membranes and multiple pregnancies were associated with preterm delivery before 32 gestational weeks [odds ratio (OR)=5.83, 95% CI: (1.20-28.36), p=0.018; OR=1.93, 95% CI:(1.37-2.72), p=0.025; OR=2.91, 95% CI:(1.88-4.61), p=0.021]. Cervical dilatation was not seen as a risk factor for preterm delivery before 32 gestational weeks (Table 3).

Table 1. The demographic characteristics of patients who underwent emergency and therapeutic cerclage procedures

	Emergency cerclage (n=22)	Therapeutic cerclage (n=16)	p value
Age	26 (range, 18-41) years	28 (range, 20-39) years	0.23
Gravida	2 (1-6)	2 (1-4)	0.49
Parity	0 (0-2)	0 (0-2)	0.91
Preterm birth history	3 (14.3%)	3 (18.8%)	0.52
IVF pregnancy	4 (18.2%)	2 (12.5%)	0.49
Multiple pregnancy	3 (14.3%)	0	0.17

IVF: *In vitro* fertilisation

Discussion

The data obtained in this study demonstrated that the cervical cerclage procedure (whether therapeutic or emergency in nature), conducted because of cervical incompetence in the second and early third trimesters, is effective in preventing extreme pre-term births. In the therapeutic cerclage group, the time from the procedure to birth was longer than when the procedure was performed under emergency conditions. Furthermore, the residual cervical length after the cerclage procedure was longer in the therapeutic group. With cervical lengths after cerclage that were shorter than 20 mm, vaginal contact with the amniotic membranes and multiple pregnancies were significantly more prevalent in patients who delivered preterm before 32 gestational weeks.

Berghella et al. [15] suggested that cervical lengths could be observed with transvaginal ultrasounds in women with a history of pre-term births because of cervical incompetence,

and that emergency cerclage should be applied only if a short cervix was determined. In contrast, Guzman et al. [16] reported that better results were obtained with therapeutic cerclage as compared with emergency cerclage. In the current study, the cerclage to birth interval was more favorable in the therapeutic cerclage group. There was a lesser need for neonatal intensive care after birth in the therapeutic cerclage group, but there was no difference between the groups in terms of the neonatal mortality rate.

Therapeutic cerclage is generally applied early in the second trimester and is a relatively low risk operation. An emergency cerclage procedure is applied more typically in the middle of the second trimester, when the cervix is significantly shortened, when there is cervical dilatation and when membranes are prolapsed into the vagina; therefore, it is an operation in which complications such as membrane rupture and chorioamnionitis are frequently seen[17]. In the current study, although the rates of membrane rupture associated with the procedure and chorioamnionitis after the procedure were higher in the emergency cerclage group, the difference between the groups was not statistically significant.

In a retrospective analysis of 158 patients treated with the McDonald method, Zhuve et al. [18] found that 10% of patients maintained the pregnancy beyond the 37th gestational week. In a study by Ohad et al. [19], the results of patients having had the McDonald procedure as a therapeutic or an emergency procedure were compared, and 64% of the therapeutic group and 59% of the emergency group gave birth after the 37th gestational week. In the current study, the rate of births in the 37th gestational week or later was 62.5% in the therapeutic group and 13.6% in the emergency group. Ohad et al. [19] applied the emergency cerclage procedure to patients with cervical lengths of less than 25 mm in the second trimester. In the current study, all patients in the emergency cerclage group had cervical dilatation and/or prolapse of the amniotic membranes into the vagina. Contact between the fetal membranes and the vagina increases the risk of chorioamnionitis and intra-amniotic infections. These infections are sometimes clinically evident, and some continue as subclinical infections and trigger pre-term labor[18,20,21]. This can be considered to be the reason for the high rate of pre-term births in the current study's emergency cerclage group. At the same time, when the cervix has shortened to an advanced degree, and the amniotic membranes have prolapsed into the vagina, it is extremely difficult to place the cerclage suture at the level of the internal cervical os. Thus, it is clear that the efficacy of sutures that have not reached

Table 3. Univariate logistic regression analysis for the association between possible risk factors and delivery before 32 gestational weeks

Factors	Odds ratio (95% CI)	p value
Cervical length after cerclage <20 mm	5.83 (1.20-28.36)	**0.018**
Cervical dilatation before cerclage >2 cm	0.31 (0.77-1.31)	0.106
Vaginal contact of amniotic membranes	1.93 (1.37-2.72)	**0.025**
Multiple pregnancy	2.91 (1.88-4.61)	**0.021**

CI: Confidence intervals

Table 2. The operative and perinatal findings of patients who underwent emergency and therapeutic cerclage

	Emergency cerclage (n=22)	Therapeutic cerclage (n=16)	p values
Operating time (minutes)	30.40±6.20	19.90±3.85	0.001
Cervical length after cerclage (mm)	20.24±3.97	29.90±4.65	0.001
Membrane rupture during the cerclage procedure	2 (9.1%)	0	0.32
Chorioamnionitis after the cerclage procedure	1 (4.5%)	0	0.57
Gestational week at the time of cerclage	21 (17-24)	19 (17-23)	0.59
Gestational week at birth	32 (25-39)	37 (33-41)	0.001
Birth after ≥37.0 weeks	3 (13.6%)	10 (62.5%)	0.002
Delivery Vaginal Caesarean	 14 (63.7%) 8 (36.3%)	 9 (56.2%) 7 (43.75%)	 0.22 0.51
Interval from cerclage to birth (days)	80 (3-130)	128 (28-170)	0.001
Requirement for tocolytic therapy	8 (36.4%)	1 (6.2%)	0.001
Requirement for neonatal intensive care	7 (31.8%)	3 (18.8%)	0.001
Neonatal mortality	2 (9.1%)	0	0.32

Values are stated as the mean ± the standard deviation and median (minimum-maximum) values as well as percentages (%)

a sufficient length will be reduced. In comparison with the therapeutic group, the fact that sutures could not be placed in the appropriate position in the emergency cerclage group can be considered to be one of the factors that prevent the pregnancy from reaching full-term.

The cervical cerclage technique has been used for more than 50 years in the prevention of pre-term births associated with cervical incompetence[22]. However, very few studies have compared the efficacy of the application of different procedures. Obido et al. [23] found no difference between the McDonald and Shirodkar procedures in the prevention of pre-term births. In contrast, Treadwell et al.[24], and more recently, Wong et al.[25], reported that the Shirodkar procedure was more effective than the McDonald procedure in terms of preventing pre-term births. In a review published by Berghella[26], it was stated that the residual cervical length after cerclage should be greater than 2 cm to be effective in the prevention of pre-term birth; therefore, the cerclage suture must be applied as close as possible to the internal cervical os. Accordingly, to get as close as possible to the cervical os, it is necessary to dissect the bladder. In this respect, the Shirodkar procedure has an advantage over the McDonald procedure in reaching a satisfactory cervical height. In the current study, the Shirodkar procedure was applied to all patients, whether therapeutic or emergency, and in comparison with the McDonald procedure, there was considered to be a greater contribution to residual cervical height. There was also better support of the cervical tissue.

Conclusion

The strength of the study is that it is one of few studies that have compared the results of emergency and therapeutic cerclage procedures. Moreover, because the procedures were applied by surgeons experienced in this field, this standardized the applications. Basic limitations of the study include that it was retrospective, that there were relatively few patients, and that there was an insufficient number of vaginal cultures taken before the cerclage procedures.

In conclusion, despite the advances that have been made in obstetrics, pre-term births remain a major problem. In the prevention of pre-term birth as a result of cervical incompetence, both therapeutic and emergency cerclage procedures are effective. However, it must be noted that with regular screening of the cervical length from the second trimester onward in high-risk pregnancies, this procedure should be applied before cervical dilatation and prolapse of the amniotic membranes into the vagina.

Ethics

Ethics Committee Approval: Approval for the study was granted by the Institutional Ethics Committee, and all procedures were performed in accordance with the 1964 Helsinki Declaration.

Informed Consent: Informed consent was obtained from all study participants.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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- Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. Lancet 2008;371:75-84.
2- Hamilton BE, Martin JA, Osterman MJ, Curtin SC, Matthews TJ. Births: Final data for 2014. Natl Vital Stat Rep 2015;64:1-64.
3- Treyvaud K. Parent and family outcomes following very preterm or very low birth weight birth: a review. Semin Fetal Neonatal Med 2014;19:131-5.
4- Cram LF, Zapata MI, Toy EC, Baker B. Genitourinary infections and their association with preterm labor. Am Fam Physician 2002;65:pp.241-8.
5- Romero R, Dey SK, Fisher SJ. Preterm labor: one syndrome, many causes. Science 2014;345;760-5.
6- Lash AF, Lash SR. Habitual abortion; the incompetent internal os of the cervix. Am J Obstet Gynecol 1950;59:68-76.
7- Frey HA, Klebanoff MA. The epidemiology, etiology, and costs of preterm birth. Semin Fetal Neonatal Med 2016;21:68-73.
8- Kassanos D, Salamalekis E, Vitoratos N, Panayotopoulos N, Loghis C, Creatsas C. The value of transvaginal ultrasonography in diagnosis and management of cervical incompetence. Clin Exp Obstet Gynecol 2001;28:266-8.
9- Kurup M, Goldkrand JW. Cervical incompetence: elective, emergent, or urgent cerclage. Am J Obstet Gynecol 1999;181:240-6.
10- McDonald IA. Suture of the cervix for inevitable miscarriage. J Obstet Gynaecol Br Emp 1957;64:346-50.
11- Shirodkar, VN. A new method of operative treatment for habitual abortion in the second trimester of pregnancy. Antiseptic 1955;52:299.
12- Benson RC, Durfee RB. Transabdominal cervico uterine cerclage during pregnancy for the treatment of cervical incompetency. Obstet Gynecol 1965;25:145-55.
13- Cerclage: Shirodkar, McDonald, and Modifications.Wood SL, Owen J - Clin Obstet Gynecol - June 1, 2016;59:302-10.
14- WongCH, ChenCP, WangKG, SunFJ, Chen CY. Comparison of two cervical cerclages forthe prevention of preterm birth and neonatal complications. J Matern Fetal Neonatal Med 2017;30:551-5.
15- Berghella V, Haas S, Chervoneva I, Hyslop T. Patients with prior second-trimester loss: prophylactic cerclage or serial transvaginal sonograms? Am J Obstet Gynecol 2002;187:747-55.
16- Guzman ER, Forster JK, Vintzileos AM, et al. Pregnancy outcomes in women treated with elective versus ultrasound-indicated cervical cerclage. Ultrasound Obstet Gynecol 1998;12:323-7.
17- Practice Bulletin No. 130: prediction and prevention of preterm birth. Obstet Gynecol 2012;120:964-73.

18- Zhu L-Q, Chen H, Chen L-B, et al. Effects of Emergency Cervical Cerclage on Pregnancy Outcome: A Retrospective Study of 158 Cases. Med Sci Monit: International Medical Journal of Experimental and Clinical Research 2015;21:1395-1401. doi:10.12659/MSM.893244.
19- Ohad G, Yossi M, Shimon G, Jacob Bar & Ron S. Obstetrical outcomes of emergency compared with elective cervical cerclage, The Journal of Maternal-Fetal & Neonatal Medicine 2016;30:1650-4.
20- Latta RA, McKenna B. Emergent cervical cerclage: predictors of success or failure. J Matern Fetal Med 1996;5:22-7.
21- Minakami H, Matsubara S, Izumi A, Kosuge S, Watanabe T, Iwasaki R, et al. Emergency cervical cerclage: relation between its success, preoperative serum level of C-reactive protein and WBC count, and degree of cervical dilatation. Gynecol Obstet Invest 1999;47:157-61.
22- Final report of the medical research Council/Royal college of obstetricians and gynaecologists multicentre randomised trial of cervical cerclage. MRC/RCOG working party on cervical cerclage. Br J Obstet Gynaecol 1993;100:516-23.
23- Odibo AO, Berghella V, To MS, et al. Shirodkar versus McDonald cerclage for the prevention of preterm birth in women with short cervical length. Am J Perinatol 2007;24:55-60.
24- Treadwell MC, Bronsteen RA, Bottoms SF. Prognostic factors and complication rates for cervical cerclage: a review of 482 cases. Am J Obstet Gynecol 1991;165:555-8.
25- Wong CH, Chen CP, Wang KG, Sun FJ, Chen CY. Comparison of two cervical cerclages for the prevention of preterm birth and neonatal complications. J Matern Fetal Neonatal Med 2017;30:551-5.
26- Berghella V, Ludmir J, Simonazzi G, Owen J. Transvaginal cervical cerclage: evidence for perioperative management strategies. Am J Obstet Gynecol 2013;209:181-92.

Clinical Investigation / Araştırma

DOI: 10.4274/tjod.galenos.2018.21298

Turk J Obstet Gynecol 2019;16:7-14

The efficacy of Ankaferd Blood Stopper® in an experimental Asherman syndrome model created in rats

Sıçanlarda oluşturulan deneysel Asherman sendromu modelinde Ankaferd Blood Stopper®'in etkinliğinin gösterilmesi

Başak Büyük[1], Fatma Beyazıt[2]

[1]*Çanakkale Onsekiz Mart University Faculty of Medicine, Department of Histology and Embryology, Çanakkale, Turkey*

[2]*Çanakkale Onsekiz Mart University Faculty of Medicine, Department of Gynecology and Obstetrics, Çanakkale, Turkey*

Abstract

Objective: Asherman syndrome (AS) is a progressive disease involving menstrual disorders, recurrent pregnancy losses, and infertility developing as a result of partial or full blockade of the uterine cavity with adhesions. AS generally develops after trauma to the basal layer of the endometrium. In spite of a variety of methods such as adhesiolysis, inserting intrauterine devices, and administering high doses of estrogen, treatments remain insufficient. This study aimed to assess the effects of local intrauterine Ankaferd Blood Stopper (ABS) administration in inducing endometrial proliferation and building a normal endometrial layer in a rat model.

Materials and Methods: AS was induced in 30 female Wistar albino rats. The rats were randomized into three groups:
Group 1: AS group
Group 2: AS + serum physiologic (SP) group
Group 3: AS + ABS group
AS model was induced in all animals. The uterine horns were harvested after 15 days of therapy and investigated for inflammation, fibrosis, and immunohistochemical (IHC) markers.

Results: Compared with the other groups, fibrosis, and inflammation were significantly reduced in group 3 (chi-square, p=19.000, 0.001 and 26.365, <0.001, respectively). The IHC assessment showed that the tumor necrosis factor-α receptor levels were not different (Kruskal-Wallis H=0.091, p=0.995), but the interleukin (IL)-1β and IL-6 expression was reduced significantly in group 3 (H, p=18.706, <0.001, and 22.114, <0.001, respectively).

Conclusion: The therapeutic effects of local administration of ABS in rats with AS model were demonstrated histopathologically and immunohistochemically. Based on these results, ABS administration in addition to the current treatments for AS may increase the treatment success and reduce the need for advanced treatment.

Keywords: Asherman syndrome, intrauterine synechiae, fibrosis, inflammation

Öz

Amaç: Asherman sendromu (AS), uterin kavitenin yapışıklıklarla kısmi veya tam tıkanıklığı sonucu gelişen amenore ve diğer menstrüel bozukluklar, tekrarlayan gebelik kayıpları ve infertilite olarak tanımlanabilir. AS genellikle endometriyumun bazal tabakasına uygulanan travmadan sonra gelişir. Adeziolizis, rahim içi araç uygulaması ve yüksek dozda östrojen tedavisi gibi çeşitli yöntemlere rağmen, bu hastalığın tedavisi hala ciddi zorluklar içermektedir ve özellikle orta ve ağır olgularda genel prognoz zayıf kalmakta, yeni terapötik yaklaşımlara duyulan gereksinim artmaktadır. Bu çalışmanın amacı, sıçan modelinde intrauterin Ankaferd Blood Stoper (ABS) uygulamasının, endometrial proliferasyonu tetikleyerek normal bir endometrial mikroçevre oluşturmadaki etkilerini değerlendirmektir.

Gereç ve Yöntemler: Bu çalışmada 30 adet dişi Wistar albino cinsi sıçan rastgele 3 gruba ayrılmıştır. Gruplar;
Grup 1: AS grubu
Grup 2: AS + serum fizyolojik (SF) grubu
Grup 3: AS + ABS grubu
Her 3 gruba ait hayvanlarda AS modeli oluşturulduktan sonra grup 1'e hiçbir işlem yapılmadı. Grup 2'ye 15 gün süreyle vajinal yoldan kateterle SF, grup 3'e ise ABS uygulanmıştır. On beş günün sonunda uterin dokular çıkarılmıştır.

PRECIS: Ankaferd Blood Stopper can ameliorate intrauterine adhesions and inflammation on Asherman syndrome rat model.

Address for Correspondence/Yazışma Adresi: Başak Büyük MD,
Çanakkale Onsekiz Mart University Faculty of Medicine, Department of Histology and Embryology, Çanakkale, Turkey
Phone: +90 505 237 27 01 **E-mail:** basakbuyuk@comu.edu.tr **ORCID ID:** orcid.org/0000-0003-1817-2241
Received/Geliş Tarihi: 24.02.2018 **Accepted/Kabul Tarihi:** 24.11.2018

Bulgular: Diğer gruplarla karşılaştırıldığında, fibrozis ve enflamasyon değerleri grup 3'te anlamlı olarak azalmış bulundu (ki-kare, sırasıyla p=19,000, 0,001 ve 26,365, <0,001). İmmünohistokimyasal değerlendirme sonuçlarına göre tümör nekroz faktör-α reseptör değerleri diğer gruplara göre fark göstermezken (Kruskal-Wallis H=0,091, p=0,995), grup 3'te interleukin (IL)-1 ve IL-6 ekspresyonları anlamlı derecede azalmıştı (sırasıyla H, p=18,706, <0,001 ve 22,114, <0,001).
Sonuç: Sıçanlarda oluşturulan AS modelinde lokal ABS uygulamasının etkinliği histopatolojik ve immünohistokimyasal olarak gösterilmiştir. Sonuçta, AS'li hastalarda mevcut tedaviye ek olarak endometrial ABS uygulaması tedavinin başarısını artırabilir ve ileri tedavi basamaklarına gereksinimi azaltabilir.
Anahtar Kelimeler: Asherman Sendromu, intrauterin sineşi, fibrozis, enflamasyon

Introduction

Asherman syndrome (AS) is defined as the complete or partial obliteration of the uterine cavity with adhesions, resulting in amenorrhea or other menstrual aberrations, recurrent pregnancy loss, and infertility[1]. Although AS usually occurs after trauma to the basal layer of the endometrium due to dilatation and curettage, it can also be observed after a miscarriage, normal delivery, or medical abortion[2]. Uterine surgeries including myomectomy, metroplasty, and uterine septum resection are other potential causes of AS[2,3].

Early detection and appropriate treatment by the removal of the adhesions could significantly improve the reproductive outcome of infertile women, promote the repair and regeneration of the destroyed endometrium, and resolve abnormal uterine bleeding complications. In this context, the actual management strategy of AS must be based on three key steps, including main treatment, re-adhesion prevention, and restoring normal endometrium[2]. Unfortunately, despite some therapeutic options, these management strategies still pose a serious challenge, and the overall prognosis (especially in moderate and severe cases) remains poor,[4] indicating the need for new therapeutic approaches.

Ankaferd Blood Stopper (ABS) is a standardized herbal extract that has been used for hemostatic purposes in Anatolia for centuries[5]. ABS comprises standardized extracts of five medicinal herbs (i.e., Thymus vulgaris, Vitis vinifera, Glycyrrhiza glabra, Alpina officinarum, and Urtica dioica) that have special and unique effects on blood cells, endothelium, cellular proliferation, angiogenesis, and vascular dynamics[6]. The hemostatic properties of ABS provide a strict balance between thrombosis and hemorrhage by inducing a protein network formation with blood cells covering the primary and secondary hemostatic systems without disturbing individual coagulation factors. Besides its hemostatic properties, ABS also has considerable therapeutic benefits including the ability to act as an anti-inflammatory, anti-oxidant, and antineoplastic agent[6].

At the cellular level, ABS has wound healing properties, which makes it a perfect candidate for mucosal disorders. Although there are no studies in the literature investigating the effect of ABS on the endometrial mucosa, experimental studies investigating the unique effects of ABS on the reduction and duration of chemotherapy-induced oral mucositis demonstrated favorable outcomes[7]. Moreover, ABS is shown to be effective in decreasing inflammatory response and accelerating wound healing in caustic esophageal injuries without any adverse effects on the gastrointestinal system mucosa[8].

Using an experimental AS model, this study aimed to assess the effects of local intrauterine ABS administration in inducing endometrial proliferation and building a normal endometrial layer. Moreover, we aimed to determine whether local administration of ABS had an effect on the endometrial inflammatory response, which is associated with intrauterine adhesions (IUA)

Materials and Methods

Trial design

This study employed an experimental design with three randomization groups. The study was approved by the Çanakkale Onsekiz Mart University Experimental Animal Research Ethics Committee (approval number: 2016-04-05). Animal procedures were performed according to the "Guide for the Care and Use of Laboratory Animals" principles[9]. All steps of the study were conducted at the experimental research center of the university, open for supervision. Study reporting was performed in accordance with the CONSORT principles[10].

Participants

In this study, 30 female Wistar albino rats weighing 220-300 g were used. The rats were supplied by the university experimental research center. All rats were housed in pairs in appropriate cages in an animal room maintained at a standard humidity (45-50%) and temperature 22±2 °C with 12 hours light and 12 hours darkness, and were fed with standard food and water ad libitum.

Randomization

The 30 rats were randomly divided into 3 groups. Randomization was performed by giving the rats sequential numbers and randomly assigning them to groups using a random numbers table. The groups were as follows:
Group 1: AS + no intervention group
Group 2: AS + serum physiologic (SP) administration group
Group 3: AS + ABS administration group (Figure 1).

Interventions

Vaginal smears were taken from all animals at the beginning of the study, and the menstrual cycles were synchronized.

All animals were anesthetized using 50 mg/kg ketamine

hydrochloride (Ketalar®, Pfizer İlaçları Ltd. Şti. İstanbul, Turkey) and 10 mg/kg Xylazine (Alfazyne 2%, Ege Vet San. Tic. İzmir, Turkey) intraperitoneally. After achieving anesthesia, the vagina was entered using a 20-gauge branule. When the uterus bifurcation was reached, the branule was directed to the left to enter the left uterine horn. Here, 0.2 mL of trichloracetic acid (TCA) (IL-33, İstanbul İlaç San. Tic. AŞ, İstanbul, Turkey) was administered. After waiting 20 seconds, the branule was retracted, inducing the AS model.

Later, rats in all three groups were left for three menstrual cycles (15 days) to ensure the formation of the IUA. At the end of the 15th day, rats in group 1 received no further intervention, whereas rats in group 2 were administered SP 2 mL/day into the left uterine horn using a 20-gauge branule via the transvaginal route under 2-3% isoflurane gas anesthesia. This intervention was repeated daily for 15 days.

At the end of the 15 days after TCA administration, rats in group 3 were administered ABS, which had a sterility statement given by Refik Saydam Hygiene Center, Ministry of Health of Turkey, at a dose of 2 mL/day into the left uterine horn using a 20 gauge branule via the transvaginal route under 2-3% isoflurane gas anesthesia for 15 days. At the end of the 15 day treatment period, a midline abdominal incision was applied to all rats under ketamine/xylazine anesthesia, followed by removal of the left uterine horn. The harvested tissues were stored in 10% neutral buffered formalin for histologic investigation.

Outcomes

Histopathologic evaluation

After the tissue processing protocol, the right and left uterine horns were embedded in paraffin blocks. Sections at a thickness of 4-5 microns were obtained from the paraffin blocks using a Leica RM 2125 RTS microtome and stained with routine hematoxylin-eosin (H&E) and Masson's trichrome methods, followed by assessments with a Zeiss AxioScope A1 light microscope. Histopathologic assessments were completed and scored according to the method of Kilic et al[1]. for fibrosis and inflammation, which employs a scale ranging from 0 to 3 (0-no fibrosis, 1-minimal/loose fibrosis, 2-moderate fibrosis, and 3-dense fibrosis or 0-no inflammation, 1-presence of occasional lymphocytes and plasma cells, 2-presence of plasma cells, eosinophils, and neutrophils, and 3-presence of many inflammatory cells and micro abscesses).

Immunohistochemical evaluation

IHC methods using anti-tumor necrosis factor (TNF) alpha receptor, anti- interleukin (IL)-1beta and anti-IL-6 primary antibodies were used to show inflammation in the uterus. After fixation in 10% neutral buffered formalin and routine histologic monitoring, the uterus tissues embedded in paraffin blocks were sliced into 4-micron sections using a Leica RM 2125 RTS microtome and mounted on adhesive slides. Antigen retrieval was applied to the sections, which were left at 65 °C for 1 hour and deparaffinized in xylene before passing through an alcohol series for rehydration. The antigen retrieval IHC method was performed at this stage.

Later, the sections were left in 10 mm EDTA (Thermo Scientific lot: Ax201208) for 20 minutes in a 200-watt microwave oven and then cooled at room temperature for 20 minutes. After cooling, each slide had sections outlined with a PAP pen. Then, 3% H_2O_2 (Thermo Scientific lot: HP31685) was dropped onto the sections and left in place for 15 minutes. Later, the samples were washed in phosphate-buffered saline with a pH of 7.4.

Lastly, all sections were incubated with anti-TNF-α (EMD Millipore Corporation, clone 13F9.1, lot: #Q2573230), anti-IL-6 (Santa Cruz Biotechnology, INC, sc-28343, lot: #I1316) and anti-IL-1β (Cell Signaling Technology, lot: #12242) primary antibodies, marked with AEC chromogen (Thermo Scientific lot: HA33805), and counterstained with Mayer's hematoxylin before being covered. IHC assessment and scoring was completed according to the method of Jiang et al.,[11] who used a formula combining both the staining intensity and the percentage of positively stained cells.

Blinding

In this study, blinding was applied at the stage of the histopathologic investigation. Histopathologic assessments were performed by a single histologist, who had no knowledge about the groups.

Statistical Analysis

Data analysis was performed using the SPSS software (version 20.0). Normal distribution of the data was checked using the Shapiro-Wilk test. Mean, standard deviation, median, maximum, and minimum values were used for descriptive data presentation. The Kruskal-Wallis test was used to compare numerical data between the groups. The chi-square test was used to compare groups for categorical data. P values of less than 0.05 were accepted as statistically significant.

Results

Participant flow

All recruited animals were followed up until analysis with no data loss (Figure 1).

Recruitment

The study was conducted from June to November 2017. No problems were encountered necessitating cessation of the study.

Outcomes and estimation

Both fibrosis and inflammation were less in group 3 compared with the other groups (chi-square, p=19.000, 0.001 and 26.365, <0.001, respectively). Although the

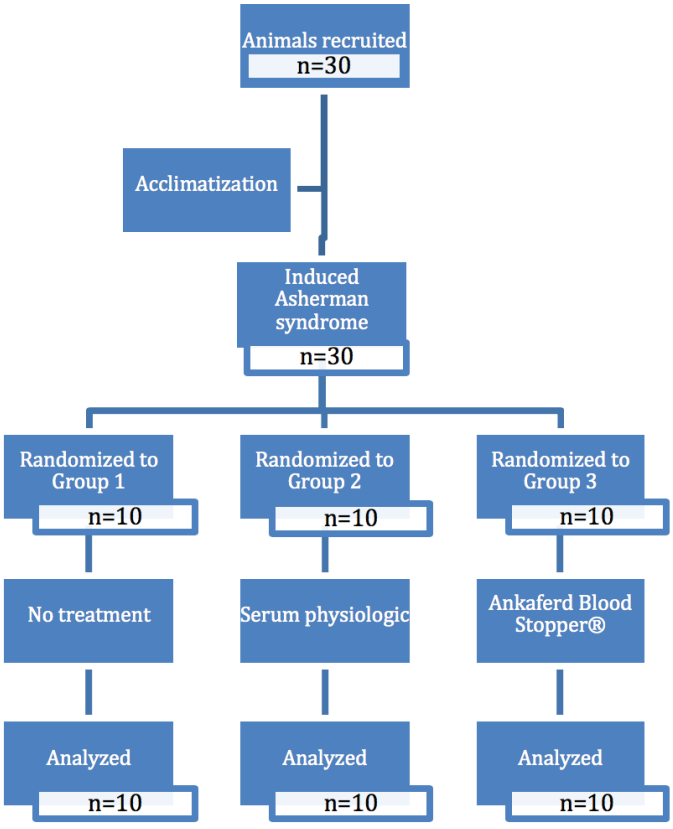
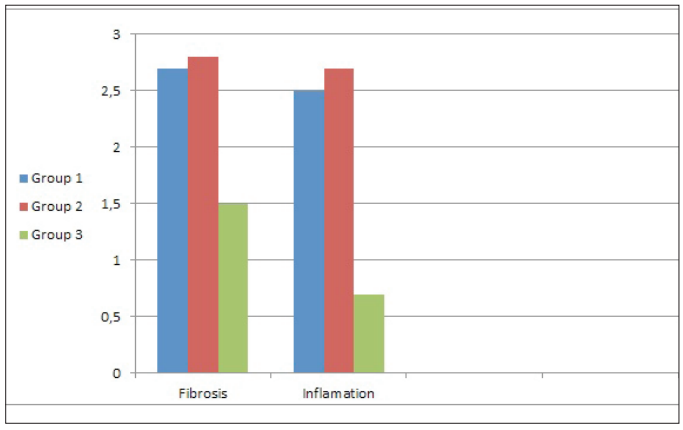


Figure 1. Randomization and participant flow



Graphic 1. Comparison of the fibrosis and inflamation levels of all groups

majority of animals in groups 1 and 2 had levels 3 fibrosis and inflammation, there were no cases of fibrosis or inflammation to this extent in group 3 (Table 1, Graphic 1).

The mean IL-1 and IL-6 scores were significantly lower in group 3, but there were no significant differences between the groups concerning the TNF-α receptor scores (Table 2, Graphic 2).

The histologic examination demonstrated that group 1 and group 2 showed significantly more fibrosis compared with group 3 (Figure 2). The fibrosis level was decreased in group 3, which received ABS. Also, group 1 and 2 showed significantly greater inflammatory cell infiltration compared with group 3 (Figure 2). Group 1 and 2 showed significantly greater histologic damage including increased cellular inflammation and fibrosis erosion compared with the ABS-treated group.

As to the IHC examination, group 3 had a less-prominent expression of IL-1 β and IL-6 compared with the other groups (Figure 3).

Discussion

In this study, we demonstrated that ABS administration in an AS rat model ameliorated uterine fibrosis and inflammation histopathologically. IL-1β and IL-6, which are acute inflammatory markers, were graded immunohistochemically, and it was shown that their levels were lower in the ABS-treated group. These findings show that local ABS administration can be helpful for treatment of AS.

AS is a health problem that is difficult to manage. Although not frequent perse, 13% of women treated for infertility are determined as having AS according to retrospective case series.[12] Causes such as pregnancy, previous infections, missed abortus, uterine surgery, and curettage have been accused in the etiology[13].

Histopathologically, the etiologic factors are thought to damage the stratum basalis layer, causing cyclic variations in the endometrium and IUA. The loss of the normal endometrial structure is accused as the underlying reason for infertility[12]. Once the basal layer of the endometrium is damaged, the stratum functionalis does not develop sufficiently during the menstrual cycle, and endometrial thickening does not occur. This large loss of the stratum basalis is called "endometrial sclerosis" and prepares the way for IUA[2].

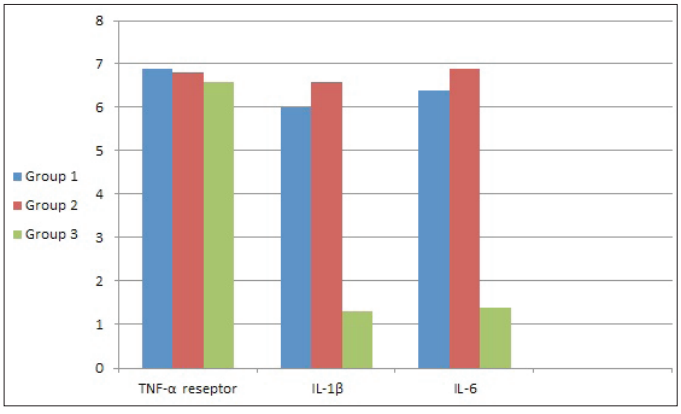
As to the medical literature, the treatment of AS generally

Table 1. Comparison of fibrosis and inflammation levels between the groups

			Fibrosis			Inflammation			
			1	2	3	0	1	2	3
Group	1	n (%)	0 (0)	3 (30.0)	7 (70.0)	0 (0)	1 (10)	3 (30)	6 (60)
	2	n (%)	0 (0)	2 (20.0)	8 (80.0)	0 (0)	0 (0)	3 (30)	7 (70)
	3	n (%)	5 (50.0)	5 (50.0)	0 (0)	3 (30)	7 (70)	0 (0)	0 (0)
Total		n (%)	5 (16.7)	10 (33.3)	15 (50.0)	3 (10)	8 (26.7)	6 (20.0)	13 (43.3)

appears to focus on opening the IUA and maintaining the opening in the uterine lumen[2,12,14]. In spite of surgically opening the IUA and administering medical treatment, adhesions recur in these patients. The resulting high infertility rates have revealed the need for the development of new treatment methods. Focusing on the endometrial stratum basalis in addition to the IUA during the treatment processes appears to be effective in ameliorating the inflammation. In this current study, ABS with previously proven hemostatic and anti-inflammatory effects were demonstrated to contribute to regulating the endometrial microenvironment, which may provide new aspects for the treatment of AS.

ABS is a medication containing standardized doses from five different plants. It has many proven effects on the hemostatic and immune system. In addition to antimicrobial, antineoplastic, antimutagenic, and antioxidant effects, it is also effective in wound healing[5,15,16]. The anti-inflammatory effect of ABS was shown in studies on cartilage tissue, gastric mucosa, pericardial tissue, and the liver[17-20]. A study on a caustic esophageal injury model, which demonstrated



Graphic 2. Comparison of the immunohistochemical results for TNF-α receptor, IL-1β and IL-6 levels of all groups

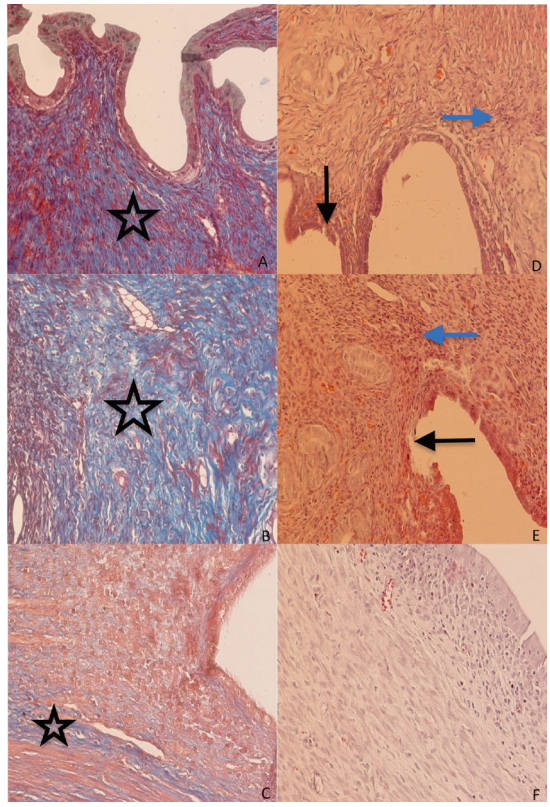


Figure 2. Microscopic comparison of fibrosis (A, B, C), and inflammation (D, E, F) in group 1 (no intervention), group 2 (serum physiologic), and group 3 (Ankaferd® Blood Stopper), respectively (x200 magnification). Group 1 and group 2 showed significantly greater fibrosis (stars) compared with group 3. Fibrosis level was decreased in group 3 with ABS treatment. Group 1 and group 2 showed significantly greater inflammatory cell infiltration (blue arrows) compared to group 3. Epithelial erosion areas that were seen in group 1 and group 2 (black arrows) were not seen in group 3. Group 1 and 2 showed significantly greater histologic damage including increased cellular inflammation, fibrosis, and erosion compared with the ABS-treated group

Table 2. Comparison of mean immunohistochemical evaluation scores between the groups

Group		Mean	SD	95% CI Lower	95% CI Upper	Min.	Max.	H*	p
TNF-α receptors	1	6.90	2.33	5.23	8.57	4	9	0.091	0.995
	2	6.80	2.04	5.34	8.26	4	9		
	3	6.60	2.36	4.91	8.29	3	9		
IL-1 Beta	1	6.00	2.90	3.92	8.08	2	9	18.706	<0.001
	2	6.60	2.22	5.01	8.19	4	9		
	3	1.30	0.82	0.71	1.89	0	3		
IL-6	1	6.40	1.50	5.32	7.48	4	9	22.114	<0.001
	2	6.90	1.44	5.86	7.94	6	9		
	3	1.40	0.96	0.71	2.09	1	4		

*Kruskal-Wallis H, SD: Standard deviation, CI: Confidence interval, Min: Minimum, Max: Maximum, TNF: Tumor necrosis factor, IL: Interleukin

improved inflammation in the esophagus and reduced stricture formation, led to the consideration that the anti-inflammatory effects of ABS may be beneficial for the adhesions in the endometrium[8]. In our study, as expected, ABS was shown to ameliorate fibrosis, largely treat adhesions, and reduce endometrial inflammation.

Different studies have revealed the therapeutic effects of ABS in fibrosis and wound healing in different tissues such as pulmonary parenchyma, skin injuries, and intraabdominal adhesions[21-23]. In particular, the regression of fibrosis after administering ABS appears hope-promising for the management of IUA due to inflammation and fibrosis. These findings coincide with the regression in fibrosis when ABS was administered in the AS model.

TNF-α is an inflammatory cytokine released mainly from monocyte-macrophages. After TNF-α is released, it affects the TNF-α receptors[24]. In an acute gastric mucosal injury model, TNF-α values increased secondary to injury, which was shown to regress with ABS administration, suggesting that ABS improved inflammation and reduced TNF-α expression[17].

In our study, the TNF-α receptor levels in the group administered ABS were not significantly different from the other groups. The TNF-α levels probably increased in response to the inflammatory process of the injury caused by TCA with an increase in TNF-α receptor expression, but the 15 day treatment may not suffice to reduce receptor expression. In fact, after ABS administration, the TNF-α levels reduced due to the anti-inflammatory effect as in all cytokines, with receptor expression reducing secondary to this decrease. Additionally, this study determined that IL-1β and IL-6 expression, increasing secondary to inflammation triggered by TCA administration to the uterus, reduced in IHC analyses. The reduction identified in the levels of the acute inflammatory markers of IL-1β and IL-6 clearly reveals the local anti-inflammatory effect of ABS.

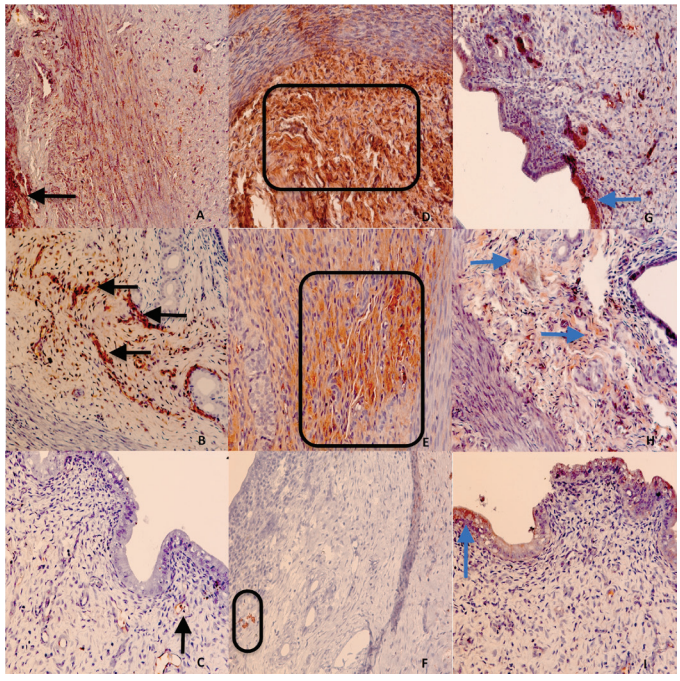


Figure 3. Immunohistochemical expression of IL-1 β in group 1 (A), group 2 (B) and group 3 (C); IL-6 in group 1 (D), group 2 (E), and group 3 (F); TNF-α receptor in group 1 (G), group 2 (H), and in group 3 (I) (x200 magnification). ABS treated group 3 demonstrated less prominent expression of IL-1 β (black arrows) and IL-6 (rectangles) compared with the other two groups (C and F, respectively). TNF-α receptor expression levels (blue arrows) were not significant between the groups (all figures taken x200 magnification)

Some researchers applied bone-marrow-derived stem cells (BMDSC) in the treatment of AS[25-29]. Autologous BMDSCs were administered with success to a patient receiving infertility treatment linked to IUA on 2011[29]. After this case report, a study was performed with six cases of grade 3 and 4 refractory AS on 2014[28]. BMDSCs were taken from iliac crest and implanted subendometrially via the transvaginal route. Patients were followed up at 3, 6, and 9 month intervals. Stem cell therapy was found efficient on AS and endometrial regeneration was obtained on patients in this study[28]. In 2016, a pilot study was performed with 16 patients whose ages ranged from 30 to 45 years[27]. In this study, researchers chose CD133+ BMDSCs, which have a neoangiogenetic effect, for stem cell therapy of refractory AS. CD133+ cells were isolated from peripheral blood and implanted subendometrially. At the end of this study, CD133+ BMDSC autologous cell therapy was found useful for treating patients with refractory AS and endometrial atrophy[27]. Apart from this, administration of adipose tissue-derived stem cells has been attempted as an alternative to BMDSCs and also here, successful results have been obtained[1].

However, as stem cell administration requires multidisciplinary work and extensive infrastructure, its application is limited to centers with advanced facilities. In our study, we demonstrated successful administration of ABS in AS requiring limited resources. Administration of ABS does not require complex laboratory conditions, but can easily be applied in a simple examination environment. Especially in the first stages of AS treatment, local uterine administration of ABS may be attempted, reserving stem cell treatment choices for advanced cases with no response to the initial treatments. Pregnancy rates in rodents receiving stem cell treatment are promising in the long term[25]. On the other hand, studies of stem cell treatment for AS have shown endometrial thickening in later periods[25-29].

Study Limitations

This study has some limitations. First, we did not attempt to induce pregnancy after treatment. Another limitation was that the duration of our experiment was 15 days. If the treatment duration had been longer, perhaps the fall in TNF-α

receptor levels would have reached statistically significant levels. However, in spite of this limitations, this is the first study in the literature to assess TNF-α receptor expression levels in an AS model. On the other hand, having included two control groups (one with no intervention and the other SF administration) is a strength of this experiment.

Generalizability

This study demonstrated the therapeutic effects of ABS on fibrosis and inflammation in Asherman syndrome. We think that the results are generalizable for further laboratory as well as clinical studies.

Conclusion

In conclusion, the ameliorative effects of ABS on fibrosis and inflammation may contribute to the treatment process of IUA. The therapeutic effects of local administration of ABS in rats with AS model were revealed histopathologically and immunohistochemically. Based on these results, ABS administration in addition to the current treatments for AS may increase the treatment success and reduce the need for advanced treatments.

Ethics

Ethics Committee Approval: The study was approved by the Çanakkale Onsekiz Mart University Experimental Animal Research Ethics Committee (approval number: 2016-04-05).
Informed Consent: It was obtained.
Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: B.B., Design: B.B., F.B., Data Collection or Processing: B.B., Analysis or Interpretation: B.B., Literature Search: B.B., F.B., Writing: B.B., F.B.
Conflict of interest: No conflict of interest was declared by the authors.
Financial Disclosure: This study was supported by Çanakkale Onsekiz Mart University Scientific Research Projects Coordination Unit (project ID: TSA-2016-1032).

References

1. Kilic S, Yuksel B, Pinarli F, Albayrak A, Boztok B, Delibasi T. Effect of stem cell application on Asherman syndrome, an experimental rat model. J Assist Reprod Genet 2014;31:975-982.
2. March CM. Management of Asherman's syndrome. Reprod Biomed Online 2011;23:63-76.
3. Conforti A, Alviggi C, Mollo A, De Placido G, Magos A. The management of Asherman syndrome: A review of literature. Reprod Biol Endocrinol 2013;11:118.
4. Yu D, Wong YM, Cheong Y, Xia E, Li TC. Asherman syndrome-one century later. Fertil Steril 2008;89:759-79.
5. Beyazit Y, Kurt M, Kekilli M, Goker H, Haznedaroglu IC. Evaluation of hemostatic effects of Ankaferd as an alternative medicine. Altern Med Rev 2010;15:329-36.
6. Haznedaroglu BZ, Beyazit Y, Walker SL, Haznedaroglu IC. Pleiotropic cellular, hemostatic, and biological actions of Ankaferd hemostat. Crit Rev Oncol Hematol 2012;83:21-34.
7. Atay MH, Aslan NA, Aktimur S, Buyukkaya P, Kelkitli E, Turgut M, et al. Safety and efficacy of ankaferd hemostat (ABS) in the chemotherapy-induced oral mucositis. UHOD - Uluslararasi Hematol Derg 2015;25:166-71.
8. Akbal E, Köklü S, Karaca G, Astarci HM, Koçak E, Taş A, Beyazıt Y, Topcu G, et al. Beneficial effects of Ankaferd Blood Stopper on caustic esophageal injuries: An experimental model. Dis Esophagus 2012;25:188-94.
9. Committee for the Update of the Guide for the Care and Use of Laboratory Animals. Guide for the Care and Use of Laboratory Animals. 2011. doi:10.17226/12910. https://www.ncbi.nlm.nih.gov/books/NBK54050/
10. Schulz KF, Altman DG, Moher D. The CONSORT Statement: Revised Recommendations for Improving the Quality of Reports of Parallel Group Randomized Trials (Turkish Translation). Euras J Fam Med 2013;2:1-10. https://www.sciencedirect.com/science/article/pii/S0140673600043373?via%3Dihub
11. Jiang JF, Deng YAN, Xue W, Zheng TP, Sun AJ. Increased Expression of Interleukin 37 in the Eutopic and Ectopic Endometrium of Patients with Ovarian Endometriosis. Reprod Sci 2016;23:244-8.
12. Capella-Allouc S, Morsad F, Rongières-Bertrand C, Taylor S, Fernandez H. Hysteroscopic treatment of severe Asherman's syndrome and subsequent fertility. Hum Reprod 1999;14:1230-3.
13. Schenker JG. Etiology of and therapeutic approach to synechia uteri. Eur. J. Obstet. Gynecol. Reprod. Biol 1996;65:109-13.
14. March CM, Israel R, March AD. Hysteroscopic management of intrauterine adhesions. Am J Obstet Gynecol 1978;130:653-7.
15. Simsek C, Selek S, Koca M, Haznedaroglu IC. Proteomic and transcriptomic analyses to explain the pleiotropic effects of Ankaferd blood stopper. SAGE Open Med 2017;5:205031211772256.
16. Uğur A, Saraç N, Çankal DA, Özle M. The antioxidant and antimutagenic activities of Ankaferd blood stopper, a natural hemostatic agent used in dentistry. Turkish J Med Sci 2016;46:657 63.
17. Hasgul R, Uysal S, Haltas H, Akyol S, Yuksel Y, Gurel A, et al. Protective effects of Ankaferd blood stopper on aspirin-induced oxidative mucosal damage in a rat model of gastric injury. Toxicol Ind Health 2014;30:888-95.
18. Nazli Y, Colak N, Alpay MF, Haltas H, Aksoy ON, Akkaya IO, et al. Assessment of the efficacy of Ankaferd blood stopper on the prevention of postoperative pericardial adhesions. Cardiovasc J Afr 2014;25:100-5.
19. Satar NYG, Akkoc A, Oktay A, Topal A, Inan K. Evaluation of the hemostatic and histopathological effects of Ankaferd Blood Stopper in experimental liver injury in rats. Blood Coagul Fibrinolysis 2013;24:518-24.
20. Taş A, Köklü S, Beyazit Y, Karaca G, Astarci HM, Akbal E, et al. Percutaneous ankaferd injection to in vivo liver tissue in comparison to ethanol in an experimental rat model. Clin Res Hepatol Gastroenterol 2011;35:549-53.
21. Metin B, Altinok T, Menevşe E, Esen H. Evaluation of the effects of ankaferd blood stopper on rabbits with paranchyme damage: An experimental study. Turkish J Thorac Cardiovasc Surg 2013;21:428-33.
22. Sezgin SÖ, Saraç GC, Samdanci E, Senol M. The effects of Ankaferd, a hemostatic agent, on wound healing. Turkderm Deri Hast ve Frengi Ars 2015;49:218-21.
23. Behçet AL, Kılıç H, Zengin S, Güler M, Taysi S, Yıldırım C, et al. Efficiency of Ankaferd Blood Stopper used in bleeding control on intraabdominal adhesions formed postoperatively. Clin Appl Thromb 2014;20:524-9.

24. Sethi G, Sung B, Aggarwal BB. TNF: a master switch for inflammation to cancer. Front Biosci 2008;13:5094-107.
25. Alawadhi F, Du H, Cakmak H, Taylor HS. Bone Marrow-Derived Stem Cell (BMDSC) transplantation improves fertility in a murine model of asherman's syndrome. PLoS One 2014;9.
26. Du H, Naqvi H, Taylor HS. Ischemia/reperfusion injury promotes and granulocyte-colony stimulating factor inhibits migration ofbone marrow-derived stem cells to endometrium. Stem Cells Dev 2012;21:3324-31.
27. Santamaria X, Cabanillas S, Cervello I, Arbona C, Raga F, Ferro J, Palmero J, Remohi J, Pellicer A, Simón C. Autologous cell therapy with CD133+ bone marrow-derived stem cells for refractory Ashermanıs syndrome and endometrial atrophy: a pilot cohort study. Hum Reprod 2016;31:1087-96.
28. Singh N, Mohanty S, Seth T, Shankar M, Bhaskaran S, Dharmendra S. Autologous stem cell transplantation in refractory Ashermanıs syndrome: a novel cell based therapy. J Hum Reprod Sci 2014;7:93.
29. Panchal S, Patel H, Nagori C. Endometrial regeneration using autologous adult stem cells followed by conception by in vitro fertilization in a patient of severe Asherman's syndrome. J Hum Reprod Sci 2011;4:43.

Clinical Investigation / Araştırma

DOI: 10.4274/tjod.galenos.2019.92260

Turk J Obstet Gynecol 2019;16:15-22

Effects of reviewing childbirth scenarios on choice of delivery type: a randomized controlled trial

Doğum senaryolarını gözden geçirmenin doğum şekli seçimine etkileri: randomize kontrollü bir çalışma

Massome Rasoli[1], Seyed Mohammad Mirrezaie[2], Ensieh Fooladi[3], Robabeh Zarouj Hosseini[4], Mahsa Fayaz[5]

[1]*Student Research Committee, School of Nursing and Midwifery, Shahroud University of Medical Sciences, Shahroud, Iran*

[2]*Shahroud University of Medical Sciences, Center for Health-Related Social and Behavioral Sciences Research, Shahroud, Iran*

[3]*Mazandaran University of Medical Sciences, School of Nursing and Midwifery, Sari, Iran*

[4]*Shahroud University of Medical Sciences, School of Nursing and Midwifery, Department of Medical Education, Shahroud, Iran*

[5]*Shahroud University of Medical Sciences, School of Public Health, Department of Epidemiology, Shahroud, Iran*

Abstract

Objective: The incidence of cesarean section (CS) was estimated as about 48% between 2000 and 2012 in Iran. This study was conducted to assess the effects of reviewing written childbirth scenarios on the selection of delivery method.

Materials and Methods: This randomized controlled trial was conducted in Shohada Women's Hospital in Behshahr, Mazandaran, Iran, from May to December 2015. A total of 223 women at 28 to 32 weeks of gestation were randomly allocated into three groups; the standard care (control), theory of planned behavior (TPB)-based education, and TPB education plus additional support via written childbirth scenarios (scenario). Participants were assessed at baseline (weeks 28-32) and intervention (week 37 of pregnancy) periods. Both intervention groups (TPB and scenario groups) participated in three learning sessions that were based on TPB, whereas the control group received routine care service.

Results: The frequencies of normal vaginal delivery (NVD) in the scenario, TPB, and control groups were 73.2%, 58.5%, and 45.7%, respectively (p=0.004). The results showed that the relative risks of CS decision in the scenario and TPB groups in comparison with the control group were both 0.87 and statistically significant (p=0.018 and p=0.013, respectively). The relative risk of choosing CS after the removal of obligatory CS cases in the scenario group compared with the control was 0.85.

Conclusion: Written childbirth scenarios that contain information on NVD and CS as additional support are effective educational tools for reducing CS rates.

Keywords: Cesarean section, theory of planned behavior, scenario, Iran

Öz

Amaç: İran'da, 2000-2012 yılları arasında sezaryen doğum (SD) insidansının yaklaşık %48 olduğu tahmin edilmektedir. Bu çalışmada, yazılı doğum senaryolarının gözden geçirilmesinin doğum yönteminin seçimine etkilerinin değerlendirilmesi amaçlanmıştır.

Gereç ve Yöntemler: Bu randomize kontrollü çalışma, Mayıs-Aralık 2015 tarihleri arasında, İran'ın Mazandaran Eyaleti Behshahr kentindeki Shohada Kadın Hastanesi'nde yürütüldü. Gebeliğinin 28 ila 32. haftasında olan toplam 223 kadın rastgele üç gruba ayrıldı. Bunlar; standart bakım (kontrol), planlanmış davranış teorisi temelli eğitim (PDT) ve PDT artı yazılı doğum senaryoları ile ek destek (senaryo) idi. Katılımcılar başlangıç (28-32. hafta) ve müdahale (gebeliğin 37. haftası) dönemlerinde değerlendirildi. Her iki müdahale grubu da (PDT ve senaryo grupları) PDT temelli üç öğrenme oturumuna katılmış, kontrol grubu ise rutin bakım hizmeti almıştır.

Bulgular: Normal vajinal doğum (NVD) sıklığı senaryo, PDT ve kontrol gruplarında sırasıyla %73,2, %58,5 ve %45,7 idi (p=0,004). Bulgular, senaryo ve PDT gruplarındaki SD kararının kontrol grubuyla karşılaştırıldığında göreceli risklerin her ikisinde 0,87 ve istatistiksel olarak anlamlı olduğunu göstermiştir (sırasıyla p=0,018 ve p=0,013). Senaryo grubundaki zorunlu SD olgularının çıkarılmasının ardından kontrole kıyasla SD seçiminde göreceli risk 0,85 idi.

Sonuç: NVD ve ek destek olarak SD hakkında bilgi içeren yazılı doğum senaryoları, SD oranlarını azaltmak için etkili eğitim araçlarıdır.

Anahtar Kelimeler: Sezaryen doğum, planlanmış davranış teorisi, senaryo, İran

PRECIS: Reviewing of normal vaginal delivery scenarios based on theory of planned behavior, in pregnant women could decrease relative risk of choosing CS compared with the control by 0.15.

Address for Correspondence/Yazışma Adresi: Mohammad Mirrezaie, MD,
Shahroud University of Medical Sciences, Center for Health-Related Social and Behavioral Sciences Research, Shahroud, Iran
Phone: +989123047255 **E-mail:** mirrezaie@shmu.ac.ir **ORCID ID:** orcid.org/0000-0001-5352-4244

Received/Geliş Tarihi: 05.10.2018 **Accepted/Kabul Tarihi:** 06.01.2019

Introduction

According to the World Health Organization (WHO), the ideal rate of cesarean section (CS) surgeries among healthy nulliparous women is 10% to 15%, but in many countries, it has continued to increase during the past three decades[1]. For example, CS rates in Latin America and the Caribbean, Europe, Asia, and Iran are 40.5%, 25%, 19.2%, and 48%, respectively[2,3].

Among pregnant Iranian women, 70% of those who opt for CS delivery do so for reasons other than medical needs, including personal request, spouse's priorities, and sometimes, inducement by physicians, so the mother's decision is one of the main factors for the increase in CS deliveries[4]. Evidence suggests that training women to develop skills of overcoming a fear of normal vaginal delivery (NVD), reducing labor pain[5], raising women's awareness regarding the advantages and disadvantages of a given delivery method[6,7], and engaging them in the process of decision-making on delivery method[8] are effective ways of reducing CS-favoring decisions. The effectiveness of health education programs is substantially related to applied educational theories and models[9]. One of the most important theories for predicting and understanding behavior is the theory of planned behavior (TPB), whose effectiveness has been confirmed in experimental studies[9,10]. The theory maintains that a particular behavior can be predicted on the basis of behavioral intention. Based on the theory of planned behavior, if a person assumes a behavior to be reflective of good conduct (positive attitude), they believe that others will regard it as valuable behavior (positive mental norm); accordingly, they will also intend to perform it.[11]

Childbirth stories or scenarios help women to select the best delivery mode because these narratives help them identify unknown aspects of delivery, reduce fear, and increase a sense of control[12]. Various studies have confirmed the importance of scenarios in learning[13,14]. Peer experiences, such as narrating childbirth stories, influence the choice of delivery method[15] because negative stories about NVD motivated pregnant women to select CS[16,17]. In a study conducted by Blainey and Slade (2015), the writing and sharing of childbirth stories by women who experienced traumatic delivery exerted positive mental health effects on the women[18].

To address this capability of scenario, the present study evaluated the effects of reviewing written childbirth scenarios on the decision of nulliparous women regarding delivery method (i.e., NVD vs. CS). The examination was based on TPB.

Materials and Methods

Study design

The study was designed as a parallel randomized controlled trial with three arms conducted in the prenatal clinic of the gynecology department of a governmental hospital, Shohada Women's Hospital of Behshahr, a northern Iranian city located in the Mazandaran province. The study received an ethics code (Ir.shmu.rec.1394.32) from Shahroud University of Medical Sciences, and was approved by Mazandaran University of Medical Sciences. The study was registered in the Iranian Center for Registration of Clinical Trials (IRCT2015052020706N2) and the study protocol is available in http://fa.search.irct.ir/search?query=IRCT2015052020706N2.

Participants

All pregnant women who visited the clinic from May through December 2015 were considered for the study. To recruit suitable participants, we approached eligible women during their first visit to the clinic. The inclusion criteria were primigravid pregnant women, single-fetus pregnancies, gestational age of 28 to 32 weeks, maternal age of 18 to 35 years, no history of frequent abortion, and no contraindication for normal childbirth. The exclusion criteria were unwillingness to participate in training courses, absence from one of the training sessions, and obstetric or medical contraindications to vaginal birth and/or trial of vaginal birth (e.g., placenta previa). All participants gave written informed consent.

Randomization and masking

Using computer-generated random blocks of six, we randomly assigned 233 eligible participants to three study groups with an allocation ratio of 1:1, namely, two intervention groups [TPB-based education (TPB group) and TPB-based education plus additional support via written childbirth scenarios (scenario group)], each consisting of 74 participants, and a control group, who received standard care (75 participants) (Figure 1). The randomization was supervised by statisticians who were not involved in the enrolment or follow-up of participants. The clinic's employees could not be subjected to masking from the group assignments because of the nature of the interventions.

Procedures

For the implementation of TPB-based education and TPB-based education plus additional support with written childbirth scenarios, we trained the healthcare staff of the prenatal and maternity departments of the intervention facility by using a WHO course[18]. Considering the lack of a standard questionnaire in this field, a study-specific instrument was developed on the basis of TPB resources and textbooks and the results of previous studies[19-21]. The questionnaire comprised 80 questions distributed across two sections. The first section, whose composition was based on the questions used by Hildingsson et al.,[20] revolves around awareness related to childbirth, socio-demographic characteristics, and obstetric background. The specific items falling under this section are those related to demographics (age, occupation, and education of the expectant mother

and her spouse, place of residence, and economic status), midwifery issues (e.g., date of last menstrual period, probable date of delivery, gestational age, gravity, parity, number of embryos in the current pregnancy), awareness (10 items on awareness about normal childbirth and CS, pros and cons of different delivery methods for the mother and the fetus, and scientific indications of CS), and results evaluation (10 items). To assess the economic situation, the questionnaire also presents questions regarding household assets. Using principal components analysis and considering three layers, we classified economic status into high, medium, and low levels. The second section of the questionnaire consists of the end-structures of TPB, including attitude toward an act (16 items), subjective norms (5 items), perceived behavioral control (3 items), behavioral intention (2 items), and behavioral performance (1 item).

Pregnant women belonging to the intervention groups were scheduled for educational TPB-based interventions in groups of 10 to 12 people; however, the control group was administered only routine services by the healthcare staff. The educational interventions were conducted at three 60-minute weekly sessions. The structures of the sessions are presented

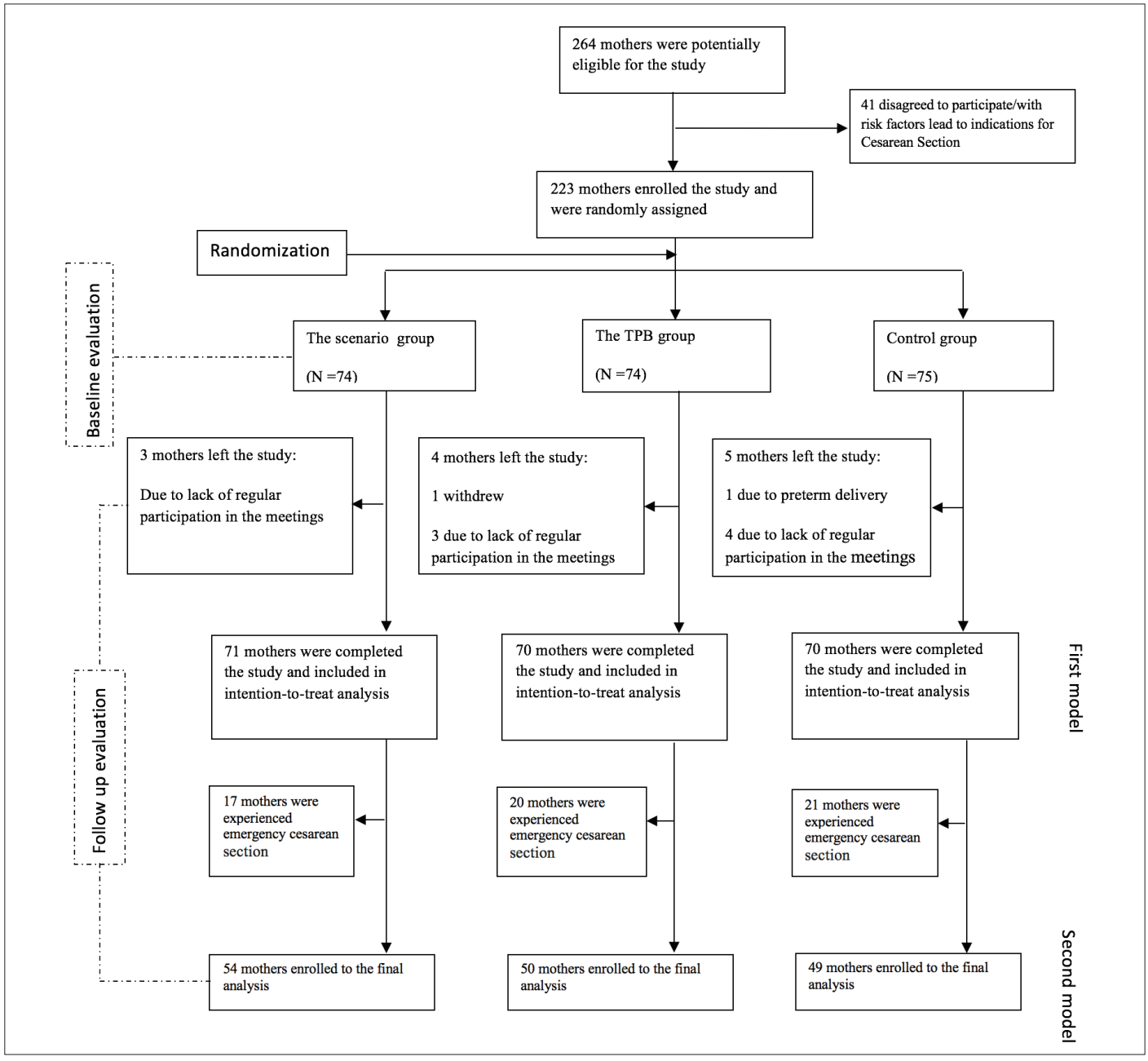


Figure 1. Trial profile

TPB: Theory of planned behavior

in Table 1 (see appendix 1 for more details regarding the training). Both the interventional groups and the control group received a training manual on the advantages of NVD and the non-pharmacologic methods of pain relief.

The written scenarios, which were composed on the basis of actual situations, consisted of six positive stories about the physiological childbirth process with non-pharmacologic pain relief methods that were helpful to mothers and two negative stories about unscientific CS deliveries that were performed at the mothers' request (appendix 2). The scenario group was asked to read the scenarios before the next meeting and identify the points that they believed were useful, interesting, and functional.

At each subsequent session, the stories were reviewed with the participants to help them improve their understanding of difficult sentences, increase the attractiveness of the stories, and create a deep connection to the narratives. The practical strategies for story review included pausing, thinking, and retelling. Encouraging individuals to pause after reading part of a story, ponder over this part, and retell it to others helps them better learn about the story; it also allows them to determine how much of the story they can recall[22]. After a review of each scenario, the participants were asked to express their feelings and impressions of each scenario.

The participants of the three groups were asked to complete a pre and post-test questionnaire at 37 weeks of pregnancy and one month after the completion of the educational interventions, respectively.

Outcomes

We assessed one primary outcome, that is, the proportion of mothers who intended to opt for NVD, within one month after the completion of the educational interventions. This decision was based on TPB's argument that intention is the main factor that determines behavior (see Introduction). We also assessed a secondary outcome, that is, the prevalence of NVD selection among the mothers.

Statistical Analysis

Mean ± SD (standard deviation) and frequency were reported as descriptive statistics. ANOVA was performed to compare the continuous outcomes of the groups. Chi-square and Fisher's exact tests were also performed for categorical variables. Log-binomial regression models were used to explore the relative risk (RR) of the decision to undergo a CS (first model) and the risk of CS delivery (second model) in the groups for which other covariates were adjusted. In the modeling, the control group was considered as a reference category. All the analyses were conducted in STATA version 12, and the significance level was considered as 0.05.

Results

As previously stated, we randomly assigned 223 participating mothers to three groups; 74, 74, and 75 women were assigned to the scenario (PTB + written scenarios), PTB (PTB-based education only), and control (routine care) groups, respectively. A total of 211 participants were included in the analysis. The loss to follow-up rate was about 5%. The mean age of the women was 24.9±4 years. Some of them had diplomas (34.6%), and many were housewives (88.6%). The baseline characteristics of the women according to groups are shown in Table 2.

The primary decision for delivery chosen by the study groups at the beginning of the study showed no significant differences (Table 2). However, the frequencies of NVD selection in the scenario, TPB, and control groups as a primary outcome were 73.2%, 58.5%, and 45.7%, respectively (p=0.004).

In the first log-binomial regression model, the regression was run on the decision for CS as an outcome variable in the presence of other covariates. The results showed that the RR of the decision to undergo CS in the scenario and TPB groups in comparison with the control group were both 0.87 and statistically significant (p=0.018 and p=0.013, respectively).

Table 1. Intervention by study groups

	What interventions were delivered and for whom		
Training session	Control group	TPB group	TPB plus written scenarios group
First session	None	Brainstorming about advantages of NVD Talking about positive outcomes	Brainstorming about advantages of NVD Talking about positive outcomes Reviewing written scenarios (1-3)
Second session	None	Role playing Group discussion	Role playing Group discussion Reviewing written scenarios (4-6)
Final session	None	Non-pharmacologic methods of labor pain relief Talking about facilitating factors Small steps of behavioral intention	Non-pharmacologic methods of labor pain relief Talking about facilitating factors Small steps of behavioral intention Reviewing written scenarios (7-8)

TPB: Theory of planned behavior, NVD: Normal vaginal delivery

Table 2. Baseline characteristics of participants

	Overall (n=211)	Scenario (n=71)	Theory (n=70)	Control (n=70)	p value
Level of education					
Elementary	6 (2.8%)	3 (4.2%)	2 (2.9%)	1 (1.4%)	
Under diploma	40 (18.9%)	14 (19.7%)	12 (17.1%)	14 (20%)	
Diploma	73 (34.6%)	24 (33.8%)	26 (37.1%)	23 (32.9%)	0.984***
Associate degree	25 (11.8%)	7 (9.9%)	9 (12.9%)	9 (12.9%)	
BSc	66 (31.3%)	23 (32.4%)	20 (28.6%)	23 (32.9%)	
MSc	1 (0.5%)	0 (0%)	1 (1.4%)	0 (0%)	
Age (mean ± SD)	24.9±4	25±4	25.3±4.4	24.4±3.8	0.421**
Occupation					
Housewife	187 (88.6%)	64 (90.1%)	60 (85.7%)	63 (90%)	0.687*
Employed	24 (11.4%)	7 (9.9%)	10 (14.3%)	7 (10%)	
Residency					
Urban	127 (60.2%)	(59.2%)	(61.4%)	(60%)	0.959*
Rural	83 (39.3%)	29 (40.8 %)	27 (38.6%)	27 (38.6%)	
Socio-economic status					
Low	88 (41.7%)	(40.8%)	32 (45.7%)	27 (38.6%)	0.834*
Middle	53 (25.1%)	(22.5%)	(24.3%)	20 (28.6%)	
High	70 (33.2%)	(36.6%)	30%)	23 (32.9%)	
Primary decision for delivery					
NVD	119 (56.4%)	(54.9%)	37 (52.9%)	43 (61.4%)	0.567*
CS	92 (43.6%)	(45.1%)	33 (47.1%)	27 (38.6%)	
Awareness	6±2.1	5.9±2.1	6±2.1	6±2.1	0.936**
Attitude	36.1±9.3	35.2±7.9	36.2±11.6	37.1±10.7	0.537**
Evaluation of results	23±5.2	22.7±4.3	23.1±6.4	23.3±4.7	0.755**
Perceived behavioral control	6.2±2.5	6.2±2.3	6±2.5	6.5±2.7	0.501**
Subjective norms	11.1±4.3	12.3±3.9	11.6±4.1	11.3±4.1	
Relative and friend's suggestion					0.609***
CS	83 (39.3%)	(40.8%)	30 (42.9%)	24 (34.3%)	
NVD	93 (44.1%)	30 (42.3%)	27 (38.6%)	36 (51.4%)	
Neutral	35 (16.6%)	(16.9%)	13 (18.6%)	10 (14.3%)	
Relative and friend's type of delivery					0.927*
CS	70 (33.2%)	(36.6%)	21 (30%)	23 (32.9%)	
NVD	58 (27.5%)	(26.8%)	(27.1%)	20 (28.6%)	
Both	83 (39.3%)	(36.6%)	30 (42.9%)	27 (38.6%)	
Mother's type of delivery					0.847***
CS	23 (10.9%)	(9.9%)	8 (11.4%)	8 (11.4%)	
NVD	177 (83.9%)	(83.1%)	60 (85.7%)	58 (82.9%)	
Both	11 (5.2%)	(7%)	2 (2.9%)	4 (5.7%)	
Husband's suggestion					0.961*

Table 2. Continued					
CS	66 (31.3%)	(33.8%)	22 (31.4%)	20 (28.6%)	
NVD	85 (40.3%)	28 (39.4%)	(38.6%)	30 (42.9%)	
Neutral	60 (28.4%)	(26.8%)	(30%)	20 (28.6%)	
Mother's suggestion					0.851*
CS	61 (28.9%)	(31%)	20 (28.6%)	19 (27.1%)	
NVD	118 (55.9%)	(54.9%)	37 (52.9%)	42 (60%)	
Neutral	32 (15.2%)	(14.1%)	13 (18.6%)	9 (12.9%)	

*Chi-square test, **ANOVA, ***Fisher exact test
SD: Standard deviation, NVD: Normal vaginal delivery, CS: Cesarean section

Table 3. Log-binomial regression on CS decision (first model) and CS delivery (second model)

	Log-binomial regression models					
	First model (n=211)			Second model (n=153)		
	Relative risk	95% CI	p value	Relative risk	95% CI	p value
Group						
Control	Reference	Reference	-	Reference	Reference	-
Scenario	0.87	0.78-0.98	0.018	0.85	0.75-0.95	0.005
TPB	0.87	0.77-0.97	0.013	0.92	0.80-1.04	0.184
Primary decision	1.26	1.18-1.35	<0.001	1.18	1.09-1.28	<0.001
Education	1.04	1.01-1.07	0.004	1.06	1.03-1.10	0.001
Knowledge score*	0.98	0.96-0.99	0.005	-	-	-
Perceived behavioral control score*	0.97	0.95-0.99	0.001	0.97	0.95-0.99	0.004

*Difference between pre and post test, TPB: Theory of planned behavior, cs: Cesarean section

The findings also indicated that the primary decision to undergo CS (RR=1.26, p<0.001) and a high level of education (RR=1.04, p=0.004) were associated with an increased RR of the decision to undergo CS. An increase in perceived behavioral control and in awareness scores decreased the RR of the decision for CS (Table 3).

The number of CS deliveries performed in this study was 86 (40.76%), among which 58 (67.44%) and 28 (32.56%) were performed for emergency and elective reasons, respectively. In the second model, women for whom obligatory CS delivery was prescribed were excluded, leaving us with a sample of 153 participants. The log-binomial regression with CS as an outcome variable in the presence of covariates was repeated. The findings showed that the RR of CS in the scenario group was 0.85, which was statistically significant (p=0.005). No statistical difference was found between the TPB and control groups. The primary decision to opt for CS (RR=1.18, p<0.001) and higher level of education (RR=1.06, p=0.001) increased the RR of CS. Increased in the difference of perceived behavioral control score between pre and post-test was decreased RR of CS decision (RR=0.97. p=0.004) (Table 3).

Discussion

Our results showed that using positive stories about childbirth in the TPB model is an effective strategy for enhancing the predilection of pregnant women to select NVD as a delivery mode. In both intervention groups, TPB strongly predicted behavioral intention. The interventions were also associated with a significantly reduced prevalence of CS decision in the intervention groups. Although the written scenarios exerted the greatest effects on the behavioral constructs, no significant difference in behavioral performance was found between the TPB and control groups.

In a study conducted in northern Iran, Besharati et al.[19] found that intervention based on TPB effectively reduced the incidence of elective CS and increased the selection of NVD. However, only half of the participants of intervention group who exhibited an intention to select NVD actually chose this type of delivery.

In the current research, after the removal of emergency CS cases, the log-binomial regression showed that the RR of CS decision significantly decreased in the scenario group compared with the risk observed in the control group (p=0.005). The RR of CS decision in this group relative to

the control and TPB groups decreased by 0.15 and 0.07, respectively. In a study performed by Regan et al.,[23] 71.2% of the women stated that the most important and valuable resource that helped them to select a method of delivery was stories of childbirth by other women.

In our study, stories as powerful educational tools had a significant effect on the behavioral tendency of the nulliparous women to reduce CS decisions. This effect seems to have originated from the characteristics of the stories, which are believable and memorable. They are believable because they originated from human and pseudo-human experiences that people are inclined to perceive as authentic scientific sources. They are memorable because they inspire involvement in the characters' intentions and performance[24]. Bruner (1986) argued that stories developed an awareness and performance outlook in humans and that these two were part of human intentions; an audience becomes involved with a story at both levels, and through this involvement, enters the minds of characters and achieves an improved understanding of story meanings[25].

Hearing negative stories about normal childbirth was one of the factors that triggered the decision to opt for CS among women in a study conducted in northern Iran[16]. In a study performed in North Carolina, Romero et al. [26] discovered that the formation of the initial fear of normal childbirth in some women was due to the fact that they heard unfavorable stories from friends and acquaintances. On the other hand, confidence in one's ability to have NVD and the belief that vaginal childbirth is a normal method of giving birth were among the principal drivers of NVD decision.

Our study showed that the intention to opt for vaginal delivery in the two intervention groups significantly differed from that in the control group. After the educational interventions, the changes in women's awareness and perceived behavioral control were associated with a reduced risk of CS decision. Additionally, a direct correlation was found between the initial decision of women to undergo CS and their levels of education and the risk of CS decision. Rahman et al.[27] (2014) and Rajabi et al. [28] (2015) in Iran and in Malaysia found that high levels of education among mothers were associated with an increase in CS delivery.

In the scenario group, projection with the heroines in the stories helped the pregnant women recognize their latent capacities and eliminate their unknown fears and concerns. Lindesmith and McWeeny[29] found that a story established a connection between women and their shared backgrounds and that sharing stories regarding childbirth, as a seminal experience, should officially be taken into consideration in childbirth training programs. In a review, Webb et al.[30] (2010) found that interventions based on TPB had a significant effect on behavior. The authors emphasized that the closer the integration of intervention with behavioral change techniques, the better the performance effects derived. In the NVD scenarios, the heroine-like roles of the characters and confidence in women's power to manage labor pains improved the perceived behavioral control capabilities of the readers. For these women, therefore, normal childbirth was viewed as achievable and realistic. A study showed that nulliparous women regarded childbirth stories as a way of facilitating the selection of normal childbirth. The study also indicated that creating new positive real stories or narratives effectively reduced the choice of CS delivery and increased the motivation to select normal childbirth[31]. In the current study, the infrastructures of the stories helped instill the truth in the mindsets of the women and improve the decision-making process underlying childbirth.

To the best of our knowledge, although childbirth stories are often used in qualitative research, in the present study we used them in a clinical trial to determine their efficacy. A limitation worth mentioning is that the effects of traumatic delivery scenarios on the choice of delivery method were not examined, also the study involved nulliparous women only.

This present study shows that a combination of childbirth scenarios and training based on TPB could be used to reduce the incidence of unnecessary CS deliveries.

Acknowledgments

We would like to thank the Shahroud University of Medical Sciences for supporting the study (grant number: 93106) and Mazandaran University of Medical Sciences who took part in this study. We thank the study mothers for their participation and time; personnel of the participating clinic for their time and efforts.

Ethics

Ethics Committee Approval: The study received an ethics code (Ir.shmu.rec.1394.32) from Shahroud University of Medical Sciences, and was approved by Mazandaran University of Medical Sciences. The study was registered in the Iranian Center for Registration of Clinical Trials (IRCT2015052020706N2) and the study protocol is available in http://fa.search.irct.ir/search?query=IRCT2015052020706N2.

Informed Consent: All participants gave written informed consent.

Peer-review: External and internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: GYN ward staff. Concept: S.M.M., M.R., Design: S.M.M., M.R., M.F.,

Data Collection or Processing: M.R., R.Z.H., E.F., Analysis or Interpretation: S.M.M., M.R., M.F., Literature Search: M.R., R.Z.H., E.F., Writing: S.M.M., M.R., M.F., R.Z.H., E.F.

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

Financial Disclosure: This study was supported by grant No 940167 from Shahroud University of Medical Sciences and granted approval by Mazandaran University of Medical Sciences.

References

1. Gibbons L, Belizán JM, Lauer JA, Betrán AP, Merialdi M, Althabe F. The global numbers and costs of additionally needed and unnecessary caesarean sections performed per year: overuse as a barrier to universal coverage. World health report 2010;30:1-31.
2. Betrán AP, Ye J, Moller A-B, Zhang J, Gülmezoglu AM, Torloni MR. The Increasing Trend in Caesarean Section Rates: Global, Regional and National Estimates: 1990-2014. PloS one 2016;11:e0148343.
3. Azami-Aghdash S, Ghojazadeh M, Dehdilani N, Mohammadi M, Asl Amin Abad R. Prevalence and Causes of Cesarean Section in Iran: Systematic Review and Meta-Analysis. Iran J Public Health 2014;43:545-55. PubMed PMID: 26060756. Pubmed Central PMCID: PMC4449402. Epub 2015/06/11. eng.
4. Eslami S, Aslani A, Tara F, Ghalichi L, Erfanian F, Abu-Hanna A. The impact of a computerized decision aid on empowering pregnant women for choosing vaginal versus cesarean section delivery: study protocol for a randomized controlled trial. Trials 2015;03;16:549. PubMed PMID: 26634922. Pubmed Central PMCID: PMC4669643. Epub 2015/12/05. eng.
5. Edmonds JK, Cwiertniewicz T, Stoll K. Childbirth Education Prior to Pregnancy? Survey Findings of Childbirth Preferences and Attitudes Among Young Women. J Perinat Educ 2015;24:93-101. PubMed PMID: 26957892. Pubmed Central PMCID: PMC4744342. Epub 2015/01/01. eng.
6. Boz I, Teskereci G, Akman G. How did you choose a mode of birth?Experiences of nulliparous women from Turkey. Women Birth 2016;29:359-67. PubMed PMID: 26846560. Epub 2016/02/06. eng.
7. Hauck YL, Stoll KH, Hall WA, Downie J. Association between childbirth attitudes and fear on birth preferences of a future geAnuesrtartaiolinan o f parents. Women Birth 2016;29:511-517. doi: 10.1016/j.wombi.2016.05.001. Epub 2016 May 24. PubMed PMID: 27233945. Epub 2016/05/29. eng.
8. McCants BM, Greiner JR. Prebirth Education and Childbirth Decision Making. International Journal of Childbirth Education 2016;31.
9. Glanz K, Rimer BK, Viswanath K. Health behavior and health education: theory, research, and practice: John Wiley & Sons; 2008.
10. Sharma M. Theoretical foundations of health education and health promotion: Jones & Bartlett Publishers; 2016.
11. Ajzen I. The theory of planned behaviour. Organizational Behaviour and Human Decision Processes, 50, 179-211. De Young 1991:509-26.
12. Nichols FH, Humenick SS. Childbirth education: practice, research and theory: WB Saunders Company; 2000.
13. Bremner MN, Aduddell K, Bennett DN, VanGeest JB. The use of human patient simulators: best practices with novice nursing students. Nurse Educ 2006;31:170-4. PubMed PMID: 16855487. Epub 2006/07/21. eng.
14. Yackley LE. Storytelling: A key to adult learning: University of Delaware; 2007.
15. Kornelsen J, Hutton E, Munro S. Influences on decision making among primiparous women choosing elective caesarean section in the absence of medical indications: findings from a qualitative investigation. J Obstet Gynaecol Can 2010;32:962-9. PubMed PMID: 21176305. Epub 2010/12/24. eng.
16. SHirvani MA, Bagheri-Nesami M, Tayebi T. Exploration of women birth experiences role in decisiding the type of next delivery. J Urmia Nurs Midwifery Fac 2014;12:286-96.
17. Rahmati N. An exploratory study of women's experiences of fear about childbirth: University of British Columbia; 2014.
18. Organization WH. Care in normal birth: a practical guide. 1996.
19. Besharati F, Hazavehei S, Moeini B, Moghimbeigi A. Effect of educational interventions based on theory of planned behavior (TPB) in selecting delivery mode among pregnant women referred to rasht health centers. ZUMSJ 2011;19:94-106.
20. Hildingsson I, Radestad I, Rubertsson C, Waldenstrom U. Few women wish to be delivered by caesarean section. BJOG 2002;109:618-
23. PubMed PMID: 12118637. Epub 2002/07/18. eng.
21. Fathian Z, Sharifirad GR, Hasanzadeh A. Study of the effects of Behavioral Intention Model education on reducing the cesarean rate among pregnant women of Khomeiny-Shahr, Isfahan, in 2006. 2007.
22. Robb L. Reading strategies that work: Teaching your students to become better readers: Scholastic Inc.; 1996.
23. Regan M, McElroy KG, Moore K. Choice? Factors That Influence Women's Decision Making for Childbirth. J Perinat Educ 2013;22:171-80. PubMed PMID: 24868129. Pubmed Central PMCID: PMC4010239. Epub 2014/05/29. eng.
24. Taylor K, Marienau C, Fiddler M. Developing Adult Learners: Strategies for Teachers and Trainers. The Jossey-Bass Higher andAdult Education Series: ERIC; 2000.
25. Minds A. Possible Worlds Cambridge. MA Harvard UP. 1986.
26. Romero ST, Coulson CC, Galvin SL. Cesarean delivery on maternal request: a western North Carolina perspective. Maternal and child health journal. 2012;16:725-34. PubMed PMID: 21400201. Epub 2011/03/15. eng.
27. Rahman M, Shariff AA, Shafie A, Saaid R, Tahir RM. Determinants of Caesarean Risk Factor in Northern Region of Bangladesh: A Multivariate Analysis. Iran J Public Health 2014;43:16-27.
28. Rajabi A, Maharlouei N, Rezaianzadeh A, Rajaeefard A, Gholami A. Risk factors for C-section delivery and population attributable risk for C-section risk factors in Southwest of Iran: a prospective cohort study. Med J Islam Repub Iran. 2015;16;29:294. eCollection 2015.
29. Lindesmith KA, McWeeny M. The power of storytelling. J Contin Educ Nurs 1994;25:186-7.PubMed PMID: 8046058. Epub 1994/07/01. eng.
30. Webb TL, Joseph J, Yardley L, Michie S. Using the internet to promote health behavior change: a systematic review and meta-analysis of the impact of theoretical basis, use of behavior change techniques, and mode of delivery on efficacy. Journal of medical Internet research. 2010:17;12:e4. PubMed PMID: 20164043. Pubmed Central PMCID: PMC2836773. Epub 2010/02/19. eng.
31. Happel-Parkins A, Azim KA. At pains to consent: A narrative inquiry into women's attempts of natural childbirth. Women and birth: journal of the Australian College of Midwives. 2016:29;310-20. PubMed PMID: 26639037. Epub 2015/12/08. eng.

Clinical Investigation / Araştırma

DOI: 10.4274/tjod.galenos.2019.02679

Turk J Obstet Gynecol 2019;16:23-8

Prenatal diagnosis of persistent left superior vena cava: a retrospective study of associated congenital anomalies

Persistan sol superior vena kavanın prenatal tanısı: İlişkili yapısal anomalilerin retrospektif değerlendirmesi

Mehmet Özsürmeli[1], Selim Büyükkurt[2], Mete Sucu[2], Erol Arslan[2], Çiğdem Akçabay[2], Selahattin Mısırlıoğlu[2], Masum Kayapınar[2], Nazan Özbarlas[3], Süleyman Cansun Demir[2], Cüneyt Evrüke[2]

[1]*University of Health Sciences, Derince Training and Research Hospital, Clinic of Obstetrics and Gynecology, Kocaeli, Turkey*

[2]*Çukurova University Faculty of Medicine, Department of Obstetrics and Gynecology, Perinatology Unit, Adana, Turkey*

[3]*Çukurova University Faculty of Medicine, Department of Pediatrics, Pediatric Cardiology Unit, Adana, Turkey*

Abstract

Objective: To evaluate persistent left superior vena cava (PLSVC) cases according to associated cardiac, extracardiac, and chromosomal anomalies in the prenatal period and to review their outcomes.
Materials and Methods: The data of patients with a prenatal diagnosis of PLSVC between January 2013 and December 2017 were reviewed retrospectively.
Results: Data of 32 cases were reviewed. Nineteen (60%) cases were associated with cardiac defects, 5 (15%) were associated with both cardiac and extracardiac defects, and 8 (25%) had no associated anomalies. Two fetuses had karyotype anomalies. All patients with isolated PLSVC survived. Among the cases associated with extracardiac anomalies, cardiac anomalies, and with both extracardiac and cardiac anomalies, the survival rate was 40%, 40%, and 25%, respectively. Outcome was more favorable in cases with isolated PLSVC (100% vs. 40%).
Conclusion: Prenatally diagnosed PLSVC is associated with cardiac and extracardiac anomalies in the majority of cases. The prognosis is good in isolated cases, but worsens when accompanied by cardiac or extracardiac anomalies.
Keywords: Congenital anomaly, superior vena cava, prenatal diagnosis, ultrasound

Öz

Amaç: Prenatal dönemde tanısı konmuş persistan sol superior vena kavalı (PSSVK) olgularla ilişkili yapısal anomalilerin değerlendirilmesi amaçlanmıştır.
Gereç ve Yöntemler: Ocak 2013-Aralık 2017 tarih aralığında tanısı konmuş PSSVK'lı olguların verileri retrospektif olarak değerlendirildi.
Bulgular: Otuz iki olgunun verileri değerlendirildi. On dokuz (%60) fetusta başka kalp anomalileri, 5 fetusta (%15) başka kalp dışı anomaliler mevcuttu. Sekiz (%25) PSSVK olgusu izoleydi. İki fetusta kromozom anomalisi mevcuttu. İzole PSSVK'lı olguların hepsi hayatta kaldı. Başka kalp anomalileri olan olgularda, başka kalp dışı anomalileri olan olgularda ve başka hem kalp hem de kalp dışı anomalileri olan olgularda hayatta kalma oranı sırasıyla %40, %40 ve %25 olarak saptandı. İzole PSSVK'lı olgularda prognoz çok daha iyiydi.
Sonuç: Prenatal dönemde tanısı konmuş PSSVK olguların çoğunda başka kalp ve kalp dışı anomaliler de mevcuttu. İzole vakalarda prognoz iyiyken, başka anomalilerin varlığında prognoz kötüleşmektedir.
Anahtar Kelimeler: Konjenital anomali, superior vena kava, prenatal tanı, ultrason

Introduction

Persistent left superior vena cava (PLSVC) is the most common variant of the thoracic venous system[1]. It was observed as 0.3% in autopsy series and in 4-8% of patients with congenital heart diseases[2]. In the embryologic period, it is thought that a defect in left cardinal vein regression results in PLSVC[3,4]. PLSVC usually drains via the coronary sinus into the right atrium. In a few cases, it drains to the left atrium[5]. There is no significant hemodynamic effect of isolated PLSVC because

PRECIS: In the present study, we evaluated prenatally diagnosed cases of persistent left superior vena cava according to associated anomalies.

Address for Correspondence/Yazışma Adresi: Mehmet Özsürmeli, MD,
University of Health Sciences, Derince Training and Research Hospital, Clinic of Obstetrics and Gynecology, Kocaeli, Turkey
Phone: +90 507 942 74 97 **E-mail:** ozsurmeli@gmail.com **ORCID ID:** orcid.org/0000-0002-8853-2815
Received/Geliş Tarihi: 05.09.2018 **Accepted/Kabul Tarihi:** 06.01.2019

systemic venous blood in the majority of cases drains to the right heart[6-8]. However, it is associated with cardiac and extra cardiac anomalies in 83% and 48% of cases, respectively[3,8]. A prenatal diagnosis of PLSVC is very important for proper management and counseling.

In this study, we aimed to identify prenatally diagnosed cases of PLSVC in our clinic, to evaluate the associated cardiac, extracardiac, and chromosomal anomalies, and to review their outcomes.

Materials and Methods

This retrospective study was conducted at Çukurova University Hospital, an academic tertiary referral center, in the Pediatric Cardiology and Prenatal Ultrasound Unit. All women who were diagnosed as having fetal PLSVC from January 2013 to December 2017 were analyzed. Data were collected from the digital patient recording system.

All sonographic evaluations were performed by one of the ten experienced authors, using a Voluson E6 and Voluson 730 with a convex volumetric transducer probe (RAB 6-D 2-7 MHz and RAB2 5L) (GE, Zipf, Austria). The study population was composed of patients who presented to our center for routine second-trimester anatomic screening and those referred to us for suspected anomalies (low risk, unselected patients). There was no family history in the study group.

Fetal echocardiography was performed by visualizing standard anatomic planes. All cases were examined by a perinatologist and a pediatric cardiologist. The diagnosis of PLSVC was made with the presence of an extra vessel seen on the left side of the pulmonary artery in the three-vessels/three-vessels trachea view (Figure 1 and 2). The diagnosis was confirmed by showing the connection of the vessel to the coronary sinuses using gray scale and color Doppler in the long axis. The patients were compared in three groups (isolated cases, PLSVC associated with cardiac anomalies, and PLSVC associated with extracardiac anomalies). All parents were counseled based on the cardiac and extracardiac pathologic findings.

Hyperechogenic cardiac focus and dilated coronary sinuses that were thought to occur due to PLSVC were not classified into cardiac anomalies. In addition, soft markers such as pelviectasis, choroid plexus cyst, and nasal hypoplasia were not considered as fetal anomalies.

An invasive prenatal procedure for karyotype analysis was offered in the presence of malformations on ultrasound. In isolated PLSVC, an invasive procedure was not offered. For the cases without prenatal karyotyping, chromosome analysis was performed postnatally according to the clinical findings. If prenatal or postnatal chromosome analysis was not performed, the karyotype was considered to be normal in healthy infants. Prenatal diagnosis was confirmed through postnatal echocardiography or cardiac surgery in all surviving patients. The findings of all the cases with neonatal loss and termination of pregnancy (TOP) were confirmed by autopsy, except for cases in which the parents did not accept post mortem examination.

The outcome was considered favorable if the infant was alive and doing well, whereas TOP, in utero death, and neonatal death constituted unfavorable outcomes. All pregnant women were informed and written content was obtained. This study was approved by the Ethics Committee of the University of Çukurova.

Statistical analysis was performed using Fisher's exact test and the Mann-Whitney U test. All values are given as mean ± SD. $P<0.05$ was considered to be significant.

Results

Over the study period, a total of 33 fetuses with PLSVC were examined. One of patients was excluded due to incomplete

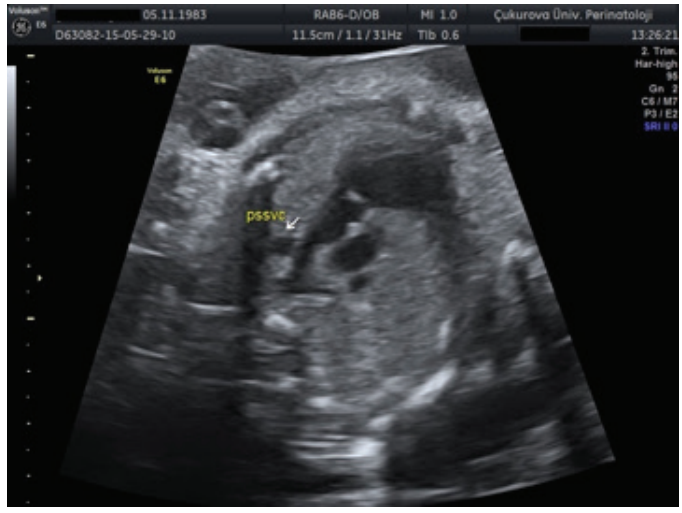


Figure 1. A persistent left superior vena cava in three-vessel view. It is seen as a fourth vessel to the left of the pulmonary artery

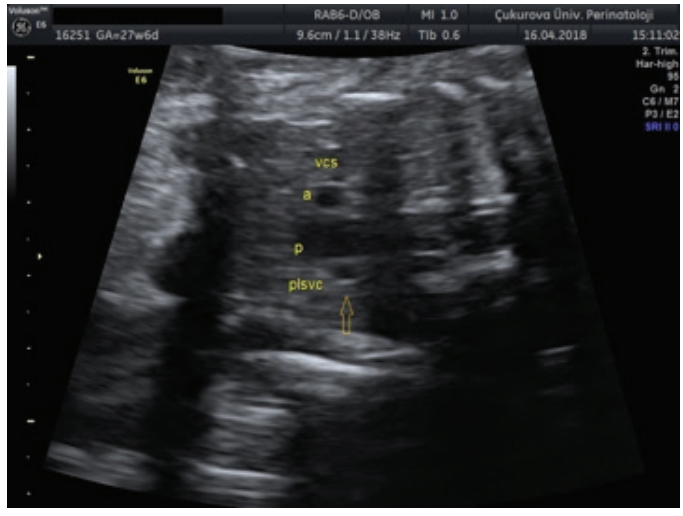


Figure 2. A persistent left superior vena cava in three-vessel trachea view

data and loss to follow-up. Thirty-one out of the 32 patients had a normal right superior vena cava. Twenty-two cases were referred due to other cardiac and extracardiac defects, and PLSVC was detected during echocardiography. In ten cases, PLSVC was detected in our clinic during routine ultrasound scanning. The demographic characteristics, associated extracardiac findings, gestational weeks at diagnosis, karyotype and outcomes of the fetuses are shown in Table 1.

Group 1 (isolated cases) consisted of eight (25%) patients. Karyotype analysis was performed in two cases and all results were normal. The karyotype was considered to be normal in six healthy infants. All of the isolated cases are alive and well.

Group 2 (associated with cardiac anomalies) comprised 19 (60%) patients. Four out of the 19 patients also had extra cardiac anomalies. Karyotype analysis was performed in eight cases in the prenatal period; trisomy 21 was detected in two cases. The genetic status of the patients who do not accept karyotype analysis and autopsy (7 cases) is unknown.

Group 3 (associated with extracardiac anomalies) was constituted by five (15%) patients. Karyotype analysis was performed in two cases and all results were normal. The genetic status of one patient who died during the newborn period was unknown. The two fetuses with additional anomalies are alive and well. The rate of aneuploidy in the total cohort was 8.3% (2/24).

The outcome was significantly more favorable in group 1 compared with groups 2 and 3 (Table 1). Table 2 shows the outcome of the cases in detail. All patients with isolated PLSVC survived and were doing well at the time of writing. On the other hand, among the cases associated with extracardiac defects, cardiac defects, and both extracardiac and cardiac defects, the survival rate was 40%, 40%, and 25%, respectively. The oldest of the 17 surviving children is 5 years old. The mean follow-up period was 29 months. Nine patients underwent surgery for extracardiac defects or cardiac defects other than PLSVC, and five died postoperatively. The features of all cases in group 1, group 2 and group 3 are summarized in Tables 3, 4, and 5, respectively.

Five cases were associated with heterotaxy syndromes. After exclusion of patients with heterotaxy, most congenital heart defects were ventricular septal defects (six cases) and hypoplasic left heart (four cases).

Table 1. Demographic and clinical features of the cases

	Group 1 (n=8)	Group 2 (n=19)	Group 3 (n=5)	Total (%) (n=32)
Age (mean ± SD, year) *	32.25±5.23	29±5.64	35.6±2.7	30.8±5.63
GAD (mean ± SD, week) *	24.3±2.26	27.9±5.5	23.4±2.6	26.3±4.88
Karyotype				
Normal	8 (100%)	10 (83%)	4 (80%)	22 (92%)
Aneuploidy	0	2 (17%)	0	2 (8%)
Unknown	0	7	1	8
Outcome				
Favorable	8 (100%)	7 (37%)	2 (40%)	17 (53%)
Unfavorable	0	12 (63%)	3 (60%)	15 (47%)

GAD: Gestational age at diagnosis (Unfavorable outcome includes neonatal death, termination of pregnancy, in utero death and aneuploidy)* (p>0.05)

Table 2. Outcome of the cases

	Isolated PLSVC (n=8)	PLSVC with extra cardiac anomalies (n=5)	PLSVC with cardiac anomalies (n=15)	PLSVC with cardiac and extra cardiac anomalies (n=4)	Total (n=32)
Well	8 (100%)	2 (40%)	6 (40%)	1 (25%)	17 (53%)
Exitus	0	3 (60%)	5 (33%)	2 (50%)	10 (31%)
TOP	0	0	2 (13%)	1 (25%)	3 (9%)
IUD	0	0	2 (13%)	0	2 (6%)

PLSVC: Persistent left superior vena cava, TOP: Termination of pregnancy, IUD: In utero death

Table 3. Features of isolated persistent left superior vena cava cases

	Age	GAD	Additional findings	Karyotype	Outcome, age
1	37	25	Coronary sinus extension	Normal	Survived, 4 years
2	32	21	Coronary sinus extension	Normal*	Survived, 3 years
3	29	27	Coronary sinus extension	Normal*	Survived, 1 years
4	26	22	Coronary sinus extension	Normal*	Survived, 1 years
5	38	24	None	Normal*	Survived, 5 years
6	26	23	Coronary sinus extension	Normal*	Survived, 2 years
7	39	27	Coronary sinus extension	Normal*	Survived, 2 years
8	31	26	Coronary sinus extension	Normal	Survived, 1 years

GAD: Gestational age at diagnosis

Table 4. Features of cases associated with cardiac anomalies

	Age	GAD	Extra cardiac anomalies	Cardiac anomalies	Karyotype	Outcome, age
1	33	21		Left ventricular hypoplasia	Normal	TOP
2	23	24		APVC, coronary sinus extension	Normal	Survived, 5 years
3	41	35		AVSD, DORV	Trisomy 21	Exitus, 3 months
4	28	34		Absent of right superior vena cava, coronary sinus extension	Normal*	Survived, 4 years
5	24	27		Left ventricular hypoplasia	Unknown	Exitus, 3 months
6	37	24		Left ventricular hypoplasia, coronary sinus extension	Unknown	NND
7	31	23		AVSD	Unknown	IUD
8	34	31		Malalignment VSD	Trisomy 21	Survived, 1 years
9	23	34		Left ventricular hypoplasia, heterotaxia	Unknown	NND
10	23	34	Hypospadias	VSD, coronary sinus extension	Normal	Survived, 2 months
11	34	34	Microcephaly	Ductal narrowing, coronary sinus extension	Unknown	Exitus, 1 years
12	28	27		VSD, aort coarctation	Normal	NND
13	24	23		Heterotaxia (left)	Unknown	TOP
14	32	32	Unilateral renal agenesis	Heterotaxia (left), coronary sinus extension	Unknown	NND
15	19	21		Heterotaxia (left), coronary sinus extension	Normal*	Survived, 4 years
16	29	37		Heterotaxia (right), coronary sinus extension, DORV, AVSD, APVC	Normal*	Survived, 3 years
17	30	21	Cerebellar hypoplasia	Coronary sinus extension, outlet VSD	Normal	TOP
18	26	25		DORV, VSD, coronary sinus extension	Normal	IUD
19	33	24		VSD	Normal*	Survived, 2 years

APVC: Abnormal pulmonary venous connection, AVSD: Atrioventricular septal defect, DORV: Double outlet right ventricul, GAD: Gestational age at diagnosis, IUD: in utero death, NND: Neonatal death, TOP: Termination of pregnancy, VSD: Ventricular septal defect. *the karyotype of healthy infants was evaluated as normal

Table 5. Features of cases associated with extra cardiac anomalies

	Age	GAD	Extra cardiac anomalies	Karyotype	Outcome, age
1	36	23	Hydrops, coronary sinus extension	Normal	NND
2	35	22	Ileojejunal atresia, coronary sinus extension	Unknown	NND
3	33	22	Ileojejunal atresia, coronary sinus extension	Normal*	Survived, 2 years
4	40	22	Laryngeal atresia	Normal	NND
5	34	28	Mega cisterna magna	Normal*	Survived, 2 years

GAD: Gestational age at diagnosis, NND: Neonatal death, * the karyotype of healthy infants was evaluated as normal

Discussion

In this study, we reviewed the prenatal and postnatal cardiac and extracardiac findings, associated chromosomal anomalies, and outcomes in 32 PLSVC cases.

PLSVC can be diagnosed easily during the perinatal period. In the three-vessel view, from the left to right side the pulmonary artery, aorta, and vena cava superior are seen with ultrasound. In addition to these three vessels in fetuses with PLSVC, a fourth vessel is seen on the left side of the pulmonary artery[4]. In addition, dilated coronary sinuses, slightly inferior of the four-chamber view, is known as an indirect sign of PLSVC[9]. It has to keep in mind that dilated

coronary sinuses can also be seen in pulmonary venous anomalies, and so other views are needed for the confirmation of PLSVC[9]. The confirmation of the diagnosis is made by showing the draining of the vessel to the coronary sinuses on the left parasagittal view. Also, a dilated coronary sinus may be misdiagnosed as an ostium primum atrial septal defect at the level of the opening of the coronary sinus into the right atrium[10]. Differential diagnosis of PLSVC includes the supracardiac type of abnormal pulmonary venous connection. Color Doppler can help differentiate PLSVC from the vertical vein in supracardiac pulmonary venous connection. Blood flow is toward the heart in PLSVC and toward the opposite direction in the vertical vein[11].

The most significant clinical implication of prenatally diagnosed PLSVC is the association with cardiac and extracardiac defects. It was reported that PLSVC was accompanied by additional cardiac anomalies in 80% of cases[3,8]. Moreover, in only 5-9% of cases, PLSVC was seen as an isolated finding. Isolated PLSVC was diagnosed more frequently in newer series[12,13]. In our series, 41% (13/32) of the cases were not associated with additional cardiac malformation and the rate of isolated PLSVC was found as 25% (8/32), which was consistent with the recent studies. The inconsistency of recent studies with previous studies may be a result of more common incorporation of the three-vessel view in routine systematic ultrasound examinations of fetal hearts in the last few years, as well the increasing awareness about diagnosing PLSVC.

In our series, septal defects were the most common cardiac defect, followed by hypoplasic left heart syndrome. Heterotaxy was observed in only 5 of the 19 cases with cardiac defects, being much lower than the other previous prenatal series in which it was reported as 41-54%[3,8]. The most frequent anomalies were reported to be ventricular septal defect and conotruncal anomalies in the study by Choi et al.[13]. However, they did not diagnose any heterotaxy syndrome in that study. Diagnosing PLSVC with minor anomalies in newer studies may support the idea of increased awareness of PLSVC.

It was thought that expansion of the lung and cardiac atriums was required for obliteration of the anterior cardinal vein[14]. However, the large variety of other associated cardiac malformations does not support this concept.

The rate of aneuploidy was 8% in our study, similar to previous series[3,12]. PLSVC is seen much more frequently in chromosomally abnormal fetuses than in normal fetuses. Du et al.[15] reported that the odds ratio for PLSVC in chromosomally abnormal fetuses compared with normal fetuses was 27.5 (95% CI: 15.8-47.8)[15]. Nevertheless, it has to be considered that either cardiac or extracardiac anomalies are seen more frequently in chromosomally abnormal fetuses. Although isolated PLSVC is thought to be a benign vascular variant and has good prognosis, some authors have asserted that fetal karyotyping should be routinely offered whenever PLSVC is detected prenatally, based on the possibility of missing some cardiac anomalies and obscure extracardiac anomalies suggesting aneuploidy[16,17]. Although it was reported that the rate of abnormal karyotype was 7% in isolated cases, it would be difficult to recommend routine prenatal invasive procedure when PLSVC is isolated[18]. The first trimester combined risk for trisomies in isolated cases is not reported in many studies, and it might be possible that undetected anomalies are included in analyses[18]. Larger studies are needed in low-risk populations to establish whether isolated PLSVC should be an indication for fetal karyotyping[13].

In our series, there were two trisomy 21 cases. Both cases had prenatally diagnosed cardiac anomalies. Trisomy 21 has been diagnosed either in isolated cases[10,13], against it we could not find any trisomy 21 in isolated cases. The number of cases reported in the literature is insufficient to draw the conclusion that PLSVC should be accepted as a marker for trisomy 21.

The outcome of PLSVC is associated with other cardiac and extracardiac findings. In previous studies, all isolated cases had a favorable prognosis. Similarly, in our cohort, all fetuses with isolated PLSVC did well after birth, whereas the survival rate declined to 40% when an additional cardiac anomaly was observed, and to 25% when an extracardiac finding was also present. These findings support the suggestion that isolated PLSVC is a benign anomaly; however, there is a need to screen for cardiac and extracardiac anomalies.

The absence of the right superior vena cava may cause difficulties during a potential invasive procedure to vessels (e.g. pulmonary artery catheterization, systemic venous cannulation) in the adulthood. Therefore, it is important to diagnose the absence of the right superior vena cava. There was only one case of absent vena cava superior in our study.

The limitations of our study are its retrospective character, the underdiagnosis of isolated PLSVC in referred cases, and the higher incidence of complicated cases due to the reference nature of our center. In a fetus with multiple anomalies, attention is given to the anomalies and therefore PLSVC may not be diagnosed and this can cause a lower incidence than the real incidence. We do not know the real rate of false-negative cases because postnatal echocardiography was not routinely performed on neonates without a prenatal diagnosis of coronary heart disease. Furthermore, the karyotype status of fetuses for whom the parents did not accept autopsy were unknown. However, despite these limitations, with this study we underline the significance of the three-vessel view during routine fetal screening and draw attention to the increasing incidence of isolated PLSVC in clinical practice.

In conclusion the prognosis is good in isolated cases, while getting worse when accompanied to cardiac or extracardiac

anomalies. The prenatal diagnosis of PLSVC is important and once it is diagnosed a detailed anatomic screening and investigation of chromosomal anomalies have to be done.

Ethics

Ethics Committee Approval: This study was approved by the Ethics Committee of the University of Çukurova (approval no: 14/05.10.2018)
Informed Consent: Retrospective study.
Peer-review: External and internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: M.Ö., S.B., M.S., E.A., Ç.A., S.M., M.K., N.Ö., S.C.D., C.E., Concept: M.Ö., S.B., S.C.D., Design: M.Ö., M.S., E.A., C.E., Data Collection or Processing: Ç.A., M.K., S.M., Analysis or Interpretation: N.Ö., S.C.D., C.E., S.B., Literature Search: S.M., Ç.A., M.S., N.Ö., Writing: M.Ö., M.K., 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. Biffi M, Boriani G, Frabetti L, Bronzetti G, Branzi A. Left superior vena cava persistence in patients undergoing pacemaker or cardioverter-defibrillator implantation: a 10-year experience Chest 2001;120:139-44.
2. Steinberg I, Dubilier W, Lukas D. Persistence of left superior vena cava Dis Chest 1953;24:479-88.
3. Berg C, Knüppel M, Geipel A, Kohl T, Krapp M, Knöpfle G, et al. Prenatal diagnosis of persistent left superior vena cava and its associated congenital anomalies. Ultrasound Obstet Gynecol 2006;27:274-80.
4. Pasquini L, Belmar C, Seale A, Gardiner HM. Prenatal diagnosis of absent right and persistent left superior vena cava. Prenat Diagn 2006;26:700-2.
5. Fraser RS, Dvorkin J, Rossall RE, Eidem R. Left superior vena cava: a review of associated congenital heart lesions, catheterization data and roentgenologic findings. Am J Med 31:711-6.
6. Pahwa R, Kumar A. Persistent left superior vena cava: an intensivist's experience and review of the literature. South Med J 2003;96:528-9.
7. Sarodia BD, Stoller JK. Persistent left superior vena cava: case report and literature review. Respir Care 2000;45:411-6.
8. Galindo A, Gutiérrez-Larraya F, Escribano D, Arbues J, Velasco JM. Clinical significance of persistent left superior vena cava diagnosed in fetal life. Ultrasound Obstet Gynecol 2007;30:152-61.
9. Chaoui R, Heling KS, Kalache KD. Caliber of the coronary sinus in fetuses with cardiac defects with and without left persistent superior vena cava and in growth-restricted fetuses with heart-sparing effect. Prenat Diagn 2003;23:552-7.
10. Barea C, Ovaert C, Moniotte S, Biard J-M, Steenhaut P, Bernard P. Prenatal diagnosis of abnormal cardinal systemic venous return without other heart defects: a case series. Prenat Diagn 2011;31:380-8.
11. Chaoui R, Heling KS, Karl K. Ultrasound of the fetal veins, part 2: veins at the cardiac level. Ultraschall Med 2014;35:302-18.
12. Esmer AÇ, Yüksel A, Calı H, Ozsürmeli M, Omeroğlu RE, Kalelioğlu I, et al. Prenatal diagnosis of persistent left superior vena cava and its clinical significance. Balkan Med J 2014;31:50-4.
13. Choi EY, Hong SK, Jeong NY. Clinical characteristics of prenatally diagnosed persistent left superior vena cava in low-risk pregnancies. Prenat Diagn 2016;36:444-8.
14. Nsah EN, Moore GW, Hutchins GM. Pathogenesis of persistent left superior vena cava with a coronary sinus connection. Pediatr Pathol 1991;11:261-9.
15. Du L, Xie HN, Zhu YX, Li LJ, Peng R, Zheng J. Fetal persistent left superior vena cava in cases with and without chromosomal anomalies. Prenat Diagn 2014;34:797-802.
16. Machevin-Surugue E, David N, Verspyck E, Labadie G, Blaysat G, Durand I, et al. Dilated coronary sinüs in prenatal echocardiography; identification, associations and outcome. Prenat Diagn 2002;22:898-902.
17. Kalache KD, Romero R, Conoscenti G, Qureshi F, Jacques SM, Chaiworapongsa T, et al. Prenatal diagnosis of dilated coronary sinüs with persistent left superior vena cava in a fetus with trisomy 18. Prenat Diagn 2003;23:108-10.
18. Gustapane S, Leombroni M, Khalil A, et al. Systematic review and meta-analysis of persistent left superior vena cava on prenatal ultrasound: associated anomalies, diagnostic accuracy and postnatal outcome. Ultrasound Obstet Gynecol 2016;48:701-8.

Clinical Investigation / Araştırma

DOI: 10.4274/tjod.galenos.2019.56255

Turk J Obstet Gynecol 2019;16:29-34

Cost-effectiveness of GnRH antagonist implementation on hCG injection day

hCG gününde GnRh antagonist yapmanın maliyet etkinliği

Ayşe Zehra Özdemir[1], Bülent Ayas[1], Davut Güven[1], Aysun Türkmen[1], Çağrı Gülümser[2]

[1]*Ondokuz Mayıs University Hospital, In vitro Fertilisation Center, Samsun, Turkey*

[2]*University of Health Science, Department of Obstetrics and Gynecology, İstanbul, Turkey*

Abstract

Objective: To compare the outcomes of antagonist stimulation protocols and to compare the cost effectiveness.
Materials and Methods: Between 2011 and December 2017, a total of 354 women who underwent intracytoplasmic sperm injection and controlled ovarian stimulation with antagonist protocols were enrolled in the study. The antagonist implementation on the day of human chorionic gonadotropin (hCG) was continued for 194 of women, whereas the antagonist was stopped 36 hours before in 160 women. The stimulation outcomes of patients and cost-effectiveness of the regimens were compared.
Results: There was a significant difference between the groups in terms of number of cryopreserved embryos, mature/immature oocyte ratio, and embryo transfer cancellations ($p<0.05$). The median value for the mature/immature oocyte ratio was 1.1 (0.2-7.5) and 1 (0.5-15) ($p=0.001$), and the ET cancellation was 5.3% vs. 1% for group 1 and 2, respectively ($p=0.037$). There was no difference between the groups in terms of pregnancy rates ($p=0.197$).
Conclusion: No difference was found in the clinical pregnancy rates between the two groups. For this reason, the cessation of antagonist implementation on the day of hCG seems more advantageous in terms of cost-effectiveness and fewer injections.
Keywords: Controlled ovarian stimulation, cost effectiveness, pregnancy rates

Öz

Amaç: Antagonist stimülasyon protokollerinin maliyet etkinliğini ve stimülasyon sonuçlarını kıyaslamaktır.
Gereç ve Yöntemler: Çalışmaya 2011-2017 yılları arasında intrastoplazmik sperm enjeksiyonu yapılan 354 hasta alınmıştır. Hastaların tamamında antagonist protokolle stimülasyon yapılmıştır. Hastaların 194 tanesinde insan koryonik gonadotropin (hCG) gününde antagonist yapılmaya devam edilmiştir. Yüz altmış hastada ise antagonist hCG gününden 36 saat önce kesilmiştir. Hastaların stimülasyon sonuçları ve iki farklı rejimin maliyet etkinliği karşılaştırılmıştır.
Bulgular: Gruplar arasında dondurulmuş embryo sayısı, matür/immatür oosit oranı ve transfer iptali açısından anlamlı farklılık bulunmuştur ($p<0,05$). Matür/immatür oosit oranı için medyan değer grup 1 ve grup 2 için 1,1 (0,2-7,5) ve 1 (0,5-15) olarak bulunmuştur ($p=0,001$). Transfer iptali grup 1 ve grup 2 için %1 ve 5,3 olarak bulunmuştur ($p=0,037$). Gebelik sonuçları açısından gruplar arasında anlamlı farklılık saptanmamıştır ($p=0,197$).
Sonuç: Klinik gebelik oranları açısından iki grup arasında anlamlı farklılık bulunmamaktadır. Bu nedenle hCG gününde antagonist dozunun atlanması maliyet etkinlik açısından ve daha az enjeksiyon yapılması nedeniyle daha avantajlı olarak görülmektedir.
Anahtar Kelimeler: Kontrollü ovaryen stimülasyon, maliyet etkinlik, gebelik oranları

Introduction

Gonadotrophin-releasing hormone (GnRH) antagonists have been used since 1999 in to prevent the luteinizing hormone (LH) peak in controlled ovarian stimulation[1,2]. GnRH antagonists suppress the release of follicle-stimulating hormone (FSH), and especially that of LH by competitively blocking the GnRH receptors in the anterior pituitary. Its effect starts rapidly and then rapidly reverts when the medication is stopped[3]. When compared with GnRH agonists, it is widely used as a safer, time-efficient, and more affordable stimulation model. In the first meta-analyses conducted on GnRH

PRECIS: Cessation of antagonist implementation on the day of hCG seems more advantageous in terms of cost-effectiveness without an effect on clinical pregnancy rate.

Address for Correspondence/Yazışma Adresi: Ayse Zehra Özdemir, MD,
Ondokuz Mayıs University Hospital, *In vitro* Fertilisation Center, Samsun, Turkey
Phone: +90 536 273 21 04 **E-mail:** aysezehra.ozdemir@hotmail.com **ORCID ID:** orcid.org/0000-0003-4232-4794
Received/Geliş Tarihi: 07.10.2018 **Accepted/Kabul Tarihi:** 06.01.2019

antagonists, the estrogen level and total number of oocytes on the human chorionic gonadotropin (hCG) day were found to be lower than with the agonists[4]. The pregnancy rates were determined to be slightly lower[5]. For this reason, many modifications have been made in the standard antagonist protocol in order to improve the efficiency of stimulation incorporating GnRH antagonists.

In recent studies[6], no difference was found between GnRH antagonists and GnRH agonists in terms of live birth rates. These recent advances suggest that the success of therapy increases as the experience with the use of antagonist increases. However, there is still no standard antagonist protocol, and significant effort is made in order to minimize the negative effects of antagonists by decreasing the number of antagonist injections.

The negative effects of GnRH antagonists are thought to originate from decreasing the LH level below the critical threshold that ensures the development of follicles. However, LH increases the aromatase activity in follicular development by having a synergistic interaction with FSH, and thus the estrogen secretion increases and ovulation and luteinization are ensured[7]. LH increase is necessary for final oocyte maturation. There are studies reporting that antagonist implementation on the hCG day might have a negative effect on the maturation of the final oocyte[7,8]. It was thought that antagonists had characteristics that negatively affected follicular development because the effects revert rapidly if the antagonist is not applied on the day of hCG.

We aimed to compare the stimulation outcomes of patients who did and did not receive antagonist on the day of hCG, and to contribute to the optimal stimulation protocol especially aspects of costs saving and reduce injection.

Materials and Methods

This is a retrospective study. Ethics committee approval was obtained from Ondokuz Mayıs University (2018/164). All subjects gave their written informed consent. Between January 2011 and December 2017, a total 354 women underwent intracytoplasmic sperm injection (ICSI) and controlled ovarian stimulation with an antagonist protocol at Ondokuz Mayıs University Reproduction Unit. Patients aged between 18 and 40 years with regular menstruation and no endocrinologic disease were enrolled in the study. The exclusion criteria were as follows: severe male factor such as oligoasthenoteratozoospermia, patients with hydrosalpinx, endometriosis, and polycystic ovary syndrome, frozen cycles, and those with no oocyte in oocyte pick up (OPU) and no follicle development in induction (no LH or progesterone increase was observed among these patients). The antagonist implementation on the day of hCG was continued for 194 of patients (group 1), whereas the antagonist was stopped for 160 participants 36 hours before hCG injections (group 2). The rationale behind this approach was to reduce the number of injections and cost. We routinely administer the GnRH antagonist in the morning so that when we administer the GnRH antagonist on the hCG day it is administered 12 hours before hCG. When we skip the GnRH antagonist we administer GnRH antagonist 36 hours before hCG.

Embryo transfer (ET) cancellation was defined as patients with no developing embryos or living embryos on the day of ET. The patients were examined on the second or third day of menstruation, and the gonadotropin FSH (Gonal-F, Serono) implementation was applied at personal doses by considering the patient's age, body mass index, and antral follicle count. GnRH Antagonist (0.25 mg cetrorelix acetate, Cetrotide, Serono) was added when the diameter of follicle reached 12 mm. When the diameters of two or more follicles reached 17 mm, recombinant hCG (Ovitrelle 250 mcg, Serono) was administered. OPU was performed 36 hours later following hCG administration and then the ICSI was performed. The ET was performed on the 3rd day of OPU. The oocyte maturation was classified as metaphase 2, intermediate, and germinal vesicles in terms of cumulus/corona morphology, cytoplasmic clarity, zona thickness, and extent of perivitelline space values. The embryonic morphology was classified into 4 grades in terms of the regularity of blastomeres, the percentage of anucleate fragments, and all dysmorphic characteristic of the embryos. Grade I: 0% anucleate fragments, regularity of blastomeres, and no apparent morphologic abnormalities; grade II: <10% anucleate fragments, regularity of blastomeres, and no apparent morphologic abnormalities; grade III: 10% to 50% anucleate fragments, irregularity of blastomeres, and no apparent morphologic abnormalities; and grade IV: ≥50% anucleate fragmentation, irregularity of blastomeres, and apparent morphologic abnormalities.

Clinical pregnancy was defined as the detection of an intrauterine gestational sac. Each patient was given 100 mg progesterone intramuscular (Progestan 50 mg, Kocak) and 6 mg estradiol orally (Estrofem 2 mg, Novo Nordisk) as luteal support from the day of OPU. The serum specimen was taken from the patients on the morning of the hCG administration day, and the LH, progesterone, and estradiol (E2) measurements were performed.

The premature LH rise was defined as 10 mIU/mL, whereas the premature progesterone rise was defined as 1 ng/mL. The combined rise of LH and progesterone was considered as premature luteinization.

Statistical Analysis

Statistical analysis was performed using the SPSS V23 software package (IBM Corp. Released 2015. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.). The normality of data distribution was tested using the Kolmogorov-Smirnov test. Non-normally distributed data were analyzed using the Mann-Whitney U test. Categorical data were analyzed using the chi-square test. The results were expressed as median (min-max) and frequency (percentage). The level of significance was set as $p<0.05$.

Results

The mean age was 30±4 years. The mean follicle count was 10 and 11 in groups 1 and 2, respectively. No statistically significant difference was observed between the groups in terms of age, number of follicles, and FSH values (Table 1, p>0.05). The most frequent reason for infertility was unexplained infertility in both groups (group 1, 54% and group 2, 63%). Poor ovarian reserve was 12.5% and 11.3%, and male factor was 33.1% and 24.7% in groups 1 and 2, respectively. There was no statistically significant difference between the groups regarding the reasons of infertility (Table 2, p=0.165).

There were no statistically significant differences between the groups in terms of estrogen (p=0.066), progesterone (p=0.127), LH (p=0.636), number of collected oocytes (p=0.088), 2PN (p=0.193), number of M2 oocytes (p=0.515), total dose of gonadotropin used in stimulation (p=0.336), and endometrium thickness (p=0.656). However, we found a significant relationship between the groups regarding the number of cryopreserved embryos, ratio of mature/immature oocytes, number of transferred embryos, and ET cancellation (p<0.05). The median mature/immature oocyte ratio was found as 1.1 (0.2-7.5) and 1 (0.5-15) in group 1 and 2, respectively (p=0.001). ET cancellation was decided in 1% and 5.3% of women in group 1 and group 2, respectively (p=0.037). Premature ovulation or premature luteinization was not seen in any woman (Table 3).

Among the patients receiving antagonist on the day of hCG, the oocyte morphology was deteriorated in 4 patients with cycle cancellation. Extensive granulation was observed in the cytoplasm and no embryo could be achieved. Embryo development was arrested in the other 5 patients. Embryo grades were statistically similar between the groups (p=0.924) (Table 4). Forty-five (45/160, 28.1%) women in group 1 and 43 (43/194, 22.2%) in group 2 became pregnant. No significant difference was observed between the groups in terms of the pregnancy rates (p=0.197) (Table 5). Ovarian hyperstimulation syndrome (OHSS) was diagnosed in 30 and 21 woman in groups 1 and 2, respectively (p=0.059).

Table 1. Pre-stimulation evaluation results

	Group 1 (n=160)	Group 2 (n=194)	p
Age	30 (20-39)	30 (19-39)	0.785
Follicle	10 (1-30)	11 (1-38)	0.269
FSH	7 (2-23)	7.8 (1.2-15)	0.055

Group 1: Patients receiving no antagonist on the day of hCG
Group 2: Patients receiving antagonist on the day of hCG
p<0.05 was considered statistically significant, FSH: Follicle-stimulating hormone

Table 2. Distribution of patients by the reason of infertility

	Group 1 (n=160)	Group 2 (n=194)	p
Unexplained infertility (%)	87 (54.4)	124 (63.9)	0.165
Poor ovarian reserve (%)	20 (12.5)	22 (11.3)	
Male factor (%)	53 (33.1)	48 (24.7)	

Table 3. Comparison of controlled ovarian hyperstimulation and IVF-ET findings

	Group 1	Group 2	p
Estrogen *	1478 (113-3339)	1297 (127-6075)	0.066
Progesterone *	0.7 (0.1-3.5)	0.5 (0.1-5.8)	0.127
LH *	1.6 (0.1-10)	1.7 (0.1-15)	0.636
Number of collected oocytes	11 (1-40)	9 (1-44)	0.088
Number of cryopreserved embryos	0 (0-8)	0 (0-12)	0.001
2PN	5 (0-24)	4,5 (0-28)	0.193
Number of M2 oocytes	8 (1-41)	8 (1-30)	0.515
Total dose of gonadotropin used in stimulation	2400 (150-6600)	2400 (225-9000)	0.336
Total duration of stimulation days	9 (1-14)	9 (6-16)	0.077
Ratio of mature/immature oocytes	1.1 (0.2-7.5)	1 (0.5-15)	0.001
Thickness of endometrium	8 (6-12)	8 (6-12)	0.656
Number of transferred embryos	1 (0-2)	1 (0-2)	0.026
ET cancellation (%) **	2 (1.0)	9 (5.3)	0.037

*Hormone levels measured on the day of hCG
**Embryo transfer cancellation (ET cancellation) was defined as the patients having no developing embryos or living embryos at the day of embryo transfer
IVF: In vitro fertilization, LH: Luteinizing hormone, hCG: Human chorionic gonadotropin

Table 4. Comparison of embryo grades

	Group 1 (n=158)	Group 2 (n=190)	p
Grade			
G1	101 (63.9)	119 (62.6)	0.924
G2	40 (25.3)	48 (25.3)	
G3	17 (10.8)	23 (12.1)	

Table 5. Comparison of the effects on gestational results

	Group 1 (n=160)	Group 2 (n=194)	
Result			p
Non-pregnant	115 (71.9)	151 (77.8)	0.197
Pregnant (%)	45 (28.1)	43 (22.2)	

All women in group 1 received one less GnRH antagonist injection. The current cost of one antagonist injection is 25,714 USD. Among the total 194 women in group 1, the cost saving was about 5003 USD compared with group 2.

Discussion

The rate of early LH surge is 20% in cycles in which no GnRH analog is used[9]. Thus, fewer oocytes are collected, fewer embryos form, and pregnancy rates decrease. GnRH analogs are widely used for preventing the increase of premature LH. In the literature, it was reported that the pregnancy rates of patients receiving controlled ovarian stimulation using an antagonist protocol were lower when compared with agonist cycles[6]. However, when compared with GnRH agonists, GnRH antagonists had significant advantages such as shorter ovarian stimulation duration and formation of fewer OHHS[10]. For this reason, it is aimed to increase pregnancy rates by applying the minimum antagonist dose that can prevent the premature increase of LH in order to protect from potential negative effects of antagonist agents.

The reason for collecting fewer oocytes and determining lower E2 levels in cycles in which the GnRH antagonists were applied might be the suppression of GnRH antagonists by decreasing LH levels below the critical threshold[4]. In natural and induced cycles, there is a phenomenon called the LH window[7]. In folliculogenesis, a certain level of LH is necessary for the proper development of oocyte. Otherwise, when the level of LH exceeds a certain limit, the aromatase activity and cellular growth are inhibited[7]. Applying competitive blockage, GnRH antagonists suppress the LH level, in addition to FSH, below the critical threshold required for follicular development.

However, GnRH antagonists suppress the pulsatile secretion of LH for 456 minutes[11] and we aimed to systematically collate evidence on the clinical efficacy of GnRH agonist triggering in patients undergoing assisted reproduction in GnRH antagonist protocols. Twenty-three publications were identified by a comprehensive literature search that included PubMed, Embase and the Cochrane Library. Three publications out of 23 fulfilled the inclusion criteria for meta-analysis, which were (i. For this reason, when no GnRH antagonist is applied on the day of hCG, this effect would disappear. This short-run LH increase will ensure more proper final oocyte maturation but not cause luteinization[7] because the process of luteinization requires time.

The antagonist given at double-dose before the day of hCG in order to block the OHSS development decreases the estrogen levels but does not affect pregnancy outcomes[12]. This outcome might be because the dose increase was not applied in the critical process of oocyte maturation. The shorter half-life of GnRH antagonists might have contributed to this result.

Another mechanism through which the GnRH antagonists can block oocyte maturation is the direct effect on the ovary through the GnRH receptors existing on granulosa cells[13,14,15]. In animal studies, it was determined that GnRH analogs were effective on the ovarian functions of in vitro granulosa luteal cells such as steroidogenesis, oocyte maturation, and follicle rupture[16].

In the present study, the mature/immature oocyte ratio of patients receiving no antagonist on the day of hCG was found to be statistically significantly higher when compared with patients receiving no antagonist on the day of hCG. Chang et al.[8] reported that the rate of mature oocytes increased significantly among patients receiving no GnRH antagonist on the day of hCG. However, Chang et al.[7] also performed a prospective study on 86 patients. They compared patients receiving GnRH antagonist on the day of hCG and those receiving no GnRH antagonist, and they reported that the controlled ovarian hyper-stimulation results were similar. When follicles reaching a size greater than critical threshold were detected, final oocyte maturation was achieved under the effect of hCG. We believe that the antagonist implementation on the day of hCG deteriorates the final oocyte maturation because of the results we achieved.

Different from other two studies[7,8], we determined that ET cancellation was statistically significantly higher among patients receiving antagonist on the day of hCG. The oocyte morphology was found to be deteriorated among 4 of these patients. Extensive granulation was observed in the cytoplasm. In the resting 5 patients, embryonic developmental arrest was observed. However, in the group that stopped receiving antagonist, two patients who were incapable of forming an embryo were found.

Embryo arrest is due to maternal factors[17]. The transcription factors of the oocyte affect the cleavage of the embryo in the early stages. These factors are formed during oogenesis. Embryos that contain low maternal transcription factors

in the early cleavage stage arrest in an inappropriate microenvironment[18]. In our study, the arrest of the embryo in the group 2 patients may be due to the negative effects of GnRH antagonist during oocyte maturation.

The oocyte quality indicates the fertilization potential. In the present study, the increase in the mature oocyte rate among patients receiving no antagonist on the day of hCG and the lower level of fertilization failure are in harmony with each other. Antagonist implementation on the day of hCG may cause lower quality and fewer mature oocytes by deteriorating the microenvironment of the oocyte. This may lead to failure in fertilization. In their study, Munoz et al.[19] reported that the protocols used in stimulation had no effect on embryonic quality but did alter the kinetics of embryonic development. Moreover, we determined no statistically significant difference between the groups in terms of the grades of embryos.

In the present study and two previous studies[7,8], no premature luteinization was observed in any patient even though the GnRH antagonist implementation on the day of hCG was stopped. Moreover, the recent studies showed that the increasing level of progesterone did not decrease pregnancy rates[20,21]. For this reason, the results of the present study and those of previous studies suggest that the implementation of GnRH antagonist in stimulation in order to prevent possible negative effects that might develop due to the premature increase of LH deteriorates formation of mature oocyte and the dynamics of embryos' development. Additionally, the cost of using GnRH antagonists on the hCG day was 5003 USD for the 194 patients in group 2. However, there was no difference in pregnancy rates and there was no premature luteinization in the patients of group 1.

We found no differences in endometrial thickness using transvaginal ultrasonography in patients who did and did not receive antagonist on the day of hCG. Similarly, Chang et al.[7,8] reported that the endometrial thickness, pattern, and implantation rates were not statistically significantly different between patients who did and did not receive GnRH antagonist on the day of hCG. However, high-dose ganirelix was observed to decrease the implantation by causing deteriorated HOXA10 expression in the endometrium[22].

Study Limitations

The limitation of the study is that the LH and E2 values of the patients were not measured after hCG administration. Thus, we might be able to show that implementation of antagonist on the day of hCG suppresses LH levels and decreases estrogen synthesis. Another limitation is the deficiency of the determination of the implantation rates because of inadequate data.

Although this was a retrospective study, it also incorporated many patients. The implementation of antagonist on the day of hCG within the scope of antagonist protocol might have negative effects on the oocyte maturation and embryonic development. As in previous studies[7,8], no negative consequence of not implementing GnRH antagonist on the day of hCG was observed in the present study.

Conclusion

In two previous studies[7,8], no difference was determined in pregnancy rates. For this reason, the administration of antagonist on the day of hCG is not acceptable both in terms of costs and excessive injection. Larger sample sized studies are required in order to clearly reveal the effects of GnRH antagonist administration on the day of hCG on embryonic development and oocyte maturation.

Ethics

Ethics Committee Approval: Ethics committee approval of was obtained from Ondokuz Mayıs University (2018/164).

Informed Consent: All subjects gave their written informed consent.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: D.G., Concept: D.G., Ç.G., Design: D.G., Ç.G., Data Collection or Processing: A.Z.Ö., Analysis or Interpretation: B.A., Literature Search: A.T., Writing: A.Z.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. Kolibianakis EM, Tarlatzis B, Devroey P. GnRH antagonists in IVF. Reprod Biomed Online [Internet] 2005;10:705-12. Available from: http://dx.doi.org/10.1016/S1472-6483(10)61113-3.
2. Copperman AB, Benadiva C. Optimal usage of the GnRH antagonists: A review of the literature. Reprod Biol Endocrinol 2013;11:1-13.
3. Gordon K, Hodgen GD. GnRH agonists and antagonists in assisted reproduction. Baillieres Clin Obstet Gynaecol 1992;6:247-65.
4. Olivennes F, Belaisch-Allart J, Emperaire JC, et al. Prospective, randomized, controlled study of in vitro fertilization-embryo transfer with a single dose of a luteinizing hormone-releasing hormone (LH-RH) antagonist (cetrorelix) or a depot formula of an LH-RH agonist (triptorelin). Fertil Steril 2000;73:314-20.
5. Johnston-MacAnanny EB, DiLuigi AJ, Engmann LL, Maier DB, Benadiva CA, Nulsen JC. Selection of first in vitro fertilization cycle stimulation protocol for good prognosis patients: gonadotropin releasing hormone antagonist versus agonist protocols. J Reprod Med [Internet] 2011;56:12-6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21366121.
6. Al-Inany H, Youssef M. Gonadotrophin-releasing hormone antagonists for assisted reproductive technology. Database Syst Rev [Internet] 2011;CD001750. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21563131%5Cnhttp://onlinelibrary.wiley.com/doi/10.1002/14651858.CD001750.pub3/pdf/standard
7. Chang HJ, Lee JR, Jee BC, Suh CS, Lee WD, Kim SH. Cessation of gonadotropin-releasing hormone antagonist on triggering day in

flexible multipledose protocol: A randomized controlled study. Clin Exp Reprod Med 2013;40:83-9.
8. Chang HJ, Lee JR, Jee BC, Suh CS, Kim SH. Cessation of gonadotropinreleasing hormone antagonist on triggering day: an alternative method for flexible multiple-dose protocol. Send to J Korean Med Sci 2009;24:262-8.
9. Edwards RG, Lobo R, Bouchard P. Time to revolutionize ovarian stimülation. Hum Reprod 1996;11:917-9.
10. Tarlatzis BC, Fauser BC, Kolibianakis EM, Diedrich K, Rombauts L, Devroey P. GnRH antagonists in ovarian stimulation for IVF. Hum Reprod Update 2006;12:333-40.
11. Griesinger G, Diedrich K, Devroey P, Kolibianakis EM. GnRH agonist for triggering final oocyte maturation in the GnRH antagonist ovarian hyperstimulation protocol: A systematic review and meta-analysis. Vol. 12, Human Reprod Update 2006;12 p. 159-68.
12. Prapas Y, Ravanos K, Petousis S, Panagiotidis Y, Papatheodorou A, Margioula-siarkou C, et al. GnRH antagonist administered twice the day before hCG trigger combined with a step-down protocol may prevent OHSS in IVF/ICSI antagonist cycles at risk for OHSS without affecting the reproductive outcomes : a prospective randomized control trial. J Assist Reprod Genet 2017;1537-45.
13. Engel JB, Riethmüller-Winzen H DK. Extrapituitary Actions of GnRH Antagonists: Prospects for in vitro Fertilization Programs. Curr Pharm Des 2012;18:264-9.
14. Yu B, Ruman J, Christman G. The role of peripheral gonadotropinreleasing hormone receptors in female reproduction. Fertil Steril 2011;95:465-73.
15. Cheng CK, Yeung CM, Chow BK, Leung PC. Characterization of a New Upstream GnRH Receptor Promoter in Human Ovarian Granulosa-Luteal Cells. Mol Endocrinol 2002;16:1552-64.
16. Humaidan P, Kol S, Papanikolaou EG. GnRH agonist for triggering of final oocyte maturation : time for a change of practice? Hum Reprod Updat 2018;17:510-24.
17. Braude P, Bolton V, Moore S. Human gene expression first occurs between the four- and eight-cell stages of preimplantation development. Nature 1988;332:459-61.
18. Betts DH, King WA. Genetic regulation of embryo death and senescence. Theriogenology 2001;55:171-91.
19. Muñoz M, Cruz M, Humaidan P, Garrido N, Pérez-Cano I, Meseguer M. The type of GnRH analogue used during controlled ovarian stimulation influences early embryo developmental kinetics: a time-lapse study. Eur J Obs Gynecol Reprod Biol 2013;168:167-72.
20. Venetis CA, Kolibianakis EM, Papanikolaou E, Bontis J, Devroey P, Tarlatzis B. Is progesterone elevation on the day of human chorionic gonadotrophin administration associated with the probability of pregnancy in in vitro fertilization? A systematic review and metaanalysis. Hum Reprod Update 2007;13:343-55.
21. Ince O, Ucuran G, Ozvatan Halksever S, Ozer M, Duymus A C , Demir M, et al. Effect of Premature Luteinizing Hormone Rise on Pregnancy Outcomes in Patients with High Trigger Day Progesterone Levels in GnRH Antagonist In Vitro Fertilization Cycles. Turkiye Klin J Gynecol Obs 2017;27:49-56.
22. Rackow BW, Kliman HJ, Taylor HS. GnRH antagonists may affect endometrial receptivity. Fertil Steril 2007;89:1234-9.

Clinical Investigation / Araştırma

DOI: 10.4274/tjod.galenos.2019.21384

Turk J Obstet Gynecol 2019;16:35-40

Fetal nuchal translucency: is there an association with birthweight and neonatal wellbeing?

Fetal ense kalınlığı: Doğum kilosu ve yenidoğan iyilik hali ile bir ilişkisi var mı?

Ziya Kalem[1], Aşkı Ellibeş Kaya[2], Batuhan Bakırarar[3], Müberra Namlı Kalem[4]

[1]*Gürgan Clinic IVF and Women Health Center, Ankara, Turkey*

[2]*Düzce University Faculty of Medicine, Department of Obstetrics and Gynecology, Düzce, Turkey*

[3]*Ankara University, Department of Biostatistics, Ankara, Turkey*

[4]*Bahcesehir University, Department of Obstetrics and Gynecology, İstanbul, Turkey*

Abstract

Objective: To evaluate the relationship between nuchal translucency (NT) values with birthweight and the wellbeing of the newborn.
Materials and Methods: This retrospective cohort study that included 508 patients made use of data on healthy full-term, singleton, live birth newborns in a university hospital between 2016 and 2018. The relationship between the NT multiple of the median (MoM) value and maternal body mass index, birthweight, sex, need for neonatal intensive care unit (NICU), and APGAR scores was evaluated. Similarly, the relationship between birthweight and NT MoM, and biochemical data in the first trimester was also evaluated.
Results: There was a positive correlation between NT and birthweight (p<0.001). The need for NICU admission increased (p=0.001), and APGAR 1st minute scores decreased (p=0.001) with increasing NT, and APGAR 5th minute scores remained unchanged (p=0.057).
Conclusion: The present study identified a positive correlation between first trimester NT and birthweight, and a negative correlation with the wellbeing of the neonate.
Keywords: Nuchal translucency, birthweight, APGAR score, fetal development

Öz

Amaç: Yenidoğan iyilik hali ve doğum kilosu ile ense kalınlığı (NT) değerleri arasındaki ilişkiyi değerlendirmek
Gereç ve Yöntemler: Bu retrospektif kohort çalışma 2016-2018 yılları arasında bir üniversite hastanesinde takipli; sağlıklı, miadında ve tekil yenidoğan ile sonuçlanan 508 gebeliği kapsamaktadır. NT-ortalamanın kat (MoM) değerleri ile maternal vücut kitle indeksi, doğum kilosu, cinsiyet, bebek yoğun bakım (NICU) ihtiyacı ve APGAR skorları değerlendirildi. Benzer şekilde ilk trimesterda da doğum kilosu, NT-MoM ve biyokimyasal data değerlendirildi.
Bulgular: NT ve doğum kilosu arasında pozitif korelasyon saptandı (p<0,001). NT değerleri arttıkça APGAR 5. dakika skorları değişmezken (p=0,057); 1. dakika skorlarının düştüğü (p=0,001) ve NICU ihtiyacının arttığı saptandı (p=0,001).
Sonuç: Bu çalışmada, birinci trimester NT ile doğum kilosu arasında pozitif korelasyon, yenidoğan iyilik hali ile negative korelasyon saptandı.
Anahtar Kelimeler: Ense kalınlığı, doğum kilosu, APGAR skoru, fetal gelişim

Introduction

Nuchal translucency (NT) is the normal fluid-filled subcutaneous space identified at the back of the fetal neck during the late first trimester and early second trimester[1]. NT is one of the parameters of first trimester screening tests for trisomies 21, 18 and 13[2], and an increased NT is thought to be related to dilated lymphatic channels and is considered a nonspecific sign of a more generalized fetal abnormality[3]. Fetuses with an increased NT are at increased risk of chromosomal anomalies, structural defects, and genetic syndromes[4,5].

PRECIS: Nuchal translucency: association with birthweight and neonatal wellbeing?

Address for Correspondence/Yazışma Adresi: Aşkı Ellibeş Kaya, MD,
Düzce University Faculty of Medicine, Department of Obstetrics and Gynecology, Düzce, Turkey
Phone: +90 505 524 83 16 **E-mail:** askiellibes@hotmail.com **ORCID ID:** orcid.org/0000-0002-1323-7416
Received/Geliş Tarihi: 09.09.2018 **Accepted/Kabul Tarihi:** 09.01.2019

Recent studies have reported that despite increased NT in the first trimester in fetuses with normal karyotypes, no problem will be experienced in the long term if ultrasonographic (USG) scans show normal findings in the second trimester; however, there is an increased probability of adverse pregnancy outcomes[6,7]. Although noninvasive prenatal diagnosis tests have largely replaced first-trimester screening tests, the measurement of NT in the first trimester will always maintain importance. A future protocol is considered to involve USG screening in the second trimester, directed by the noninvasive prenatal tests and NT measurements in the first trimester[8].

Although there are ongoing studies in literature attempting to determine cut-off values for NT in healthy fetuses with no chromosomal anomalies, researchers have also noticed that NT values vary depending on race and fetal development[9-12]. It is now thought that differences in fetal body measurements, even NT, can be observed from the first trimester onwards[13]. The present study investigated whether NT values measured in the first trimester varied such as other measurements regarding the body or if it indicated adverse pregnancy events. To this end, the study investigated the relationship between NT values with birthweight and the wellbeing of the newborn.

Materials and Methods

This retrospective cohort study made use of data on healthy full-term, singleton, live birth newborns in a university hospital between 2016 and 2018. The study included 508 patients for whom postnatal and first trimester screening test findings were available. Newborns with congenital anomalies or systemic diseases diagnosed in the intrauterine or postpartum period, and those underwent karyotype analysis for any reason during pregnancy or postpartum and who were found to have an abnormal karyotype were excluded from the study. Patients with indefinite last menstrual periods and those with USG measurements that were inconsistent with the last menstrual period, *in vitro* fertilization (IVF) patients, patients who received exogenous progesterone in the first trimester, multiple pregnancies, patients delivering their babies before 37 weeks of gestation, newborns with known intrauterine viral infections, patients with preeclampsia/eclampsia, and diabetic mothers were also excluded from the study. The maternal age, height, weight, data on previous pregnancies, crown-rump-length measurements (mm), pregnancy-associated plasma protein A multiples of median (PAPP-A MoM) values, free beta human chorionic gonadotropin (hCG) MoM values, and NT MoM values were used for abnormal karyotype screening tests in the first trimester, maternal weight gain, newborn's APGAR scores, need for neonatal intensive care unit (NICU), sex, and birthweight of those involved in the study were recorded. The relationship between the NT MoM values and maternal body mass index (BMI), birthweight, sex, and APGAR scores was evaluated. Similarly, the relationship between birthweight and NT MoM and biochemical data in the first trimester was also evaluated.

Statistical Analysis

All statistical analyses were performed using SPSS for Windows 11.5 software program (SPSS Inc., Chicago, IL). Compatibility of data with normal distribution was examined graphically and using the Kolmogorov-Smirnov test. Mean ± standard deviation and median (min-max) were used for the quantitative variables, an numbers (percentage) were used as descriptors for categorical variables in the study. When to look whether there was a statistically significant difference between the categories of a qualitative variable with two categories in terms of a quantitative variable, Student's t-test was used if the normal distribution assumption was met; if not, Mann-Whitney U test was used. The chi-square test was used to examine the relationship between two categorical variables. Covariance analysis (ANCOVA) was used to see whether one or more continuous independent parameters had any impact on the dependent parameter. Receiver operating charcteristics curve analysis was used to find the discriminative factors between the groups. Significance level was set at p=0.05.

Results

The study included 508 subjects, the demographic characteristics of whom and the parameters evaluated in the study are presented in Table 1.

Of these subjects, 25 (4.9%) were current smokers. The nasal bone could not be visualized in 22 subjects (4.3%). Of the newborns, 267 (52.5%) were male and 241 (47.4%) were female. Of the 21 (4.1%) babies that were required to be admitted to the NICU, 15 (71.4%) had respiratory problems and five (23.8%) had other system problems (gastrointestinal, cardiovascular); four babies (0.7%) died in the neonatal period.

Multiple linear regression analysis was performed to evaluate the relationship between NT MoM values and maternal BMI, and the parameters of the newborn (Table 2). In the multiple linear regression model: NT MoM: 0.687+0.001 * newborn's weight + 0.503 * need for NICU + (-0.025) * newborn's APGAR 1 min, and the p values for the variables were <0.001, <0.001, and 0.015, respectively. When the variables were incorporated into the model together, they accounted for 15.6% of the variance in the NT MoM variable.

Linear regression analysis was made to evaluate the relationship between newborn weight with maternal parameters and NT MoM (Table 3). In the multiple linear regression model: newborn weight: 2896.277 + 16.031 * weight gain + (-331.941) * smoking + 248.250 * Nt MoM, and the p values for the variables were 0.003, 0.001, and <0.001,

Table 1. Demographic data, parameters related to the pregnancy and the newborn

Variables	n	Mean ± SD	Median (Min-Max)
Maternal Age (year)	508	30.18±5.07	30.00 (17.00-44.00)
Pregestational BMI (kg/m2)	508	26.53±5.14	25.78 (12.54-44.53)
Number of previous pregnancies	507	2.27±1.42	2.00 (1.00-8.00)
Number of previous livebirths	297	0.86±1.06	1.00 (0.00-7.00)
Number of previous pregnancy losses	293	0.38±0.77	0.00 (0.00-5.00)
Gestational week at screening	503	12.18±0.69	12.20 (11.00-13.85)
CRL (mm)	508	59.83±9.56	59.20 (45.00-84.00)
PAPP-A MoM	505	1.21±0.84	1.05 (0.17-7.59)
Free beta hCG MoM	503	1.20±0.80	1.05 (0.14-6.00)
NT MoM	508	0.86±0.32	0.82 (0.50-2.15)
Maternal weight-gain in pregnancy (kg)	502	10.83±4.31	10.00 (5.00-33.00)
APGAR min 1	499	8.58±1.37	9.00 (3.00-9.00)
APGAR min 5	503	9.65±1.51	10.00 (2.00-10.00)
Gestational week at birth	508	39.±2.23	38.20 (37.00-42.00)
Birthweight (g)	508	3240.56±505.43	3235.00 (1625.00-4850.00)

SD: Standard deviation, Min: Minimum, Max: Maximum, BMI: Body mass index, CRL: Crown-rump-length, PAPP-A: Pregnancy-associated plasma protein A, MoM: Multiples of median, hCG: Human chorionic gonadotropin, NT: Nuchal translucency

Table 2. Nuchal translucency MoM-when the variables are included in the model one at a time

Independent Variables	β	S.E.	R2	p value	95% Confidence interval	
					Lower limit	Upper limit
Newborn's weight	0.001	0.001	0.038	<0.001	0.001	0.002
Need for NICU	0.580	0.074	0.113	<0.001	0.435	0.725
Fetal sex	-0.048	0.029	0.006	0.097	-0.105	0.009
Newborn's Apgar 1 min	-0.042	0.010	0.034	<0.001	-0.062	-0.022
Newborn's Apgar 5 min	-0.019	0.009	0.008	0.057	-0.037	-0.001
BMI	0.002	0.003	0.001	0.440	-0.003	0.008

MoM: Multiples of median, NICU: Neonatal intensive care unit, BMI: Body mass index

Table 3. Newborn's Weight-when the variables were included in the model one at a time

Independent variables	β	S.E.	R^2	p value	95% Confidence interval	
					Lower limit	Upper limit
Age	1.204	4.507	0.001	0.789	-7.651	10.059
Number of previous pregnancies	7.961	30.490	0.001	0.794	-52.049	67.971
Weight gain	17.201	5.023	0.027	0.001	7.327	27.076
Smoking	-335.883	108.009	0.021	0.002	-548.135	-123.632
BMI	5.785	4.578	0.003	0.207	-3.210	14.780
PAPP-A MoM	13.460	26.788	0.001	0.616	-39.171	66.092
Free beta hCG MoM	-7.023	28.434	0.001	0.805	-62.891	48.844
NT MoM	310.047	70.918	0.038	<0.001	170.703	449.391

BMI: Body mass index, PAPP-A: Pregnancy-associated plasma protein A, MoM: Multiples of median, hCG: Human chorionic gonadotropin, NT: Nuchal translucency

respectively. When these variables were incorporated into the model together, they accounted for 9.2% of the variance in the newborn weight.

The variables were added individually to the model in a logistic regression analysis performed to evaluate the effects of maternal and fetal factors on the need for admission to the NICU (Table 4). The logistic regression model was statistically significant (p<0.001), and explained 30.4% (Nagelkerke *R2*) of the variance in the need for NICU, and accurately classified 96.7% of patients. Increasing maternal age and NT MoM were associated with an increase in the likelihood of requiring admission to the NICU.

Discussion

The present study evaluates the relationship between NT in the first trimester and newborn weight, and found a significant positive correlation between the two. The study also evaluates the relationship between NT in the first trimester and the wellbeing of the newborn, and found that an increase in NT was associated with an increase in the need for admission to the NICU and a decrease in the APGAR 1st minute score.

It is currently acknowledged in the literature that variations in fetal weight occur from the first trimester onwards, rather than the traditionally accepted second half of the second trimester, fetal measurements other than NT have been used in these studies[13-16]. Studies in the literature have linked increased first trimester NT to adverse pregnancy outcomes after being linked initially to fetal birthweight over complications accruing during pregnancy. Krantz et al.[17] showed that PAPP-A and increased NT could be associated with adverse pregnancy outcomes, and particularly with intrauterine growth retardation (IUGR)[17], and another study suggested that increased NT together with impaired glucose intolerance could reflect fetal macrosomia[18].

In later years, studies began to report an association between NT and birthweight in the absence of adverse pregnancy outcomes. In a study of the non-diabetic population, it was shown that an increased first trimester NT was linked to large-for-gestational-age (LGA) neonates[19]. In a study published in 2017 by Hackmon et al.[20], it was reported that early fetal measurements and NT were correlated with term birthweight[20]. Given that previous studies faced problems in accurately determining gestational age, this study involved IVF patients so as to ensure the accuracy of gestational age.

The present study established a positive association between first trimester NT and birthweight, although our study includes non-IVF patients. Some studies in literature demonstrated increased NT with the use of exogenous progesterone in the first trimester[21,22], and it is widely known that progesterone is used in the luteal phase and the first trimester as a worldwide standard procedure in IVF patients[23,24]. Accordingly, the current study population was selected from non-IVF patients and those who did not use progesterone in the first trimester. For an accurate determination of gestational age, patients with a last menstrual period that was consistent with USG measurements were selected.

Plasencia et al.[25] reported an association between birthweight and NT, serum PAPP-A, beta hCG, and uterine artery Doppler PI measurements, and suggested that these findings could lead to the early recognition of LGA infants. The present study did not include Doppler USG findings; however, we identified no association between parameters other than NT (PAPP-A and beta hCG) and birthweight.

The findings of the present study identified no relationship between NT and fetal sex, although there have been studies in literature in which such a relationship was demonstrated. Spencer et al. reported that NT was 3.4% lower in female fetuses[26], whereas Weismann-Brenner et al. [27], in contrast, found that NT was higher in the male sex, and that the correlation between NT and birthweight was independent of sex. Such a relationship could not be reliably established in the present study due to the small number of participants.

Aside from its relationship with NT, the present study identified a positive correlation between birthweight and maternal weight gain, and a negative correlation with smoking, and both of these findings are consistent with literature[28-30].

Table 4. Need for NICU-When the variables are included in the model one at a time

Independent variables (reference category)	β	S.E.	OR	p value	95% Confidence interval	
					Lower limit	Upper limit
PAPP-A MoM	0.260	0.206	1.297	0.206	0.867	1.940
Free beta hCG MoM	-0.031	0.316	0.970	0.923	0.522	1.802
Fetal sex (male)	0.476	0.501	1.610	0.342	0.602	4.300
Smoking (no)	0.988	0.785	2.686	0.208	0.576	12.517
Maternal age	0.126	0.052	1.135	0.015	1.025	1.257
Gravida	-0.263	0.357	0.769	0.462	0.382	1.547
NT MoM	4.047	0.683	57.198	<0.001*	15.003	218.064

*p<0.05 statistical significance, NICU: Neonatal intensive care unit, OR: Odds ratio, PAPP-A: Pregnancy-associated plasma protein A, MoM: Multiples of median, hCG: Human chorionic gonadotropin, NT: Nuchal translucency

In the present study, it was observed that the need for NICU admission increased and APGAR 1st minute scores decreased with increasing NT, but APGAR 5th minute scores remained unchanged. Previous studies suggest that if U scans in the second trimester and afterwards are normal in babies with increased first trimester NT and normal karyotypes, there will be no adverse outcome in the long term, although there is an increased likelihood of adverse pregnancy outcomes[6,7,31]. Studies have also shown that the long-term neurodevelopmental outcome of children after increased fetal NT is reassuring[32].

The APGAR 5-minute score is also a good indicator of a long-term neurodevelopmental outcome[33], and its lack of variance to NT in the present study is an expected finding. The APGAR 1st minute score determines how well the baby tolerated the birthing process[34], and the relationship between increased NT and the APGAR 1st minute score identified in the present study suggests that babies with increased NT may have poorly tolerated the birthing process. This indicates that a relationship may exist between increased NT and placental insufficiency. In a recently published study, Lee et al. [35] evaluated the relationship between NT and placental dysfunction and reported higher first trimester NT in babies exhibiting signs of placental insufficiency, although this was not statistically demonstrated. It would seem that this topic deserves further comprehensive research.

The increased need for NICU admission with increasing NT values in the present study indicates that placental insufficiency develops and tolerance to the birthing process decreases with increasing NT MoM values. Our study has demonstrated two factors that affect the need for NICU admission, namely increasing maternal age and increasing NT. It is, however, unknown whether a relationship exists between increasing maternal age and increasing NT in babies with normal karyotypes. The increasing rate of miscarriage with increasing maternal age complicates a joint evaluation of these two factors, but it is clear that this area merits more comprehensive research.

Studies in the literature have reported an increased likelihood of submicroscopic chromosomal abnormalities in fetuses with increased NT and normal karyotypes, and that this could cause structural defects in the second and third trimesters. The placenta may be affected by these microscopic chromosomal abnormalities, and accordingly, may cause adverse pregnancy outcomes such as preeclampsia and IUGR[36].

The present study is the first in the literature to investigate the relationship between the first trimester NT MoM values of healthy newborns with both the birthweight and the wellbeing of the newborns. The most important limitation of the current study is that no karyotype analysis was available for all of the newborns in the study. Furthermore, no long-term data related to the newborns in the study were included, other than the neonatal period. The retrospective nature, the lack of details for the indications of NICU admission, and lack of a retrospective power analysis also limits of this study. In conclusion, the present study identified a positive correlation between first trimester NT and birthweight, and a negative correlation with the wellbeing of the neonate. A number of studies in literature have aimed at determining cut-off levels for NT; these studies should take into account the variations in NT values. It is not yet understood whether different NT values indicate a variation in fetal development or an adverse perinatal outcome, and so further studies are required to clarify this issue.

Ethics

Ethics Committee Approval: The study was approved by Liv Hospital Ankara (approval no: 2017/2-003).
Informed Consent: Retrospective study.
Peer-review: External and internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Z.K., A.E.K., Concept: Z.K., M.N.K., A.E.K., Design: Z.K., B.B., Data Collection or Processing: A.E.K., B.B., Analysis or Interpretation: B.B., M.N.K., Literature Search: Z.K., A.E.K.
Writing: Z.K., M.N.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. Bunduki V, Zugaib M. The Fetus at the First Trimester. In Atlas of Fetal Ultrasound 2018 (pp. 15-28). Springer, Cham.
2. Kagan KO, Wright D, Nicolaides KH. First-trimester contingent screening for trisomies 21, 18 and 13 by fetal nuchal translucency and ductus venosus flow and maternal blood cell-free DNA testing. Ultrasound Obstet Gynecol 2015;45:42-7.
3. Burger NB, Bekker MN, Kok E, De Groot CJ, Martin JF, Shou W, et al. Increased nuchal translucency origins from abnormal lymphatic development and is independent of the presence of a cardiac defect. Prenat Diagn 2015;35:1278-86.
4. Taipale P, Hiilesmaa V, Salonen R, Ylöstalo P. Increased nuchal translucency as a marker for fetal chromosomal defects. N Engl J Med 1997;337:1654-8.
5. Souka AP, Snijders RJ, Novakov A, Soares W, Nicolaides KH. Defects and syndromes in chromosomally normal fetuses with increased nuchal translucency thickness at 10-14 weeks of gestation. Ultrasound Obstet Gynecol 1998;11:391-400.
6. Lithner CU, Kublickas M, Ek S. Pregnancy outcome for fetuses with increased nuchal translucency but normal karyotype. J Med Screen 2016;23:1-6.
7. Äyräs O, Eronen M, Tikkanen M, Rahkola-Soisalo P, Paavonen J, Stefanovic V. Long-term outcome in apparently healthy children with increased nuchal translucency in the first trimester screening. Acta Obstet Gynecol Scand 2016;95:541-6.
8. Lichtenbelt KD, Diemel BD, Koster MP, Manten GT, Siljee J, Schuring-Blom GH, et al. Detection of fetal chromosomal anomalies: does

nuchal translucency measurement have added value in the era of non-invasive prenatal testing? Prenat Diagn 2015;35:663-8.
9. Maya I, Yacobson S, Kahana S, Yeshaya J, Tenne T, Agmon-Fishman I, et al. Cut-off value of nuchal translucency as indication for chromosomal microarray analysis. Ultrasound Obstet Gynecol 2017;50:332-5.
10. Sharifzadeh M, Adibi A, Kazemi K, Hovsepian S. Normal reference range of fetal nuchal translucency thickness in pregnant women in the first trimester, one center study. J Res Med Sci 2015;20:969.
11. Huang T, Wang F, Boucher K, O'Donnell A, Rashid S, Summers AM. Racial differences in first trimester nuchal translucency. Prenat Diagn 2007;27:1174-6.
12. Chen M, Lam YH, Tang MH, Lee CP, Sin SY, Tang R, et al. The effect of ethnic origin on nuchal translucency at 10-14 weeks of gestation. Prenat Diagn 2002;22:576-8.
13. Salomon LJ, Hourrier S, Fanchin R, Ville Y, Rozenberg P. Is firsttrimester crown-rump length associated with birthweight?. BJOG 2011;118:1223-8.
14. Pardo J, Peled Y, Yogev Y, Melamed N, Ben-Haroush A. Association of Crown-Rump Length at 11 to 14 Weeks' Gestation and Risk of a Large-for-Gestational-Age Neonate. J Ultrasound Med 2010;29:1315-9.
15. Pardo J, Peled Y, Yogev Y, Melamed N, Ben-Haroush A. Association of Crown-rump Length at 11 to 14 Weeks' Gestation and Risk of a Large-for-gestational-age Neonate. J Ultrasound Med 2011;66:10-1.
16. Smith GC, Smith MF, McNay MB, Fleming JE. First-trimester growth and the risk of low birth weight. N Engl J Med 1998;339:1817-22.
17. Krantz D, Goetzl L, Simpson JL, Thom E, Zachary J, Hallahan TW, et al. Association of extreme first-trimester free human chorionic gonadotropin-β, pregnancy-associated plasma protein A, and nuchal translucency with intrauterine growth restriction and other adverse pregnancy outcomes. Am J Obstet Gynecol 2004;191:1452-8.
18. Kelekci S, Yilmaz B, Savan K, Sonmez S. Can increased nuchal translucency in the first trimester of pregnancy predict gestational diabetes mellitus. J Obstet Gynaecol 2005;25:579-82.
19. Weissmann-Brenner A, Weisz B, Lerner-Geva L, Gindes L, Achiron R. Increased nuchal translucency is associated with large for gestational age neonates in singleton pregnancies. J Perinat Med 2011;39:305-9.
20. Hackmon R, Librach C, Burwick R, Rodrigues N, Farine D, Berger H. Do Early Fetal Measurements and Nuchal Translucency Correlate With Term Birth Weight?. J Obstet Gynaecol Can 2017;399:750-6.
21. Namlı Kalem M, Kalem Z, Bakırarar B, Ergün A, Gürgan T. The effect of progesterone use in the first trimester on fetal nuchal translucency. J Turk Ger Gynecol Assoc 2018;19: 29.
22. Giorlandino C, Cignini P, Padula F, Giannarelli D, d'Emidio L, Aloisi A, et al. Effects of exogenous progesterone on fetal nuchal translucency: an observational prospective study. Am J Obstet Gynecol 2015;212:335-e1.
23. Griesinger G, Meldrum D. Introduction: Management of the luteal phase in assisted reproductive technology. Fertil Steril 2018;109:747-8.
24. Child T, Leonard SA, Evans JS, Lass A. Systematic review of the clinical efficacy of vaginal progesterone for luteal phase support in assisted reproductive technology cycles. Reprod Biomed Online 2018;36:630-45.
25. Plasencia W, González Dávila E, Tetilla V, Padrón Pérez E, García Hernández JA, & González Maternal NL. First-trimester screening for large-for-gestational-age infants. Ultrasound Obstet Gynecol 2012;39:389-95.
26. Spencer K, Ong CY, Liao AW, Papademetriou D, Nicolaides KH. The influence of fetal sex in screening for trisomy 21 by fetal nuchal translucency, maternal serum free β-hCG and PAPP-A at 10-14 weeks of gestation. Prenat Diagn 2000;20:673-5.
27. Weissmann-Brenner A, Weisz B, Lerner-Geva L, Gindes L, Achiron R. Increased nuchal translucency is associated with large for gestational age neonates in singleton pregnancies. J Perinat Med 2011;39:305-9.
28. Siega-Riz AM, Viswanathan M, Moos MK, Deierlein A, Mumford S, Knaack J, et al. A systematic review of outcomes of maternal weight gain according to the Institute of Medicine recommendations: birthweight, fetal growth, and postpartum weight retention. Am J Obstet Gynecol 2009;201:339-e1.
29. Kalem MN, Kamalak Z, Kosus N, Kosus A, Kalem Z. Prenatal multivitamin supplementation increases birth weight. Int J Reprod Contracept Obstet and Gynecol 2017;6:2148-53.
30. Ko TJ, Tsai LY, Chu LC, Yeh SJ, Leung C, Chen CY, et al. Parental smoking during pregnancy and its association with low birth weight, small for gestational age, and preterm birth offspring: a birth cohort study. Pediatr Neonatol 2014;55:20-7.
31. Bakker M, Pajkrt E, Bilardo CM. Increased nuchal translucency with normal karyotype and anomaly scan: What next?. Best Pract Res Clin Obstet Gynaecol 2014;28:355-66.
32. Äyräs O, Eronen M, Tikkanen M, Rahkola-Soisalo P, Paavonen J, Stefanovic V Long-term neurodevelopmental outcome of children from euploid pregnancies with increased nuchal translucency in the first trimester screening. Prenat Diagn 2015;35:362-9.
33. Thorngren-Jerneck K, HerbstA. Low 5-minute Apgar score: a population-based register study of 1 million term births. Obstet Gynecol 2001;98:65-70.
34. Rüdiger M, Braun N, Aranda J, Aguar M, Bergert R, Bystricka, et al. (2015). Neonatal assessment in the delivery room-Trial to Evaluate a Specified Type of Apgar (TEST-Apgar). BMC pediatrics 2015;15:18.
35. Lee H, Han S, Lee Y, Kim S. The correlation of nuchal translucency and maternal serum aneuploidy markers with placental dysfunction. Ultrasound in Obstetrics & Gynecology 2017;50:335-335. EP16.13.
36. Yang X, Li R, Fu F, Zhang Y, Li D, Liao C. Submicroscopic chromosomal abnormalities in fetuses with increased nuchal translucency and normal karyotype. J Matern Fetal Neonatal Med 2017;30:194-8.
Yang X, Li R, Fu F, Zhang Y, Li D, Liao C. Submicroscopic chromosomal abnormalities in fetuses with increased nuchal translucency and normal karyotype. J Matern Fetal Neonatal Med 2017;30:194-8.

Clinical Investigation / Araştırma

DOI: 10.4274/tjod.tjod.galenos.2019.92300

Turk J Obstet Gynecol 2019;16:41-9

Inflammation-mediated fetal injury by maternal granulocyte-colony stimulating factor and high-dose intra-amniotic endotoxin in the caprine model

Maternal granülosit koloni stimülan faktör ve yüksek doz intra-amniyotik endotoksin ile enflamasyon aracılı fetal beyin hasarı keçi modeli

Mekin Sezik[1], Afşin Köker[2], Özlem Özmen[3], Mehmet Halıgür[4], Duygu Kaşıkçı[5], Ahmet Aydoğan[4], Orhan Özatik[6]

[1]*Süleyman Demirel University Faculty of Medicine, Department of Obstetrics and Gynecology, Isparta, Turkey*

[2]*Mehmet Akif Ersoy University Faculty of Veterinary Medicine, Department of Obstetrics and Gynecology, Burdur, Turkey*

[3]*Mehmet Akif Ersoy University Faculty of Veterinary Medicine, Department of Pathology, Burdur, Turkey*

[4]*Çukurova University Faculty of Ceyhan Veterinary Medicine, Department of Pathology, Adana, Turkey*

[5]*Isparta University of Applied Sciences Faculty of Agricultural Sciences and Technologies, Department of Animal Science, Isparta, Turkey*

[6]*Kütahya Health Sciences University Faculty of Medicine, Department of Histology and Embryology, Kütahya, Turkey*

Abstract

Objective: To define a novel experimental model with maternal intravenous (i.v.) granulocyte-colony stimulating factor (G-CSF) followed by a single- and high-dose of 20 mg intra-amniotic (IA) endotoxin to induce fetal brain injury in the preterm fetal goat.

Materials and Methods: Pregnant goats (n=4) were given 50 microg/day G-CSF into the maternal jugular vein through gestational days 110-115 (term, 150 days). At gestational day 115, 20 mg of IA endotoxin was administered. Following preterm delivery at day 120 by cesarean section umbilical cord, fetal lung and brain tissues were harvested for histopathology, immunohistochemistry, and electron microscopy. Inflammatory markers were evaluated in the amniotic fluid and fetal plasma.

Results: Necrotizing funisitis with abundant leukocyte infiltration and fetal brain injury was induced in all the fetuses in the experimental group.

Conclusion: Maternal i.v. G-CSF for 5 days followed by 20 mg of IA endotoxin is a feasible caprine model to exacerbate intrauterine inflammation.

Keywords: Animal model, endotoxins, cerebral palsy, chorioamnionitis, inflammation

Öz

Amaç: Preterm gebe keçide fetal beyin hasarı oluşturmak için maternal intravenöz (i.v.) granülosit koloni stimulan faktör (G-CSF) sonrasında amniyon içine tek ve yüksek-doz 20 mg endotoksin verilmesini içeren yeni deneysel modelin tanımlanmasıdır.

Gereç ve Yöntemler: Gebeliğin 110-115. günlerinde (term: 150 gün) keçilere (n=4) jugular ven yoluyla 50 mikrog/gün G-CSF verildi. Gebeliğin 115. gününde, 20 mg IA endotoksin uygulandı. Gebeliğin 120. gününde sezaryen ile preterm doğumu takiben umblikal kord, fetal akciğer ve beyin örnekleri histopatoloji, immünhistokimya ve elektron mikroskobu incelemeleri için elde edildi. Amniyon sıvısı ve fetal plazmada enflamatuvar belirteçler değerlendirildi.

Bulgular: Deney grubundaki fetüslerin tümünde, belirgin lökosit infiltrasyonu ile şekillenen nekrotizan funisit ve fetal beyin hasarının geliştiği saptandı.

Sonuç: Şiddetli intrauterin enflamasyon oluşturmak için 5 gün maternal i.v. G-CSF ve takiben 20 mg IA endotoksin uygulamalarını içeren keçi modelinin uygulanabilir olduğu sonucuna varıldı.

Anahtar Kelimeler: Hayvan modeli, endotoksinler, serebral palsi, koryoamniyonit, enflamasyon

PRECIS: Using low-dose maternal G-CSF and high-dose IA endotoxin, we have defined a feasible animal model of inflammation-mediated fetal injury in the preterm goat.

Address for Correspondence/Yazışma Adresi: Mekin Sezik, MD,
Süleyman Demirel University Faculty of Medicine, Department of Obstetrics and Gynecology, Isparta, Turkey
Phone: +90 541 433 71 57 **E-mail:** msezik@yahoo.com **ORCID ID:** orcid.org/0000-0002-6989-081X

Received/Geliş Tarihi: 25.11.2018 **Accepted/Kabul Tarihi:** 20.01.2019

Data was partially presented as an abstract at the 24th World Congress on Ultrasound in Obstetrics Gynecology, 14-17 September 2014, Barcelona, Spain.

Introduction

Fetoplacental inflammation secondary to intra-amniotic (IA) microbial colonization and subclinical chorioamnionitis provide a basis for the development of preterm birth and cerebral palsy (CP) by preferentially affecting the fetal brain tissue. Increased number of experimental and clinical work previously focused on fetoplacental inflammation and fetal brain injury. Therefore, animal models to aggravate intrauterine inflammation were often required and used[1]. Within this context, the preterm small ruminant (such as sheep and goat) models that exclusively used IA endotoxin were accepted as relatively suitable designs to induce fetal lung and brain injury[2,3].

Granulocyte-colony stimulating factor (G-CSF) is a hemopoietic growth factor involved in the control of neutrophil numbers. G-CSF expression is increased in response to infection or injury. Therefore, a proinflammatory role of G-CSF has been suggested, secondary to increased neutrophil production and migration to the inflamed site[4].

Here, we aim to define our experience with intravenous (i.v.) recombinant (G-CSF) administrations for 5 days followed by a single-dose of 20 mg of IA endotoxin to induce IA inflammation, necrotizing funisitis, and fetal brain/lung injury in the preterm goat model. Previous small ruminant models for this purpose have not previously used combined IA high-dose endotoxin and systemic G-CSF administrations[1-3]. Moreover, maternal i.v. bolus injections of G-CSF have not been utilized before. Hence, we hypothesized that our novel and relatively simple experimental model that does not require chronic maternal or fetal instrumentation would be able to produce exacerbated inflammation in utero, at least to an extent of that observed in previous experiments[1-3,5-7].

Materials and Methods

The current study includes data on preliminary experimental groups (model and controls) formed prior to the main intervention study testing the effects of pentoxifylline on fetal brain injury[8]. The study protocol was subject to animal ethics committee approval by Süleyman Demirel University Animal Experimentation Local Ethics Committee (approval date and no, 23.08.2011/03). Principles of laboratory animal care (NIH publication No. 86-23, revised 1985) were followed, as well as specific national laws where applicable. Eight date-mated singleton pregnant hair goats (Capra hircus) at age of 4-5 years and prepregnancy body weight of 40±5 kg were included. Term pregnancy in hair goats is around 150 days. A singleton structurally normal ongoing pregnancy and accurate fetal biometry was confirmed by ultrasonography (BCF Easi Scan, Dundalk, Ireland) at days 28, 43, 58, 73, and 88 of pregnancy. The does were sheltered in a semi-open pen and reared on pasture and/or standard food. Water and mineral salts were provided ad libitum.

At day 110 of gestation, animals were transported to the animal clinics at the Faculty of Veterinary Medicine, Mehmet Akif Ersoy University. Randomization into 2 groups was carried out: Control (group 1, n=4) and the experimental model (group 2, n=4) groups. At gestational days 110 through 115, animals in the model group were given 50 microg/day i.v. bolus injections of G-CSF (Neupogen Roche, F. Hoffmann-La Roche Ltd, Basel, Switzerland) solubilized in 2 ml of normal saline for 5 days, whereas the controls received 2 ml of i.v. normal saline only. Jugular vein was used for all i.v. administrations.

At day 115, amniocentesis was performed as defined previously[9]. Following sterilization of the abdomen with povidine-iodine, a 20-gauge amniocentesis needle was inserted into the amniotic cavity under ultrasound guidance, and 10 ml of amniotic fluid was sampled. Erroneous access into the allantois was excluded by the color and viscosity of the sample[10]. Following sampling in the model group, 20 mg of endotoxin solution (Lipopolysaccharides from Escherichia coli 055:B5, L 2880, Sigma-Aldrich, Missouri, USA) was administered intra-amniotically, using the same needle under strict ultrasound guidance. The controls received identical amount of normal saline into the amniotic cavity.

Cesarean sections were performed with minor modifications to our previously reported technique[11,12]. Approximately 120 h after the amniocentesis procedures (day 120) corresponding to 0.80 of gestation, preterm birth was induced by cesarean section. The does were sedated with 0.25 mg/kg xylazine (Rompun, Bayer, Germany) and given epidural anesthesia with 25 mg lidocaine and 0.016 mg epinephrine (Jetokain, Adeka, Samsun, Turkey) into the sacrococcygeal space. Local infiltrative anesthesia with 25 mg lidocaine into the presumed incision line was also used. Following a 10-cm paralumbar skin incision, uterus was opened from its dorsal curvature. Prior to amniotomy, 10 mL of amniotic fluid was aspirated with a sterile injector, followed by delivery of the neonate. Tissue samples from fetal membranes, umbilical cord, and placenta were also harvested appropriately. Postoperatively, the does received i.m. antibiotics (200 mg procain penicillin plus 250 mg dihydrostreptomycin sulfate; Diperinisol, Bayer, İstanbul, Turkey) and analgesia with i.m. metamizole sodium (Novalgin ampoule, PharmaVision, Istanbul, Turkey).

Following drying and weighing, the kids were given 50 mg/kg intraperitoneal sodium thiopental (Pental Sodyum, IE Ulugay, Istanbul, Turkey) for euthanasia[9,11]. Then, intracardiac blood was sampled via the transthoracic route and chest opened followed by en bloc dissection of the lungs and brain[11]. Pulmonary parenchyma and cerebral white matter were sampled.

For routine histopathology and immunohistochemistry (IHC), tissue samples were fixed in 10% buffered formaldehyde and embedded into paraffin with routine processing for further histopathological and immunohistochemical staining. For

transmission electron microscopy (TEM), tissue samples were carefully sliced into approximately 1-mm^3 pieces on a petri dish that contained buffered glutaraldehyde (2.5%, pH, 7.2) as a fixative. Sliced tissues were transferred into fixative-containing dark-colored bottles, kept at 4 °C for 24 h and transported to the TEM laboratory.

For IHC evaluations fetal lung samples were stained with surfactant proteins A, B, C, and D (Santa Cruz Biotechnology Inc, USA), prosurfactant protein B (Abcam, UK), interleukin-1, interleukin-4, interleukin-6, interleukin-10, tumor necrosis factor (TNF)-alpha, caspase 3, caspase 5, caspase 7, COX-1, COX-2, interferon-alpha, and interferon-beta (Abcam, UK).

Fetal brain samples were immunostained with interleukin-1, interleukin-4, interleukin-6, interleukin-10, TNF- alpha, caspase 3, caspase 5, caspase 7, COX-1, COX-2, interferon-alpha and interferon-beta, neuron specific enolase (NSE), glial fibrillary acidic protein (GFAP) (Abcam, UK), apoptosis protease activating factor (Biosensis, Australia), vimentin (Abcam, UK), anti-neurofilament protein (NFP) (Abcam, UK), and anti-myelin basic protein (MBP) (Abcam, UK).

Placentae and fetal membranes were stained for interleukin-1, interleukin-4, interleukin-6, interleukin-10, TNF-alpha, caspase 3, caspase 5, caspase 7, COX-1, COX-2, interferon-alpha, and interferon-beta. Commercial kits were used for IHC examinations, using a routine streptavidine-biotin peroxidase technique.

To evaluate the severity of the IHC reactions semiquantitative analyses were performed, using an arbitrary visual scale with a grading score ranging from 0 to 3 as follows: (0) = negative, (1) = weak staining, (2) = moderate staining, and (3) = diffuse staining. Olympus CX41 light microscope and the Database Manual Cell Sens Life Science Imaging Software System (Olympus Corporation, Tokyo, Japan) were used for examinations. The pathologists were blinded to the experimental groups.

Samples were bleached in propylene oxide and placed into 1/1 propylene-oxidized araldehyde for 2 h and soaked in pure araldehyde overnight. Afterwards, the specimens were embedded in araldehyde and polymerized at 60 °C for 48 h. The 700 nm sections were cut at ultra-microtome (Leica Ultracut R, Leica Microsystem, Austria), and stained with toluidine blue and examined by light microscope (Olympus BX50, Olympus, Tokyo, Japan). Selected areas were trimmed and 60-nm thin sections were stained with uranyl acetate and lead citrate, and examined by JEOL JEM1220 Transmission Electron Microscope (Nippon Denshi Co, Tokyo, Japan).

On lung sections, ultra-structural changes such as increase in goblet cells, secretory components of goblet cells, endoplasmic reticulum in goblet cells, protein synthesis, cilial degenerations, neutrophil, lymphocyte and plasma cells infiltrations, increase in intracellular ribosomes, and thickness of noncellular basal membranes were examined. In brain tissues, increase in polymorphonuclear leukocyte in perivascular areas and demyelination were evaluated.

Interleukin and TNF-alpha levels in plasma and amniotic fluid samples were evaluated by the double antibody sandwich enzyme-linked immunosorbent assay (ELISA) method. Commercial kits for goat serum provided from Eastbiopharm (Hangzhou, China) were used for TNF-alpha, interleukin-1, interleukin-4, interleukin-6, and interleukin-10. Results were evaluated at 450 nm, and optic density (OD) values were calculated and standardized accordingly.

Statistical Analysis

Variables were expressed as median and interquartile ranges given within brackets. Mann-Whitney U test was used for comparisons across the two groups. Wilcoxon signed-rank test was used to compare amniotic fluid measurements (at day 115 and 120) within the groups A two-sided $p<0.05$ was considered as significant for all analyses.

Results

Macroscopically, the umbilical cords from group 1 (controls) were normal, whereas hemorrhage and edema were evident in the model group. Microscopically, funisitis and vasculitis characterized by extensive inflammatory reaction including the Wharton jelly were present in endotoxin-exposed animals. This was characterized by necrotic arcs of inflammatory debris around all vessels made up of degenerated neutrophils in the Wharton's jelly, showing areas of inflammatory debris and neovascularization. Most of the infiltrating cells were neutrophils, while lymphocytes and plasma cells were also observed. Thrombi were observed in some of the umbilical vessel sections. Fetal membranes from group 1 were normal, whereas extensive inflammatory reaction and desquamation at the epithelial cells with areas of hemorrhage were present in membranes from the model animals. In some specimens, aggregates of bacteria presumably due to cervical dilatation secondary to inflammation in fetal membranes specific to goats or contamination were also evident (Figure 1, Figure 2).

Pulmonary sections from group 1 revealed thin septal walls, concordant with normal preterm lungs. G-CSF plus endotoxin-exposed kids had thickened and edematous septal walls accompanied by increased alveolar macrophages and neutrophilic infiltrations. Routine histopathology did not reveal any distinctive findings in the brain tissues except hyperemia and mild gliosis in a kid from group 2.

IHC results were in parallel with histopathology. Comparisons of the staining intensities across the groups are given in Table 1, revealing significant differences for all the studied parameters. Inflammatory markers including various interleukins were increased in all tissues. Moreover, specimens from the model group generally stained heavily for the apoptosis markers. Fetal brain injury was apparent in the model animals, shown by decreased NSE, NFP, GFP, and MBP staining compared to controls (Table 1).

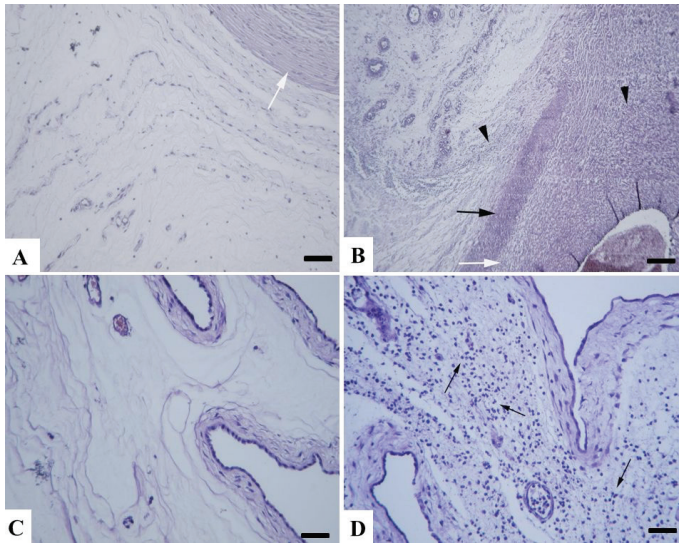


Figure 1. Umbilical cord and fetal membrane histopathology. (A) Normal appearance of umbilical cord from a kid in the control group, umbilical vessel (white arrow) (B) Severe funisitis characterized by inflammatory cell infiltrations (arrow heads) and necrotic arcs (black arrow) of inflammatory debris around the umbilical vessel (white arrow) and neovascularization (upper left side of the picture) from the model group. (C) Histology of normal fetal membranes from the control group. (D) Inflammation of fetal membranes showing numerous inflammatory cells (arrows) from model group, HE, Bar=100 µm

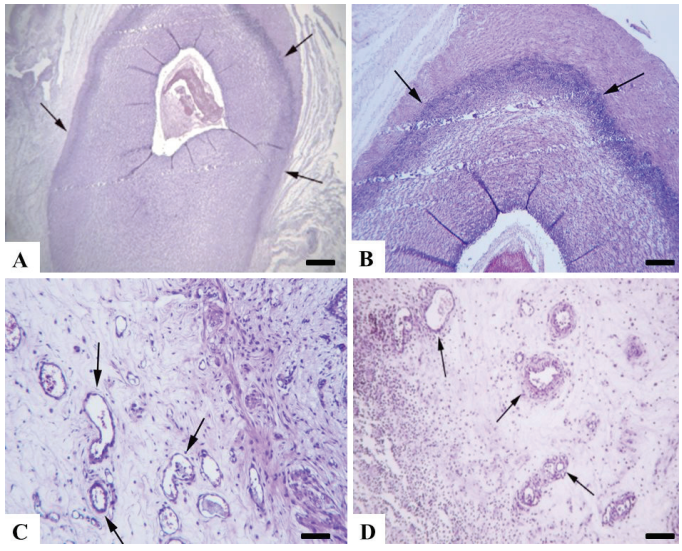


Figure 2. Umbilical vessel histopathology. (A) Marked necrotic arc (arrows) in vascular wall of the umbilical vessel, Bar=200 µm. (B) Higher magnification of the necrotic arc, Bar=100 µm. (C and D) Neovascularization (arrows) in umbilical cord, HE, Bars=100 µm

Selected IHC sections showing staining properties are shown in Figure 3 and Figure 4. Brain IHC revealed decreased immunoreaction in brain markers in the model group. While surfactant protein expirations were decreased, apoptotic

Table 1. Comparisons of immunohistochemical staining intensities across the groups

Marker	Group 1 controls (n=4)	Group 2 model (n=4)	p-value
Fetal brain			
Interleukin-1	0 (0-0.75)	3 (3-3)	0.011
Interleukin-4	0 (0-0.75)	2 (2-2.75)	0.015
Interleukin-6	0 (0-0.75)	3 (1.5-3)	0.022
Interleukin-10	0 (0-0.75)	3 (2.25-3)	0.015
Interferon-alpha	0 (0-0.75)	3 (2.25-3)	0.015
Interferon-beta	0 (0-0.75)	3 (2.25-3)	0.015
Tumor necrosis factor-alpha	0 (0-0.75)	3 (3-3)	0.011
Caspase 3	0 (0-0.75)	3 (2.25-3)	0.015
Caspase 5	0 (0-0.75)	2 (2-2.75)	0.015
Caspase 7	0 (0-0.75)	2 (2-2.75)	0.015
Cyclooxygenase-1	0.5 (0-1)	2.5 (2-3)	0.018
Cyclooxygenase-2	0 (0-0.75)	2.5 (1.25-3)	0.025
Vimentin	3 (3-3)	1.5 (1-2)	0.013
Neuron specific enolase	3 (3-3)	0.5 (0-1)	0.013
Neurofilament protein	3 (3-3)	1 (0.25-1.75)	0.013
Glial fibrillary acidic protein	3 (3-3)	1 (1-2.5)	0.04
Myelin basic protein	3 (3-3)	1 (1-2.5)	0.04
Apoptotic protease activating factor 1	2.5 (2-3)	0 (0-0.75)	0.017
Fetal lung			
Interleukin-1	0 (0-0.75)	3 (3-3)	0.011
Interleukin-4	0 (0-0.75)	3 (2.25-3)	0.015
Interleukin-6	0.5 (0-1)	2.5 (2-3)	0.018
Interleukin-10	0 (0-0.75)	2.5 (2-3)	0.017
Interferon-alpha	0 (0-0.75)	3 (3-3)	0.011
Interferon-beta	0 (0-0.75)	3 (2.25-3)	0.015
Tumor necrosis factor-alpha	0 (0-0.75)	3 (2.25-3)	0.015
Caspase 3	0 (0-0.75)	2.5 (2-3)	0.017
Caspase 5	0 (0-0.75)	2 (2-2.75)	0.015
Caspase 7	1 (0.25-1)	3 (1.5-3)	0.04
Cyclooxygenase-1	0 (0-0.75)	3 (2.25-3)	0.015
Cyclooxygenase-2	1 (0.25-1)	3 (1.5-3)	0.04
Surfactant protein A	2.5 (2-3)	0 (0-0.75)	0.017
Surfactant protein B	2.5 (2-3)	0.5 (0-1)	0.018

Continued Table 1			
Surfactant protein C	3 (2.25-3)	0 (0-0.75)	0.015
Surfactant protein D	3 (3-3)	0.5 (0-1.75)	0.013
Pro-surfactant protein B	3 (3-3)	0.5 (0-1)	0.013
Placenta			
Interleukin-1	0 (0-0.75)	3 (3-3)	0.011
Interleukin-4	0.5 (0-1)	3 (2.25-3)	0.017
Interleukin-6	0.5 (0-1)	3 (2.25-3)	0.015
Interleukin-10	0 (0-0.75)	2.5 (2-3)	0.017
Interferon-alpha	0 (0-0.75)	3 (2.25-3)	0.015
Interferon-beta	0 (0-0.75)	3 (1.5-3)	0.022
Tumor necrosis factor-alpha	0 (0-0.75)	3 (2.25-3)	0.015
Caspase 3	0 (0-0.75)	3 (3-3)	0.011
Caspase 5	0 (0-0.75)	2.5 (2-3)	0.017
Caspase 7	0 (0-0.75)	2.5 (2-3)	0.017
Cyclooxygenase-1	1 (0.25-1)	2.5 (2-3)	0.017
Cyclooxygenase-2	0 (0-0)	3 (2.25-3)	0.011

Data are expressed as medians with interquartile ranges within brackets. Staining intensity was graded as follows: 0=negative, 1=weak staining, 2=moderate staining, and 3=diffuse staining

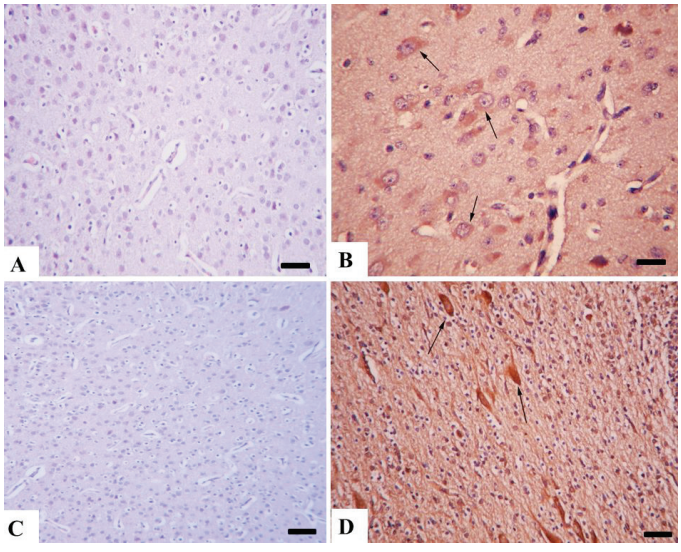


Figure 3. Brain immunohistochemistry. (A) Negative caspase-5 immunoreaction in the control group, Bar=100 µm. (B) Increased caspase-5 immunoreaction in neurons (arrows) from the model group, Bar=50 µm. (C) Negative COX-1 immunoreaction in the control group, Bar=100 µm. (D) Marked COX-1 reaction in the model group, Bar=100 µm

markers' expirations were increased in the lungs of endotoxin-exposed kids (Figure 5, Figure 6).

In the model group, brain and lungs tissues were severely affected (Figure 7). Ultrastructural examination of the brain specimens revealed chromatin margination at the nuclei accompanied by membrane damage and tissue lysis. Pulmonary specimens revealed blebbing at alveolar and bronchiolar cells. Mitochondrial damage and accompanying blebbing formations were also observed (Figure 7).

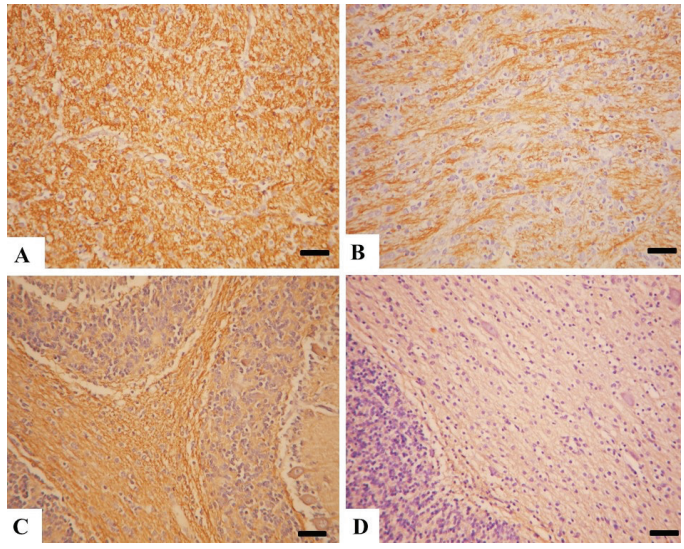


Figure 4. Brain tissue markers immunohistochemistry. (A) Marked myelin basic protein immunoreaction in a control brain, Bar=100 µm. (B) Decreased myelin basic protein expiration in the model group, Bar=100 µm. (C) Marked neurofilament protein immunopositive reaction in the control group, Bar=100 µm. (D) Markedly reduced neurofilament protein expiration in the model group, Bar=100 µm, streptavidin biotin peroxidase method

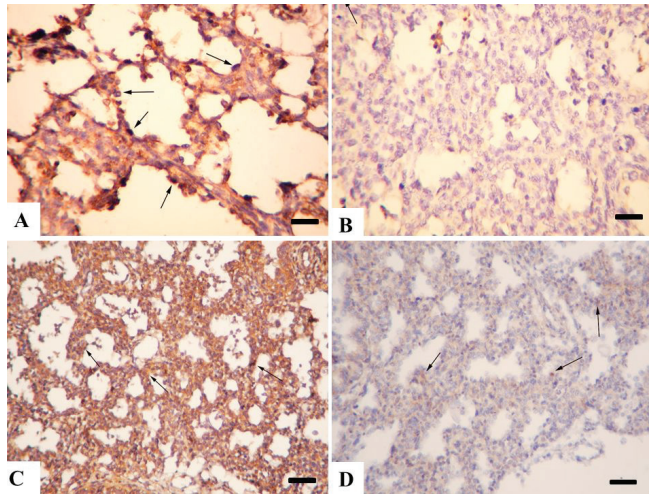


Figure 5. Lung immunohistochemistry (A) Strong SP-A expiration from alveolar cells (arrows) in a lung from the control group, Bar=100 µm. (B) Decreased SP-A expiration from alveolar cells (arrows) in the model group, Bar=100 µm. (C) Marked SP-B reaction in lung epithelial cells (arrows) in the control group, Bar=200µm. (D) Decreased SP-B immunoreaction in alveolar cells in lungs from the model group, Bar=100 µm

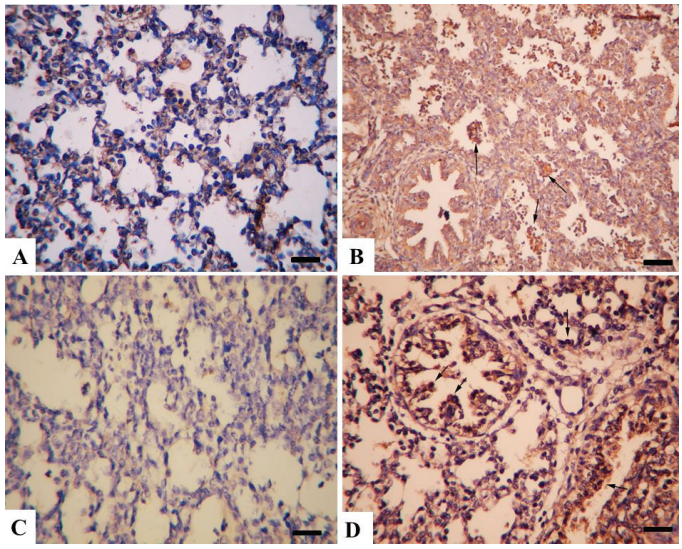


Figure 6. Lung interleukin (IL)-1 and caspase-5 immunohistochemistry. (A) Very slight IL-1 immunoreaction in the control group, Bar=100 µm. (B) Marked increase in IL-1 expiration from alveolar macrophages (arrows) in the model group, Bar=100 µm. (C) Negative caspase-5 expression in the control group, Bar=100 µm. (D) Increased caspase-5 immunoreaction in bronchiolar cells (arrows) in a kid's lung from the model group, Bar=100 µm, streptavidin biotin peroxidase method

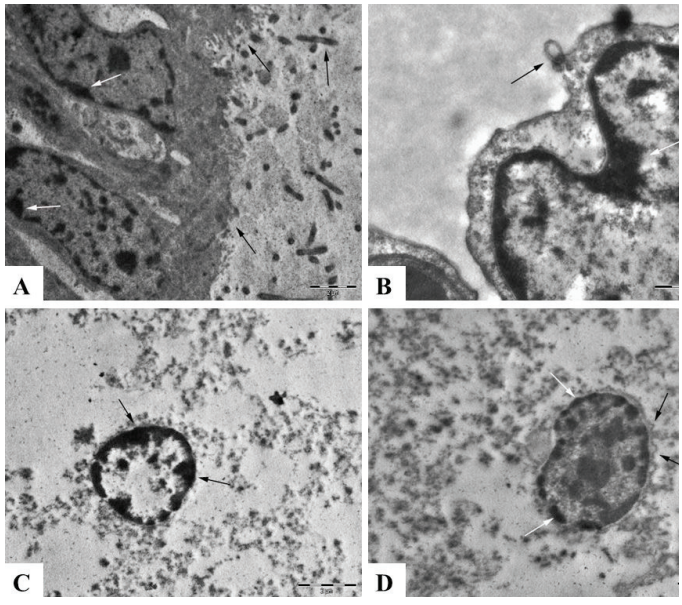


Figure 7. Electron microscopic findings in the model group, (A) Marked ciliary loss (black arrows) and chromatin marginations (white arrows) from lung cells, Bar=2 µm, (B) Blebbing at nuclear membrane (black arrow) and chromatin margination (white arrow) in the nucleus of a lung cell, Bar=1µm, (C) Severe necrotic changes, organelle lysis and chromatin marginations (arrows) in a neuron, Bar=2 µm, (D) Blebbings at nuclear membrane (black arrows) and chromatin marginations (white arrows) in a neuron, Bar=2 µm

Comparisons of interleukin (IL) and TNF-alpha levels measured by ELISA in the fetal serum and amniotic fluid samples are summarized in Table 2. Pregnancies in the model group had higher mean IL-1, 4, 6, 10 and TNF-alpha in the fetal plasma and amniotic fluid samples that were retrieved at day 115 (before endotoxin administration) and at day 120 (during preterm delivery). However, comparisons of differences over time (day 120 minus day 115) across the groups revealed no significant differences (Table 2).

IL-1, IL-4, IL-6, IL-10, and TNF-alpha levels showed non-significant increments both in the control (p=0.141, p=0.461, p=0.066, p=0.066 and p=0.066, respectively) and the study groups (p=0.461, p=0.461, p=0.461, p=0.18 and p=0.066, respectively).

Discussion

According to our findings, the use of maternal G-CSF without fetal catheterization followed by high-dose IA endotoxin is effective to induce fetal funisitis as well as fetal brain and lung injury. The suggested model can be used in experimental research to test the efficacy of potential drugs for the prevention of inflammation-related preterm labor.

Certain models to induce subchronic chorioamnionitis associated with preterm delivery have been used in the medical literature. The most common animal model includes the injection of endotoxin, i.e. lipopolysaccharides (LPS) usually isolated from *E. coli*[1-3,5-7,13]. Although endotoxin has been injected into the maternal peritoneum[14] or directly into the uterine horns[15] of small animals such as rat and rabbit, IA administration generates one of the most plausible models, similar to the human disorder. Therefore, many experimental models used small ruminant models such as pregnant sheep and goat to induce chorioamnionitis and subsequent fetal injury by injecting endotoxin into the amniotic fluid under ultrasound guidance[13]. Fetal administrations of IA endotoxin to induce inflammation are technically difficult in smaller animals, but are feasible in the pregnant sheep and goat. Moreover, small ruminant models are convenient for evaluating novel fetal therapy modalities against fetal injury, as tissue harvesting and adequate sampling of amniotic fluid or fetal plasma are easier[16].

Ureoplasma species, especially Ureoplasma pavum serovars of up to $2x10^7$ Colony Forming Units have also been used to induce chorioamnionitis in pregnant sheep[17]. However, Ureoplasma alone seems to cause modest responses and may down-regulate LPS-induced proinflammatory cytokines[18].

The endotoxin dose given into the amniotic cavity of the ewes has mostly been 10 mg LPS. However, different doses such as 1 mg, 4 mg, 20 mg, and 100 mg were also evaluated and were reported not to alter birth weight or umbilical arterial blood pH and partial carbon dioxide values relative to controls[19]. Moreover, even ultra-high IA doses (100 mg) were not associated with fetal deaths in the sheep

Table 2. Comparisons of enzyme-linked immunosorbent assay results across the groups

Marker	Group 1 controls	Group 2 model	p-value
Fetal plasma			
Interleukin-1 (pg/mL)	63 (55- 91)	141 (125-161)	0.02
Interleukin-4 (pg/mL)	84 (80-98)	489 (467-499)	0.0001
Interleukin-6 (pg/mL)	44 (42-48)	164 (123-180)	0.005
Interleukin-10 (pg/mL)	23 (16-26)	100 (69-104)	0.005
Tumor necrosis factor-alpha (pg/mL)	84 (64-89)	162 (139-180)	0.001
Amniotic fluid (day 115)			
Interleukin-1 (pg/mL)	76 (68-93)	200 (153-240)	0.012
Interleukin-4 (pg/mL)	72 (69-135)	437 (429-593)	0.002
Interleukin-6 (pg/mL)	56 (49-58)	158 (136-200)	0.008
Interleukin-10 (pg/mL)	28 (24-29)	100 (83-103)	0.001
Tumor necrosis factor-alpha (pg/mL)	91 (77-93)	284 (264-285)	0.0001
Amniotic fluid (day 120)			
Interleukin-1 (pg/mL)	95 (87-102)	207 (200-245)	0.002
Interleukin-4 (pg/mL)	105 (83-144)	475 (462-579)	0.0001
Interleukin-6 (pg/mL)	60 (52-79)	180 (173-236)	0.003
Interleukin-10 (pg/mL)	31 (28-34)	105 (100-117)	0.0001
Tumor necrosis factor-alpha (pg/mL)	112 (109-118)	302 (294-303)	0.0001
Change in amniotic fluid (day 120 minus day 115)			
Interleukin-1 (pg/mL)	19 (-6-35)	7 (-40-92)	0.686
Interleukin-4 (pg/mL)	14 (-17-58)	25 (-110-143)	0.686
Interleukin-6 (pg/mL)	4 (3-20)	15 (-16-96)	0.686
Interleukin-10 (pg/mL)	3 (3-5)	3 (0-33)	1.0
Tumor necrosis factor-alpha (pg/mL)	25 (19-33)	18 (17-30)	0.2

Data are expressed as median values with interquartile ranges within brackets

model[19]. Interestingly, relatively low doses of IA endotoxin were associated with increased fetal pulmonary maturation (instead of injury) in some investigations[5,6]. It is possible that IA endotoxin stimulates lung maturation by a mechanism distinct from glucocorticoids[19].

The finding that low doses of IA endotoxin may paradoxically prevent fetal lung injury led investigators to modify animal models of chorioamnionitis and fetal injury. Watanabe at al.[7] suggested that fetal G-CSF pretreatment before activation in utero by an IA infusion is 100% effective to induce necrotizing funisitis. This assumption depends on the observation that preterm neonates born following chorioamnionitis had significantly higher umbilical cord G-CSF levels than those without chorioamnionitis[20]. Moreover, fetuses with fetal inflammatory syndrome were found to have high median fetal plasma G-CSF concentrations[21,22].

It is known that low-grade systemic inflammatory response is an important component of fetal brain injury[1]. Therefore, pretreatment with fetal G-CSF may exacerbate inflammation in the umbilical cord and the fetus. Furthermore, necrotizing funisitis has been reported as an important risk factor for the development of chronic lung disease in the human[23]. Therefore, the induction of necrotizing funisitis in an experimental animal model has the potential to simulate the severe form of neonatal injury. Overall, these data[7,20-23] support the notion that exacerbated inflammation by G-CSF and high-dose endotoxin causes substantial fetal lung and brain injury, as opposed to fetal pulmonary maturational or protective effects observed with low-dose IA endotoxin injections.

Depending on these previous data, we developed our model in goat pregnancy by modifying the setting described by Watanabe at al.[7]. These authors performed a laparotomy and hysterotomy to catheterize fetal carotid arteries chronically. At least 48 h after surgery, catheterized fetuses received daily bolus infusions of G-CSF from day 125 to day 129 of gestation. While on fetal G-CSF pretreatment, 20 mg of endotoxin was

administered intra-amniotically at day 127. The investigators reported 100% success rate with the model, with all animals developing necrotizing funisitis[7].

We modified the experimental model by Watanabe et al.[7] and preferred giving G-CSF to the doe (mother) instead of performing chronic fetal arterial catheterization, a technically demanding intervention. G-CSF was shown to cross the placenta in previous human and animal studies[24-26]. G-CSF was measurable in the amniotic fluid and fetal plasma of pregnant mice 30 min after injection, with a peak concentration reached at 2 to 4 h. Relatively high concentrations revealed that a functional placental receptor was not essential for the transfer of G-CSF across the placenta[25]. Similar results were obtained in rat models, demonstrating that maternally administered G-CSF crosses the placenta and has myelopoietic effects even at low concentrations in the fetus[26].

G-CSF is generally considered safe in pregnancy. Although we were not able to locate any information on potential side effects of G-CSF particularly in pregnant goats, data from other species including humans demonstrate no significant adverse effects during pregnancy[7,27]. In a recent report[28], rates of spontaneous terminations, preterm births, maternal infections, and neonatal adverse events were similar in women with and without G-CSF therapy during pregnancy.

These results[24-28] led us to administer G-CSF directly into the venous circulation of the does. We believe this has two advantages. First, a low-grade inflammation was induced in the maternal compartment. This conforms to the human preterm birth cascades associated with early maternal infection/inflammation. Second, G-CSF in the maternal compartment is expected to cross the placenta, obviating the need for direct fetal arterial administrations. Another modification in our design was the administration of IA endotoxin by the completion of the 5-day course of maternal G-CSF injections. We suppose this is more coherent with the fetal inflammatory response syndrome, in which low-grade preclinical systemic maternal and IA inflammation is followed by a relatively abrupt insult that leads to fetal brain injury and preterm delivery.

Inflammatory markers including interleukin-1, 4, and 6 and COX-1 and COX-2 were increased in the fetal tissues and placenta of our model pregnancies. Apoptosis in terms of caspase 3, 5, and 7 was also prominent in all those tissues. Fetal brain injury was shown in the model kids, as expressions of markers such as vimentin, NSE, NFP, GFAP, MBP, and APAF-1 were all decreased. Significant fetal inflammation was additionally proved by increased interleukin and TNF concentrations in the fetal plasma and amniotic fluid.

An interesting finding of our study was lack of significant change in certain amniotic fluid inflammatory markers between day 115 and day 120. This held true for both of the groups, suggesting that IA interleukin-1, 4, 6, 10 and TNF-alpha levels were comparable 5 days after IA endotoxin exposure. In fact, these data are in line with previous findings indicating maximally induced cytokine mRNAs within only 5 h after IA endotoxin administration and decreasing to control values by 2 days[29]. Therefore, our design with a sampling interval of 5 days probably did not allow detecting the early and rapid elevation of IA interleukins.

Interleukin-10 is an anti-inflammatory cytokine, capable of inhibiting synthesis of pro-inflammatory cytokines. We found increased interleukin-10 staining in the fetal brain, fetal lung, and the placenta of exposed animals compared to controls. Interleukin-10 plasma concentrations were also higher in the model animals. Although these results seem contradictory, they support previous human and animal data, denoting increased amniotic fluid and fetal tissue levels of interleukin-10 during IA infection and/or inflammation. In a recent ovine study that used IA endotoxin[30], interleukin-1, 6, 8, TNF-alpha, and interleukin-10 mRNA were all reported to be increased similar to our findings. Overall, our data support increased reflex anti-inflammatory processes in our design and the involved setting.

Study Limitations

We did not specifically evaluate physiological parameters such as fetal heart rates and arterial or amniotic pressures, as these were not part of our original experimental design. We were not able to track changes in leukocyte count or activity during the study period in the groups. Due to ethical reasons, our design did not include groups that were given only G-CSF and given only endotoxin to observe independent effects on inflammation separately. Despite these drawbacks, our robust design proved that the present novel and customized experimental model leads to profound IA and fetal inflammation as well as fetal injury, shown repeatedly by ELISA, immunohistochemistry, and electron microscopy results. Moreover, our model consistently resulted in necrotizing funisitis, which is a predictor of chronic lung disease and impaired neurological outcome.

Conclusion

The supposed small ruminant experimental design, which includes maternal i.v. G-CSF followed by 20 mg of IA endotoxin is a feasible model to aggravate intrauterine inflammation and fetal lung/brain injury and will have potential use for research in that specific area.

Ethics

Ethics Committee Approval: The study protocol was subject to animal ethics committee approval by Süleyman Demirel University Animal Experimentation Local Ethics Committee (approval date and no, 23.08.2011/03).

Informed Consent: Experimental study.

Peer-review: External and internal peer-reviewed.

Authorship Contributions

Concept: M.S., A.K., Ö.Ö., M.H., D.K., A.A., O.Ö. Design: M.S., A.K., D.K. Data Collection or Processing: M.S., A.K., Ö.Ö., M.H., D.K., A.A., O.Ö. Analysis or Interpretation: M.S., Ö.Ö, M.H, O.Ö. Literature Search: M.S., A.F, O.Ö., Writing: M.S., Ö.Ö.

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

Financial Disclosure: The present study was supported by funds from the Scientific and Technological Research Council of Turkey (research project no, 111S424).

References

1. Hagberg H, Peebles D, Mallard C. Models of white matter injury: comparison of infectious, hypoxic-ischemic, and excitotoxic insults. Ment Retard Dev Disabil Res Rev 2002;8:30-8.
2. Gavilanes AW, Strackx E, Kramer BW, Gantert M, Van den Hove D, Steinbusch H, et al. Chorioamnionitis induced by intraamniotic lipopolysaccharide resulted in an interval-dependent increase in central nervous system injury in the fetal sheep. Am J Obstet Gynecol 2009;200:437.e1-8.
3. Miura A, Yoneyama Y, Sawa R, Araki T. Fetal plasma prostaglandin F(2alpha) and cortisol responses to high-dose endotoxin administration in fetal goats. J Nippon Med Sch 2003;70:151-6.
4. Hamilton JA, Achuthan A. Colony stimulating factors and myeloid cell biology in health and disease. Trends Immunol 2013;34:81-9.
5. Moss TJ, Newnham JP, Willett KE, Kramer BW, Jobe AH, Ikegami M. Early gestational intra-amniotic endotoxin: lung function, surfactant, and morphometry. Am J Respir Crit Care Med 2002;165:805-11.
6. Kuypers E, Collins JJ, Kramer BW, Ofman G, Nitsos I, Pillow JJ, et al. Intra-amniotic LPS and antenatal betamethasone: inflammation and maturation in preterm lamb lungs. Am J Physiol Lung Cell Mol Physiol 2012;302:L380-9.
7. Watanabe T, Matsuda T, Hanita T, Okuyama K, Cho K, Kobayashi K, et al. Induction of necrotizing funisitis by fetal administration of intravenous granulocyte-colony stimulating factor and intra-amniotic endotoxin in premature fetal sheep. Pediatr Res 2007;62:670-3.
8. Sezik M, Koker A, Ozmen O, Haligur M, Ince D, Aydogan A, et al. Antenatal pentoxifylline therapy to prevent endotoxin-induced fetal injury in the preterm goat model. In: Ultrasound in Obstetrics & Gynecology Supplement: Abstracts of the 24th World Congress on Ultrasound in Obstetrics Gynecology; 14-17 September 2014; Barcelona, Spain; International Society of Ultrasound in Obstetrics Gynecology; 2014. p. 310.
9. Sezik M, Ozkaya O, Arslanoglu E, Koker A, Cetin H, Ozbasar D, et al. Evaluation of intra-amniotic surfactant administration for lung maturation in preterm sheep. J Exp Anim Sci 2007;43:301-7.
10. Frandson RD, Wilke WL, Fails AD. Anatomy and physiology of farm animals. 7th ed. Singapore: Wiley-Blackwell; 2009.
11. Sezik M, Antalyali M, Ozmen O, Haligur M, Koker A, Ozkaya O, et al. Ultra-high dose of intra-amniotic or direct fetal intramuscular betamethasone for lung maturation in the preterm goat model. Eur J Obstet Gynecol Reprod Biol 2012;164:15-23.
12. Antalyalı M, Ozmen O, Halıgür M, Sezik M. Fetal pulmonary injury following single high-dose intra-amniotic betamethasone treatment in preterm goat kids. J Turk Ger Gynecol Assoc 2012;13:242-6.
13. Kemp MW, Saito M, Newnham JP, Nitsos I, Okamura K, Kallapur SG. Preterm birth, infection, and inflammation advances from the study of animal models. Reprod Sci 2010;17:619-28.
14. Cai Z, Pan ZL, Pang Y, Evans OB, Rhodes PG. Cytokine induction in fetal rat brains and brain injury in neonatal rats after maternal lipopolysaccharide administration. Pediatr Res 2000;47:64-72.
15. Debillon T, Gras-Leguen C, Vérielle V, Winer N, Caillon J, Rozé JC, et al. Intrauterine infection induces programmed cell death in rabbit periventricular white matter. Pediatr Res 2000;47:736-42.
16. Barry JS, Anthony RV. The pregnant sheep as a model for human pregnancy. Theriogenology 2008;69:55-67.
17. Kramer BW. Chorioamnionitis - new ideas from experimental models. Neonatology 2011;99:320-5.
18. Snyder CC, Wolfe KB, Gisslen T, Knox CL, Kemp MW, Kramer BW, et al. Modulation of lipopolysaccharide-induced chorioamnionitis by Ureaplasma parvum in sheep. Am J Obstet Gynecol 2013;208:399.e1-8.
19. Jobe AH, Newnham JP, Willet KE, Moss TJ, Gore Ervin M, Padbury JF, et al. Endotoxin-induced lung maturation in preterm lambs is not mediated by cortisol. Am J Respir Crit Care Med 2000;162:1656-61.
20. Li Y, Ohls RK, Rosa C, Shah M, Richards DS, Christensen RD. Maternal and umbilical serum concentrations of granulocyte colony-stimulating factor and its messenger RNA during clinical chorioamnionitis. Obstet Gynecol 1995;86:428-32.
21. Chaiworapongsa T, Romero R, Berry SM, Hassan SS, Yoon BH, Edwin S, et al. The role of granulocyte colony-stimulating factor in the neutrophilia observed in the fetal inflammatory response syndrome. J Perinat Med 2011;39:653-66.
22. Goldenberg RL, Andrews WW, Mercer BM, Moawad AH, Meis PJ, Iams JD, et al. The preterm prediction study: granulocyte colonystimulating factor and spontaneous preterm birth. National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network. Am J Obstet Gynecol 2000;182:625-30.
23. Matsuda T, Nakajima T, Hattori S, Hanatani K, Fukazawa Y, Kobayashi K, et al. Necrotizing funisitis: clinical significance and association with chronic lung disease in premature infants. Am J Obstet Gynecol 1997;177:1402-7.
24. Calhoun DA, Rosa C, Christensen RD. Transplacental passage of recombinant human granulocyte colony-stimulating factor in women with an imminent preterm delivery. Am J Obstet Gynecol 1996;174:1306-11.
25. Calhoun DA, Gersting JA, Lunøe M, Du Y, Christensen RD. Transfer of recombinant human granulocyte colony stimulating factor (rhGCSF) from the maternal to the fetal circulation is not dependent upon a functional G-CSF-receptor. Placenta 2001;22:609-12.
26. Medlock ES, Kaplan DL, Cecchini M, Ulich TR, del Castillo J, Andresen J. Granulocyte colony-stimulating factor crosses the placenta and stimulates fetal rat granulopoiesis. Blood 1993;81:916-22.
27. Pessach I, Shimoni A, Nagler A. Granulocyte-colony stimulating factor for hematopoietic stem cell donation from healthy female donors during pregnancy and lactation: what do we know? Hum Reprod Update 2013;19:259-67.
28. Boxer LA, Bolyard AA, Kelley ML, Marrero TM, Phan L, Bond JM, Newburger PE, Dale DC. Use of granulocyte colony-stimulating factor during pregnancy in women with chronic neutropenia. Obstet Gynecol 2015;125:197-203. 29.
29. Kallapur SG, Willet KE, Jobe AH, Ikegami M, Bachurski CJ. Intraamniotic endotoxin: chorioamnionitis precedes lung maturation in preterm lambs. Am J Physiol Lung Cell Mol Physiol 2001;280:L527-36.
30. Schmidt AF, Kannan PS, Kemp MW, Kramer BW, Newnham JP, Jobe AH, et al. Intra-amniotic LPS modulates expression of antimicrobial peptides in the fetal sheep lung. Pediatr Res 2014;76:441-7.

Clinical Investigation / Araştırma

DOI: 10.4274/tjod.galenos.2019.84665

Turk J Obstet Gynecol 2019;16:50-4

Neural and cardiac injury markers in fetal growth restriction and their relation to perinatal outcomes

Fetal büyüme kısıtlılığında kardiyak ve nöral hasar belirteçlerinin perinatal sonuçlarla ilişkisi

Betül Yakıştıran[1], Doruk Cevdi Katlan[2], Tuncay Yüce[1], Acar Koç[3]

[1]*University of Health Sciences, Ankara Zekai Tahir Burak Woman's Health Training and Research Hospital, Clinic of Obstetrics and Gynecology, Ankara, Turkey*

[2]*İstanbul Süleymaniye Women's Health Training and Research Hospital, Clinic of Obstetrics and Gynecology, İstanbul, Turkey*

[3]*Ankara University Faculty of Medicine, Department of Obstetrics and Gynecology, Ankara, Turkey*

Abstract

Objective: To compare the levels of umbilical cord blood Neuron-Specific Enolase (NSE) and troponin T and venous blood gas samples between healthy newborns and growth-retarded fetuses with impaired Doppler velocity or low APGAR scores.

Materials and Methods: This study was a prospective cohort study. The study group comprised 26 patients with intrauterine growth restriction and pathologic Doppler symptoms, and the control group included 24 healthy fetuses. Umbilical cord blood and blood gas samples were taken from all patients. The blood samples were centrifuged and sent to a laboratory to study NSE and troponin T Perinatal outcomes were evaluated from the medical records of the newborns.

Results: Both groups were similar in terms of demographic characteristics. Fetuses with fetal growth restriction (FGR) were born earlier and had lower APGAR scores than the study group. Chronic hypoxemic fetuses in the study group had lower cord pH and HCO_3 levels. Further, troponin T levels were higher in the study group than in the control group. There were no major differences in Doppler velocity measurements.

Conclusion: It has been understood that cardiac and neuronal injury detection on fetuses with FGR, troponin T, and NSE are indicators that can be used. In the literature there are studies with heterogeneous paradigms using different indicators to find neuronal injury. As a result of this study, it is clear that to assess neonatal prognosis, wider-scoped and comparative studies will provide more information about the subject.

Keywords: Fetal growth disorders, NSE, Troponin T, pregnancy outcome

Öz

Amaç: Bu çalışmadaki amacımız, bozulmuş Doppler akımı olan veya düşük APGAR skoru ile doğan gelişme geriliği olan fetuslar ile sağlıklı fetusların umbilikal kord kanında Neuron-Specific Enolase (NSE), troponin T seviyeleri ve venöz kan gazı parametrelerini karşılaştırmaktır.

Gereç ve Yöntemler: Bu çalışma prospektif kohort çalışmasıdır. Çalışma grubu olarak patolojik Doppler bulgularına sahip fetal büyüme kısıtlılığı olan 26 olgu ve kontrol grubu olarak 24 sağlıklı fetus çalışmaya dahil edilmiştir. Bütün olgulardan, umbilikal kord kanı ve kan gazı örnekleri alınmıştır. Kan örnekleri santrifüjlendikten sonra, NSE ve troponin T çalışılması için laboratuvara gönderilmiştir. Yenidoğanların medikal kayıtlarından perinatal sonuçları kaydedilmiştir.

Bulgular: Her iki grup da demografik özellikler açısından benzerdi. Fetal büyüme kısıtlılığı olan fetuslar, kontrol grubuna göre daha erken haftada ve daha düşük APGAR skoru ile doğdular. Çalışma grubundaki kronik hipoksik fetuslar, daha düşük kord pH ile HCO_3 düzeylerine sahipken; troponin T düzeyi kontrol grubuna göre daha yüksek saptandı. Doppler akım ölçümlerinde belirgin farklılık izlenmedi.

Sonuç: Büyüme kısıtlılığı olan fetuslarda kardiyak ve nöral hasarın saptanmasında troponin T ve NSE kullanılabilir. Literatür incelendiğinde, nöral hasarın saptanması için farklı indikatörlerin kullanıldığı görülebilir. Bu konuda yapılacak geniş hasta grupları ile karşılaştırmalı çalışmalar neonatal sonuçların değerlendirilmesinde daha kapsamlı sonuçlar verecektir.

Anahtar Kelimeler: Fetal büyüme-gelişme bozuklukları, NSE, Troponin T, gebelik sonuçları

PRECIS: NSE and troponin T in fetal growth restriction.

Address for Correspondence/Yazışma Adresi: Betül Yakıştıran, MD,
University of Health Sciences, Ankara Zekai Tahir Burak Woman's Health Research and Training Hospital, Clinic of Obstetrics and Gynecology, Ankara, Turkey
Phone: +90 546 429 63 21 **E-mail:** btlengin@gmail.com **ORCID ID:** orcid.org/0000-0002-3993-4017

Received/Geliş Tarihi: 22.12.2018 **Accepted/Kabul Tarihi:** 07.02.2019

Introduction

Fetal growth restriction (FGR) is defined as a fetus's failure to achieve its previously planned growth potential. In most cases, it is secondary to placental insufficiency, and these cases refer to late-onset growth restriction[1]. Although the common belief is that placental insufficiency causes FGR, it may be a result of different, unknown etiologic factors.

Fetuses with growth restriction respond to inadequate nutrient and oxygen uptake with abnormal functioning of the endocrine, cardiovascular, hematologic, and neuronal systems. The fetus can experience numerous complications in the neonatal period, including mortality, necrotizing enterocolitis, neonatal asphyxia, meconium aspiration, hypoglycemia, and other metabolic abnormalities[2]. Among these complications, impaired cognitive function is the most important because of its hazardous effect on the neonate's life. Furthermore, altered cardiovascular function in fetal life is a significant risk factor for chronic hypertension and ischemic heart disease for the subsequent life[3]. Therefore, to predict and prevent these complications, neurologic and cardiac biomarkers may be helpful for management.

In FGR, the transplacental nutrient flow is diverted through the ductus venosus, initially reaching the brain and heart; this is a brain-sparing effect to protect the blood supply to the vital organs. A fall-off in the interval growth of the abdominal circumference because of altered blood distribution is one of the earliest finding in FGR. Moreover, consumption of different energy substrates is associated with decreased myelination, neurotransmitters, and new synapses[4]. Intrauterine hypoxia is the main reason for neural damage in cases of FGR. When neural damage occurs, enzymes are released by the injured neurons and other neuronal system cells, such as astrocytes and Schwann cells. The enzymes are released by villous and intermediate trophoblasts. The most studied markers are neuron-specific enolase (NSE) and S-100B protein[5].

In addition to the neural injury, in growth-retarded fetuses with increased placental resistance, the lengths of the aortic and pulmonary systolic and pulmonary peak velocities shorten, but the length of the aortic peak velocity extends. The ejection fractions of both ventricles are decreased, and this is associated with poor neonatal outcomes and fetal acidosis. Myocardial hypertrophy without ventricular dilatation results from the increased ratio of fetal heart weight to body weight. The cardiac-sparing effect includes all these changes[6] and cardiac ischemia and myocardial necrosis may occur in asphyxiated fetuses[7]. Increased levels of troponin T, a cardiac enzyme, are related to cardiac injury. The aim of this study was to compare the levels of umbilical cord blood NSE and troponin T and venous blood gas samples between healthy newborns and growth-retarded fetuses with impaired Doppler velocity or low APGAR scores.

Materials and Methods

This prospective cohort study was approved by the Clinical Research Ethics Committee of Ankara University (Number of IRB: 05-216-15/23.03.2015). Informed consent was provided by all participants prior to the baseline interview in the original study.

Patients

We investigated 50 fetuses, 26 of which had been diagnosed as havig growth retardation in the Department of Obstetrics and Gynecology, Ankara University School of Medicine, from 2014 to 2016. The pregnant mothers attended routine antenatal obstetric care throughout their pregnancies and delivered in the same hospital. In the study group, 26 fetuses were diagnosed as having FGR by a perinatologist. The control group included 24 healthy fetuses with no signs of intrauterine hypoxemia or growth abnormalities, as shown by ultrasonography.

Inclusion and exclusion criteria

The study group's inclusion criteria were as follows: 1) women who had agreed to participate in the study and had given informed consent, 2) fetuses with signs of chronic hypoxemia and pathologic arterial/venous Doppler velocity measurements and that were born with low APGAR scores, 3) oligohydramnios, 4) gestational age more than 24 weeks, and 5) delivery by cesarean section. The exclusion criteria for both groups were as follows: 1) fetuses with congenital abnormalities and aneuploidy, 2) vaginal/operative births, and 3) preterm rupture of membranes.

Study parameters

In this study, the patients' characteristics, and medical and obstetric histories were recorded. In the third trimester of pregnancy, biometric measurements and biophysical profiles were assessed using high-resolution grayscale ultrasonography, and umbilical artery ductus venosus and middle cerebral artery Doppler velocity indices were measured with pulsed-waved and color Doppler sonography. The decision concerning delivery time was made on impaired biophysical profiles and Doppler velocity. Cesarean section was selected as the delivery mode for avoiding the influence of vaginal birth on the blood parameters. Immediately after the baby was delivered by cesarean section, the umbilical cord was clamped and blood gas samples were taken using a heparinized injector. The blood gas samples from the umbilical cord vein were collected and sent for analyses. At the same time, approximately 10 mL of umbilical blood was taken into two empty biochemistry tubes (Isotherm, clot activator/6 mL; Hongyu Med Devices Co Ltd.; Weihai, China), and after 30 minutes, the umbilical blood samples were centrifuged at 3500 rpm for 5 minutes, and the serums were frozen at -80 °C in a deep freezer to be kept at -20 °C. The samples were sent to Düzen Laboratory to study the NSE and troponin T, and they

were investigated using electroluminescence immunoassays. The pO_2, pCO_2, HCO_3, and base excess values were studied; gestational age at delivery, birth weight, APGAR 1/5 scores and necessity for neonatal intensive care unit stays were also recorded.

Statistical Analysis

Data were calculated using the SPSS 11.5 software package for Windows (SPSS Inc., USA). A value of p<.05 was considered to indicate statistical significance. Patients from both groups were compared using the Mann-Whitney U/Student *t*-test, depending on the character of distribution. Categorical variables were compared using the chi-square test and Pearson/Spearman analyses. Linear regression was used to identify the correlation between dependent variables (NSE and troponin T) and independent quantitative parameters.

Results

A total of 50 fetuses were included in this analysis. Among the pregnancies, for the study group, 26 (52%) fetuses were followed with a diagnosis of FGR or pathologic Doppler symptoms, and for the control group, 24 (48%) healthy fetuses were included. The maternal characteristics, gestational age at delivery, birth weight, and APGAR 1/5 scores are shown in Table 1. Both groups were similar in terms of maternal age and numbers of gravida, livebirths, and abortus. Fetuses with FGR were born earlier and had lower APGAR scores than the study group.

Umbilical cord gas analyses, Doppler velocity indices, and NSE and troponin T levels are summarized in Table 2. Chronic hypoxemic fetuses in the study group had lower cord pH and HCO_3 levels. Further, troponin T levels were higher in the study group than in the control group. There were no major differences in Doppler velocity measurements.

There was no correlation between NSE and birth-weight, APGAR scores, cord gas analysis, UA, DV, and melting curve analysis Doppler PI. Further, there was a statistically significant difference between troponin T and APGAR scores, pH, HCO_3, and UA-PI (Table 3).

NSE, hemolysis index, and the DV-PI correlation are summarized in Table 4. NSE and hemolysis index had a positive correlation.

Table 1. Maternal characteristics and parameters of birth among women with and without FGR

Characteristics	FGR (n=26)	No FGR (n=24)	p value
Maternal age	30.23±5.5	32.04±5.02	0.128
Gravida	1.35±0.629	2.58±1.28	0.059
Parity	0.23±0.114	1.13±0.947	<0.001
Abortus	0.12±0.026	0.42±0.317	0.225
Livebirth	0.19±0.091	0.96±0.806	0.324
Gestational age at delivery	34.84±4.18	38.4±1.07	<0.001
Birth weight	1845±833.6	3252±532	0.026
APGAR (1st min)	6.96±1.732	8.04±0.825	0.039
APGAR (5th min)	8.42±1.172	9.30±0.559	0.004

FGR: Fetal growth restriction

Discussion

Findings from our study indicated that the FGR group had higher troponin T levels and lower values of pH, HCO_3, APGAR scores, birth weight, and gestational age at birth than in the healthy control group. No significant difference was found for NSE levels between both groups.

It is known that placental insufficiency causes altered fetal cardiac output distribution[8]. This alteration is with increased placental resistance and cardiac output volume from the right ventricle, and decreased systemic vasoconstriction and normal/elevated left ventricle cardiac output[6,8]. In healthy pregnancies, right ventricle output increases with gestational age. This physiologic alteration for growth-restricted fetuses causes a decrease in ejection of the right ventricle, increase of systemic venous pressured pulsatility of the inferior vena cava, hepatic veins, and atrial contraction of the ductus venosus[9]. In our results, increased troponin T levels in the study group had a relation between myocardial injury that developed secondary to cardiac alterations and possible cardiac dysfunction. Makikallio et al.[10] reported elevated troponin T levels in fetuses who had umbilical vein atrial pulsatility. Nomura et al.[11] reported a significant relation between troponin T levels and increased ductus venous PIV in Doppler measurements.

Table 2. Blood gas analyses and Doppler velocity measurements in groups with and without FGR

	FGR (n=26)	No FGR (n=24)	p value
Blood gas parameters			
pH	7.259±0.059	7.35±0.045	<0.001
pO2	18.29±7.307	27.732±7.36	0.23
pCO2	47.067±7.74	40.536±5.09	0.36
HCO3	19.75±1.89	21.338±1.06	0.001
Doppler measurements			
UA PI	1.57±0.617	0.8±0.13	<0.001
MCA PI	1.447±0.37	1.53±0.308	0.468
DV-PI	0.656±0.91	0.836±0.272	0.232
DV PVIV	0.268±2.23	0.817±0.274	0.134
DV S/a	3.107±1.94	3.06±1.737	0.541
NSE (µg/L)	31.250 (8.8-184.2)	22.350 (7.5-354.3)	0.162
Troponin T (ng/L)	61.62±30.478	40.88±27.738	0.015

FGR: Fetal growth restriction, MCA: Melting curve analysis, NSE: Neuron-specific enolase

Acidemy (pH<7) and elevated base-excess values in cord blood gas analysis is a predictive laboratory test to evaluate neonatal encephalopathy for acute intrapartum hypoxia[12]. Fetal acidemia causes neuronal necrosis, apoptosis, and impaired cognitive functions that affects the whole life of newborn. Sheikh and Cantu's study found that venous pH, base-excess were more predictive than other blood gas parameters for fetal acidemia[12,13]. Our results are similar to those of previous studies. Similarly, lower umbilical cord venous pH values and increased HCO_3 levels were related with increased troponin T levels. However, Yıldırım et al[7]. reported that HCO_3 had no effect on troponin T, but gestational age at delivery and pCO_2 were associated with increased troponin T levels. In the literature, elevated troponin T levels have been studied on pregnancies treated with long-term tocolytics and fetuses with respiratory distress syndrome, but it has been investigated in a limited number of studies to predict cardiac injury. Creatine kinase, troponin I and T have been studied to evaluate cardiac dysfunction in hypoxemic-ischemic encephalopathy. Yıldırım et al[7]. reported that there was a correlation between troponin T and creatine kinase-MB (CKMB), but troponin T was more specific than CKMB.

Table 3. Correlation of NSE and troponin T between birth parameters

	NSE		Troponin T	
	Correlation coefficient	p value	Correlation coefficient	p value
Birth weight	-0.251	0.082	-0.640	0.001
APGAR (1st min)	-0.108	0.459	-0.283	0.049
APGAR (5th min)	-0.047	0.748	-0.328	0.021
pH	-0.254	0.059	-0.450	0.002
HCO3	-0.101	0.500	-0.233	0.119
MCA PI	0.062	0.688	-0.232	0.125
UA PI	0.154	0.318	0.334	0.027
DV-PI	-0.219	0.153	-0.119	0.445

NSE: Neuron-specific enolase, MCA: Melting curve analysis

Table 4. Correlation of NSE, DV, PI, and hemolysis index

	NSE	DV-PI	Hemolysis index
Pearson's correlation			
NSE (µg/L)	1.000	-0.392	0.940
DV PI	-0.392	1.000	-0.293
Hemolysis index	0.940	-0.293	1.000
p values			
NSE	0	0.004	0.000
DV-PI	0.004	0	0.027
Hemolysis index	0.000	0.027	0

NSE: Neuron-specific enolase

Although our study supports the existing data linking troponin T and gestational age at delivery, birth weight, APGAR scores, and umbilical artery Doppler PI, Karadeniz et al[14]. certified no relationship between troponin T and gestational age or birth weight in fetuses with mild pre-eclampsia. As they mentioned in their report, this could be considered there was insufficient time for elevation of the troponin T plasma concentration.

Perinatal asphyxia and hypoxic ischemic encephalopathy are associated with increased morbidity and mortality rates. As discussed above, several biomarkers (S100, CK-BB, GFAP, NFp) have been investigated to identify neuronal damage in FGR[5,15-18]. However, even today, the most specific and optimal biomarker remains controversial. We observed higher values of NSE in fetuses complicated by FGR, but there was no statistically significant difference. Velipaşaoğlu et al[19]. published a cohort study with an association between neuronal injury markers and intrauterine growth restriction; they found no significance for NSE, but they observed a positive correlation for umbilical artery PI, RI, ductus venosus RI, S/d ratio with NSE. We found an association between ductus venosus PI, the hemolysis index, and NSE; also, higher NSE levels were associated with lower APGAR scores. Çeltik et al[20]. found that NSE concentrations ascended six hours after delivery for newborns complicated by grade 3 hypoxic ischemic encephalopathies. This results can explain why NSE levels did not increase significantly in our study. It is a fact that premature and small fetuses have lower blood volume and flow than term fetuses. Also, using an injector to take blood samples can result with hemolysis and elevated NSE levels from erythrocytes. The association between NSE and the hemolysis index can explain this. To exclude this determinant, Zinsmeyer et al[21]. studied amniotic fluid and reported that amniotic NSE increased in acute hypoxemia. Berger and Richichi[22] concentrated on a formula to exclude the hemolysis index and clinical use of NSE for hemolyzed serum. Our study group contained only a small number fetuses so we did not use this formula.

Some disadvantages exist in our study. First, only small number of fetuses met all criteria for inclusion in the study, and second, only two biomarkers were studied to investigate cardiac and neuronal injury. However previous studies concentrated on the progression of these markers after delivery in newborns, a limited number of studies used these markers on fetuses.

In light of our findings, it has been understood that troponin T and NSE can be used as indicators for cardiac and neuronal injury detection in fetuses with FGR. According to the literature, it can be seen that there are studies with heterogeneous paradigms using different indicators to find neuronal injury. As a result of this study, it is clear that to assess neonatal prognosis, wider-scoped and comparative studies will provide more information about the subject.

Ethics

Ethics Committee Approval: This prospective cohort study was approved by the Clinical Research Ethics Committee of Ankara University (approval of IRB: 05-216-15/23.03.2015).
Informed Consent: Informed consent was provided by all participants prior to the baseline interview in the original study.
Peer-review: External and internal peer-reviewed.

Authorship Contributions

Concept: D.C.K., Design: B.Y., Data Collection or Processing: B.Y., Analysis or Interpretation: T.Y., Literature Search: A.K., Writing: B.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. Miller J, Turan S, Baschat AA. Fetal Growth Restriction. Seminars in Perinatol 2008;32:274-80.
2. Flood K, Unterscheider J, Daly S, Geary MP, Kennely MM, McAuliffe FM, et al. The role of brain sparing in the prediction of adverse outcomes in intrauterine growth restriction: results of the multi center PORTO study. Am J Obstet Gynecol 2014;211:288.e1-5.
3. Kanık E, Ozer EA, Bakiler AR, Aydınlıoğlu H, Dorak C, Doğrusöz B, et al. Assesment of myocardial dysfunction in neonates with hypoxic-ischemic encephalopathy: is it a significant predictor of mortality? J Matern Fetal Neonatal Med 2009;22:239-42.
4. Baschat AA. Neurodevelopment after Fetal Growth Restriction. Fetal Diagn Ther 2014;36:136-42.
5. Ramaswamy V, Horton J, Vandermeer B, Buscemi N, Miller S, Yager J. Systematic review of biomarkers of brain injury in term neonatal encephalopathy. Pediatr Neurol 2009;40:215-26.
6. Bahtiyar M, Copel J. Cardiac changes in the intrauterine growth-restricted fetus. Semin Perinatol 2008;32:190-193.
7. Yıldırım A, Ozgen U, Alataş O, Tekin N, Kılıç Z. The diagnostic value of Troponin T level in the determination of cardiac damage in perinatal asphyxia newborns. Fetal and Pediatric Pathology 35:1,29-36.
8. Society for Maternal-Fetal Medicine Publications Committee, Berkley E, Chauhan S, Abuhamad A. Doppler assessment of the fetus with intrauterine growth restriction. Am J Obstet Gynecol 2012;206:300-8.
9. Figureas F, Puerto B, Martinez JM, Cararach V, Vanrell JA. Cardiac function monitoring of fetuses with growth restriction. Eur J Obtet Gynecol Reprod Biol 2003;110:159-63.
10. Makikallio K, Vuolteenaho O, Jouppila P, Räsänen J. Association of severe placental insufficiency and systemic venous pressure rise in the fetus with increased neonatal cardiac Troponin T levels. Am J Obstet Gynecol 2000;183:726-31.
11. Nomura R, Cabar F, Costa VN, Miyadahira S, Zugaib M. Cardiac Troponin T as a biochemical marker of cardiac dysfunction and ductus venosus Doppler velocimetry. Eur J Obstet Gynecol Reprod Bio 2009;147:33-6.
12. Cantu J, Szychowski J, Li X, Biggio J, Edwards RK, Andrews W, et al. Predicting fetal acidemia using umbilical venous cord gas parameters. Obstetrics and Gynecology 2014;124:926-32.
13. Sheikh M, Zoham M, Hantoushzadeh S, Shariat M, Dailili H, Amini E. Umbilical blood gas analysis in preeclamptic versus healthy pregnancies with preterm birth. J Matern Fetaal Neonatal Med 2016;29;2549-54.
14. Karadeniz L, Coban A, Ince Z, Türkoğlu U, Can G. Cord blood cardiac Troponin T and non-protein bound iron levels in newborns of mild pre-eclamptic mothers. Neonatology 2010;97:305-10.
15. Gazzalo D, Marioni E, Dı Irorio R, Lituania M, Marras M, Bruschettini P, et al. High maternal blood S100B concentrations in pregnancies complicated by intrauterine growth restriction and intraventricular hemorrhage. Clinical Chemistry 2006;52:5:819-26.
16. Constantine M, Weiner S, Rouse D, Hirtz DG, Vader MW, Spong CY, et al. Umbilical cord blood biomarkers of neurologic injury and the risk of cerebral palsy or infant death. Int J Devl Neuroscience 2011;29:917-22.
17. Mazarico E, Llurba E, Cumplido R, Valls A, Melchor JC, Iglesias M, et al. Neural injury markers in intrauterine growth restriction and their relation to perinatal outcomes. Pediatr Res 2017;82:452-7.
18. Mazarico E, Llurba E, Cabero L, Sanchez O, Valls A, Martin-Ancel A, et al. Associations between neural injury markers of intrauterine growth-restricted infants and neurodevelopment at 2 years of age. J Matern Fetal Neonatal Med 2018;18:1-7.
19. Velipaşaoğlu M, Yurdakök M, Özyüncü Ö, Portakal O, Deren Ö. Neuronal injury markers to predict neonatal complications in intrauterine growth restriction. J Obstet Gynecol 2015;35:555-60.
20. Çeltik C, Acunaş B, Öner N, Pala O. Neuron-spesific enolase as a marker of the severity and outcome of hypoxic ischemic encephalopathy. Brain Dev 2004;26(6):398-402.
21. Zinsmeyer J, Marangos PJ, Issel EP, Gross J. Neuron-spesific enolase in amniotic fluid- a possible indicator for fetal distress and brain implication. J Perinat Med 1987;15:199-202
22. Berger R, Richichi R. Derivation and validation of an equation for adjustment of neuron- specific enclave concentrations in hemolyzed serum. Pediatr Crit Care Med 2009;10:260-63.

Clinical Investigation / Araştırma

DOI: 10.4274/tjod.galenos.2019.24571

Turk J Obstet Gynecol 2019;16:55-62

Role of less commonly agreed risk factors on disease recurrence in endometrial cancer: a propensity score-matched comparison

Endometrial kanser rekürrensinde daha az kabul gören risk faktörlerinin rolü: eğilim puan eşleştirme karşılaştırması

Hülya Ayık Aydın[1], Gülgün Erdoğan[2], Hatice Elif Pestereli[2], Tayup Şimşek[1]

[1]*Akdeniz University Faculty of Medicine, Department of Obstetrics and Gynecology, Division of Gynecological Oncology, Antalya, Turkey*

[2]*Akdeniz University Faculty of Medicine, Department of Medical Pathology, Division of Gynecopathology, Antalya, Turkey*

Abstract

Objective: To compare the clinicopathologic features of patients with endometrial cancer (EC) with recurrent disease with a primary surgery, stage, grade, and tumor histotype-matched cohort of patients without recurrence.

Materials and Methods: Patients with EC who were surgically treated at a single tertiary care institution between 2005 and 2015 were enrolled in this study. The dataset included 381 consecutive patients with EC, of which 31 (8.1%) had disease recurrence. Data consisting of age at surgery, CA-125 concentration at diagnosis, number of lymph nodes harvested, growth pattern of the primary tumor, location of the primary tumor within the endometrium, tumor histotype, tumor grade, disease stage, adjuvant therapy, disease recurrence, time to recurrence, CA-125 concentration at recurrence, clinical and imaging findings at recurrence, and treatment modalities used for recurrent disease were extracted from the institutional database.

Results: After 1-to-1 propensity-score matching of patients with and without recurrence, the clinicopathologic features of 26 patients from each group were compared. Patients with recurrent disease were found to have a significantly higher CA-125 concentration at initial diagnosis ($p<0.001$) and different tumor growth pattern ($p=0.019$) than patients without disease recurrence. The papillary growth pattern of the primary tumor was significantly associated with disease recurrence as compared with polypoid and infiltrative patterns. Omental involvement, papillary tumor growth, and advanced age were associated with increased mortality.

Conclusion: Our results indicated that higher CA-125 concentrations at initial diagnosis and papillary growth pattern of primary tumors were found to be associated with disease recurrence.

Keywords: Endometrial cancer, recurrence, risk factor, CA-125

Öz

Amaç: Rekürren hastalığı olan endometrial kanser (EC) hastalarının, primer cerrahi, evre, grade ve rekürrens saptanmayan tümör histotipi eşleştirilmiş kohortu ile klinikopatolojik özelliklerini karşılaştırmak.

Gereç ve Yöntemler: 2005 ve 2015 yılları arasında üçüncü basamak hastanede cerrahi olarak tedavi edilen hastalar bu çalışmaya dahil edildi. Veri seti, EC'si olan 381 ardışık hastayı içermekte olup, bunların 31'inde (%8,1) hastalık nüksü vardır. Cerrahi esnasındaki yaş, tanı sırasında CA-125 seviyesi, çıkarılan lenf nodu sayısı, primer tümörün büyüme paterni, endometriumda primer tümörün yeri, tümör histotipi, tümör derecesi, hastalığın evresi, adjuvan tedavi, hastalık rekürrensi, rekürrens zamanı, rekürrensde CA-125 düzeyi, nükste klinik ve görüntüleme bulguları ve tekrarlayan hastalık için kullanılan tedavi yöntemleri kurumsal veri tabanından çıkarıldı.

Bulgular: Nüks olan ve olmayan hastaların 1'e 1'lik eğilim skoru eşleştirmelerinden sonra, her gruptan 26 hastanın klinikopatolojik özellikleri karşılaştırıldı. Rekürren hastalığı olan hastalarda, olmayanlarla karşılaştırıldığında tümör büyüme paterninde ($p=0,019$) ve başlangıçtaki CA-125 seviyesinde anlamlı bir artış saptandı. Primer tümörün papiller büyüme paterni, polipoid ve infiltratif modellere kıyasla hastalık nüksü ile anlamlı olarak ilişkiliydi. Omental tutulum, papiller tümör büyümesi ve ileri yaş artmış mortalite ile ilişkiliydi.

Sonuç: Bulgularımız, ilk tanıdaki yüksek CA-125 seviyesinin ve primer tümörün papiller büyüme paterninin hastalık nüksüyle ilişkili olduğunu göstermiştir

Anahtar Kelimeler: Endometrial kanser, rekürrens, risk faktör, CA-125

PRECIS: Role of less commonly agreed risk factors on disease recurrence in endometrial cancer: A propensity score- matched comparison.

Address for Correspondence/Yazışma Adresi: Hülya Ayık Aydın,
Akdeniz University Faculty of Medicine, Department of Obstetrics and Gynecology, Division of Gynecological Oncology, Antalya, Turkey
E-mail: hulya_ayik@hotmail.com **ORCID ID:** orcid.org/0000-0002-3028-7247

Received/Geliş Tarihi: 13.09.2018 **Accepted/Kabul Tarihi:** 07.02.2019

Introduction

Endometrial cancer (EC) is the most common cancer of the female genitourinary system in the United States of America and the 4th most common tumor in Western countries, with more than 280.000 patients appearing yearly, globally[1]. Nevertheless, data reveal that the mortality rate is rising more quickly than the incidence, possibly because of an improved rate of advanced-stage tumors, high-risk histopathologies, and cases being identified in the elderly[2]. Consequently, 13-17% of patients will develop disease recurrence, usually within three years of initial treatment[3,4].

Although most with EC are diagnosed with early-stage disease and have a favorable prognosis, approximately 15% of these cases relapse[5]. Treatment for recurrence includes local treatment (radiotherapy or surgery), systemic chemotherapy or relevant combinations. It differs according to the involved site, recurrent disease extent, and types of previous therapy. The 3-year survival rate in patients with EC recurrence is reported to be between 8% and 73%[6].This broad range indicates that patients with EC recurrence represent a heterogeneous group with different prognostic factors for survival. Thus, there is a need to better discriminate these patients depending on prognosis after relapse. Numerous features have been examined to evaluate their effect on recurrence; patient's age at diagnosis, the stage, histologic type, cell type, cervical involvement, depth of myometrial invasion, and lymph node metastasis at the time of treatment were identified as the most significant prognostic factors in patients with EC[7,8]. Nevertheless, these factors are not satisfactory to precisely predict the prognosis of these patients. There is a common consensus that disease stage, tumor histotype, and tumor grade are significantly associated with risk of recurrence in EC. To examine the roles of less commonly agreed risk factors on disease recurrence, we compared the clinicopathologic features of patients with recurrent disease with a primary surgery, stage, grade,and tumor histotype-matched cohort of patients without recurrence.

Materials and Methods

Study design

The study was approved by the Akdeniz University Faculty of Medicine Ethics Committee (no: 15/03.01.2018). All patients gave written informed consent, which allowed the participating institution to use their clinical data. Patients with EC who were surgically treated at a single tertiary care institution between 2005 and 2015 were enrolled in this study.

In the present study, recurrence was defined as any documented reappearance of cancer, either locally or systemically, after a disease-free interval of ≥3 months. Local-regional recurrence was defined as relapse at the vaginal vault or in the regional lymph nodes including pelvic and paraaortic nodes. Abdominal/peritoneal recurrence was defined as relapse at the peritoneum, omentum or serosal surfaces of the abdominal viscera, or occurrence of malignant ascites. Distant metastasis was defined as any relapse at extraabdominal organs and lymph nodes, or in the parenchyma of the intraperitoneal organs.

Routine post-remission surveillance at our institution was to follow up patients every three months for two years, every six months for the next three years, and then annually. At follow-up visits, a physical examination, ultrasonography, complete blood count, and blood chemistry including serum CA-125 level were performed. Thoracic and abdominal computed tomography scans were used six-monthly for two years and then annually. In the event of equivocal clinical or radiologic findings, positron emission tomography combined with computed tomography (PET-CT) was performed. Image-guided biopsy was used whenever possible, and there was a high suspicion of disease recurrence before the treatment of recurrence was started.

Outcome parameters

Data consisting of age at surgery, CA-125 concentration at diagnosis, time from diagnosis to primary treatment, type of surgical procedure performed, number of lymph nodes harvested, growth pattern of the primary tumor, location of the primary tumor within the endometrium, tumor histotype, tumor grade, disease stage, adjuvant therapy, disease recurrence, time to recurrence, CA-125 concentration at recurrence, clinical and imaging findings at recurrence, largest recurrent tumor size, extent of recurrent disease, number of recurrent lesions, and treatment modalities used for recurrent disease were extracted from the institutional database.

Statistical Analysis

The dataset included 381 consecutive patients with EC, of which 31 (8.1%) had disease recurrence. A 1-to-1 propensity score-matched analysis was conducted to provide matched cohorts of patients with and without recurrence, and thus, to reduce the impact of covariate bias. Multivariate logistic regression models were used to develop separate propensity scores. Disease stage, tumor grade, tumor histotype, and primary surgery [total hysterectomy/bilateral salpingo-oophorectomy (TH/BSO) alone vs. TH/BSO plus lymphadenectomy] were selected as potential confounding covariates because they are known to be independent factors for risk of recurrence and to be the main determinants of prognosis in patients with EC. For tight matching, nearest-neighbor matching was performed with a caliper width (the match tolerance) equal to 0.01, which resulted in successful matching of 26 patients from each group. A simple logistic regression analysis was then performed using the matched groups to assess independent associations of clinicopathologic factors and disease recurrence. The differences between

two groups were tested using the Mann-Whitney U test for nonparametric data. The chi-square or Fisher's exact tests were used for the comparison of categorical variables.

Statistical analyses were performed using NCSS (Number Cruncher Statistical System) 2007 statistical software (NCSS LLC, Kaysville, Utah, USA). A two-tailed $p<0.05$ was considered statistically significant.

The cut-off point for CA-125 was determined using receiver operating characteristics (ROC) analysis based on the survival data. Survival analysis was performed to investigate the survival probabilities of variables. The mean durations of survival, 95% confidence intervals, as well as hazard ratios are presented.

Results

Table 1 shows the baseline characteristics of 381 patients with EC. Eighty percent of patients had endometrioid tumor histotype. According to the International Federation of Obstetricians and Gynecologists (FIGO) grading system, 52% of patients had grade 1 tumor, 30.2% had grade 2, and 17.6% had grade 3. The patients treated with TH/BSO plus lymphadenectomy accounted for 90.6%. The median number of lymph nodes harvested was 30. The $FIGO_{2009}$ stages of the patients were as follows: stage IA, 179 patients (47.0%); stage IB, 110 patients (28.9%); stage II, 25 patients (6.6%); stage IIIA, 8 patients (2.1%); stage IIIB, 1 patient (0.3%); stage IIIC, 46 patients (12.1%); and stage IVB, 12 patients (3.1%). Adjuvant therapy was given to 55.4% of the patients. Thirty-one patients (8.1%) developed disease recurrence during a median follow-up time of 68 months.

The characteristics of the 31 patients with recurrent disease are presented in Table 2. The median time to recurrence was 15 (range, 3 to 46) months. Only five patients (16.1%) first presented with abnormal clinical signs and/or symptoms, but none had positive physical findings alone. At the time of the diagnosis of recurrence, all patients had positive imaging findings and 26 (83.9%) had elevated CA-125 concentrations. On the other hand, only 2 (6.5%) patients were incidentally diagnosed throuhg routine imaging studies alone. The majority (74.2%) of patients developed multiple or disseminated metastases. Recurrent disease was limited to the vaginal vault and/or regional lymph nodes in only 19.4% of patients. The liver was the most common site of distant metastasis, found in 22.6% of patients.

After 1-to-1 propensity-score matching of patients with and without recurrence based on stage, grade, histotype, and primary surgery, the clinicopathologic features of 26 patients from each group were compared (Table 3). Study groups were comparable for age at surgery, time from diagnosis to primary treatment, number of lymph nodes harvested, location of the primary tumor within the endometrium, lymphovascular space involvement, cervical invasion, positive peritoneal cytology, and adjuvant therapies received.

Table 1. Initial surgical and pathologic characteristics of 381 patients with endometrial cancer

Variables	Values
Age, median (range), years	58 (24-88)
CA-125 concentrations at diagnosis, median (range), U/mL	30 (3.2-1172)
Time from diagnosis to primary treatment, median (range), days	12 (1-45)
Primary surgery, n (%)	
TH/BSO alone	36 (9.4)
TH/BSO plus lymphadenectomy (pelvic ± paraaortic)	345 (90.6)
No. of lymph nodes harvested, median (range)	30 (0-107)
Growth pattern of the primary tumor, n (%)	
Polypoid	150 (39.4)
Infiltrative	123 (32.3)
Papillary	108 (28.3)
Tumor location within the endometrium, n (%)	
Fundus	70 (18.4)
Corpus	159 (41.7)
Isthmus	19 (5.0)
Involving the whole cavity	133 (34.9)
Histotype, n (%)	
Endometrioid	306 (80.3)
Non-endometrioid	75 (19.7)
FIGO grade, n (%)	
Grade 1	199 (52.2)
Grade 2	115 (30.2)
Grade 3	67 (17.6)
FIGO stage, n (%)	
IA	179 (47.0)
IB	110 (28.9)
II	25 (6.6)
IIIA	8 (2.1)
IIIB	1 (0.3)
IIIC	46 (12.1)
IVB	12 (3.1)
Adjuvant therapy, n (%)	211 (55.4)
Radiotherapy alone (EBRT and/or brachytherapy)	137 (36.0)
Chemotherapy ± radiotherapy	74 (19.4)
Follow-up time, median, (95% CI), months	68 (1-144)
Disease recurrence, n (%)	31 (8.1)

TH/BSO: Total hysterectomy bilateral salpingo-oophorectomy, FIGO: International Federation of Gynecology and Obstetrics, EBRT: External beam radiotherapy, CI: Confidential interval

Table 2. Characteristics of 31 patients with recurrent disease

Variables	Values
Time to recurrence, median (range), months	15 (3-46)
CA-125 concentration at recurrence, median (range), U/mL	66 (9.6-962)
Diagnosis of recurrence, n (%)	
Positive clinical findings	5 (16.1)
Elevated CA-125 concentrations	26 (83.9)
Positive imaging findings	31 (100)
With normal CA-125 concentration and negative clinical findings	2 (6.5)
Largest recurrent tumor size, median (range), cm	3.5 (0.7-12)
No. of recurrent lesions, n (%)	
Isolated metastasis	8 (25.8)
Multiple/disseminated metastases	23 (74.2)
Disease extent at recurrence, n (%)	
Local-regional disease	6 (19.4)
Confined to the vaginal cuff	4 (12.9)
Abdominal/peritoneal disease	14 (45.2)
Distant metastasis	11 (35.5)
Liver	7 (22.6)
Lung	3 (9.7)
Brain	1 (3.2)
Musculoskeletal soft tissue	1 (3.2)
Extraabdominal lymph nodes	2 (6.5)
Treatment modalities used for recurrent disease, n (%)	
Surgical resection	2 (6.5)
Radiotherapy	2 (6.5)
Chemotherapy	29 (93.5)

However, patients with recurrent disease were found to have a significantly greater tumor size (p=0.026), higher CA-125 concentration at initial diagnosis (p<0.001), and different tumor growth pattern (p=0.019) than patients without disease recurrence. The papillary growth pattern of the primary tumor was significantly associated with disease recurrence as compared with polypoid and infiltrative patterns.

Elevated CA-125 concentration and papillary growth pattern did not exhibit a significant difference between type I and type II cancers (p=0.267 and p=0.429, respecetively). Table 4 demonstrates a comparative overview for histological grade, histological type, and staging data for tumors with and without recurrence. As would be expected, advanced grade and tumor stage were significantly associated with increased recurrence.

As shown in Table 5, analysis of the impacts of parameters on survival indicated that patients with tumoral involvement of omentum were at 5.712-times greater risk for mortality. Papillary tumors were 4.67 times more lethal compared with non-papillary neoplasms. Mortality was 1.041 times more likely for every advanced year.

ROC analysis was used to determine the cut-off point for CA-125 concentrations. Taking mortality into account as the gold standard, the cut-off point was determined as 44.1 with a sensitivity and specificity of 72.6% and 60%, respectively. The ROC curve is demonstrated in Figure 1.

Kaplan-Meier analyses for omental involvement and papillary tumor patterns are indicated in Figures 2 and 3, respectively.

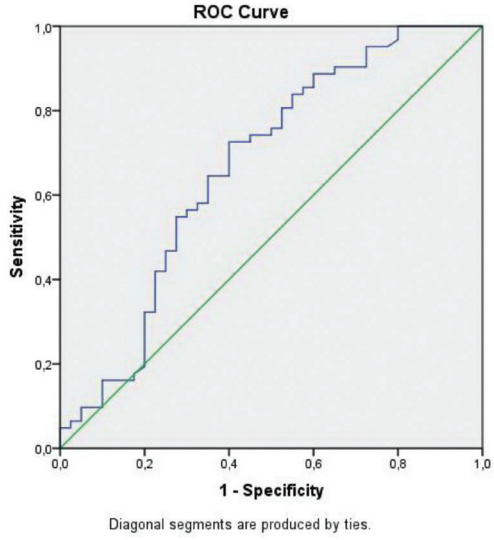


Figure 1. ROC curve for CA-125 levels

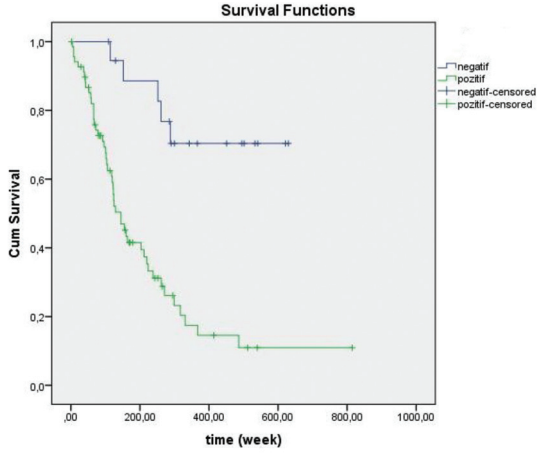


Figure 2. Kaplan-Meier analysis for omental involvement

Discussion

In the present study, we aimed to compare the clinicopathologic features of patients with recurrent disease with a primary

Table 3. Comparison of clinicopathologic features of matched cohorts with and without recurrence

	Recurrent disease		p
	Yes (n=26)	No (n=26)	
Age at surgery, median (range), years	63 (48-74)	62 (44-74)	0.219
Tumor size (cm), mean ± SD	5.2±4.2	3.3±1.9	0.026
CA-125 level at initial diagnosis, median (range), U/mL	71.5 (30-1172)	29 (8.5-57)	<0.001
Time from diagnosis to primary treatment, median (range), days	12.5 (3-27)	10 (3-25)	0.212
Number of lymph nodes harvested, median (range)	36 (10-79)	29 (11-52)	0.084
Growth pattern of the primary tumor, n (%)			
Polypoid	10 (38.5)	15 (57.7)	0.019
Infiltrative	7 (26.9)	10 (38.5)	
Papillary	9 (34.6)	1 (3.8)	
Primary tumor location, n (%)			
Fundus	5 (19.2)	4 (15.4)	0.856
Corpus	7 (26.9)	9 (34.6)	
Isthmus	5 (19.2)	3 (11.5)	
Involving the whole cavity	9 (34.6)	10 (38.5)	
Lymphovascular space involvement, n (%)	8 (30.8)	5 (19.2)	0.337
Cervical invasion	9 (34.6)	7 (26.9)	0.548
Positive peritoneal cytology, n (%)	2 (7.7)	3 (11.5)	0.999
Adjuvant therapy, n (%)	23 (88.5)	25 (96.2)	0.610

Bold values indicate statistical significance at p<0.05

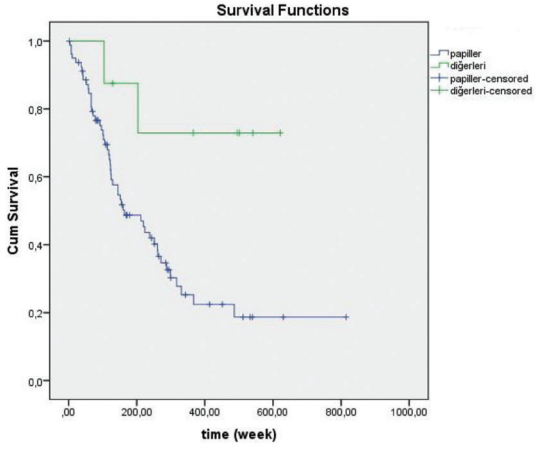


Figure 3. Kaplan-Meier analysis for papillary tumor growth pattern

surgery, stage, grade, and tumor histotype-matched cohort of patients without recurrence. We found that higher CA-125 concentrations at initial diagnosis and a papillary growth pattern of the primary tumor were associated with disease recurrence.

In the present study, the ratio of recurrence after the initial treatment of EC was 8.1%. This rate is slightly lower than previously described in other reports[4-6]. The low recurrence rates obtained in our study may be due to the patient characteristics in the study group or may be a result of the extensive surgical interventions and/or adjuvant radiotherapy, and chemotherapy being applied. The relation of EC recurrences and the interval passed from initial treatment for EC indicates that time to recurrence was detected with a median of 15 months. These data are in accordance with ratios described by other reports[6-8].

In the present study, our data confirmed a tendency described by the mainstream of reports that distant recurrences are more frequent than local recurrences after the initial treatment of EC. This is possibly due to pelvic adjuvant radiotherapy following surgical procedures. The present study demonstrated findings that are very comparable to the other reports[9,10]. Sohaib et al.[9] reported 34.4% local recurrences, 46.9% distant recurrences, and 18.8% in both sites, and Fung-Fee-Fung et al.[10] reported 61% of distant metastases including multifocal relapses and 39% of local recurrences.

Although serum concentrations of CA-125 have been used as a worthy tumor marker for EC diagnosis and recurrence detection after the initial treatment, their role as a useful tumor marker in EC is the subject of debate[11]. Several reports

Table 4. Comparison of histologic grade and type, and staging in patients with and without tumor recurrence

Variable	Subgroup	Recurrence		p
		No (n, %)	Yes (n, %)	
Histological grade	1	202 (57.7)	2 (6.4)	<0.001*
	2	78 (22.3)	7 (22.6)	
	3	19 (5.4)	22 (71)	
	Data unavailable	51 (14.6)	0	
Stage	1A	167 (47.7)	7 (22.6)	<0.001*
	1B	88 (25.1)	7 (22.6)	
	2	23 (6.6)	2 (6.5)	
	3A	7 (2.0)	1 (3.2)	
	3B	1 (0.3)	0 (0)	
	3C	7 (2.0)	1 (3.2)	
	3C1	13 (3.7)	1 (3.2)	
	3C2	19 (5.4)	5 (16.1)	
	4B	5 (1.4)	7 (22.6)	
Histological type	Endometrioid	292 (83.4)	14 (45.2)	N/A
	Mucinous	6 (1.7)	0 (0)	
	Serous	6 (1.7)	5 (16.1)	
	Clear cell	9 (2.6)	3 (9.7)	
	Mixed	19 (5.4)	3 (9.7)	
	Undifferentiated	3 (0.9)	0 (0)	
	MMMT	14 (4.0)	6 (19.4)	
	Other	1 (0.3)	0 (0)	

*: Statistically significant, MMMT: Malignant mixed Müllerian tumor, N/A: Analysis not applicable due to small number of cases in some subgroups, bold values imply statistical significance

have revealed that serum levels of CA-125 are critical for preoperative diagnosis and prediction of the recurrence, and that their elevation was associated with advanced-stage EC[12]. Yildiz et al.[13] showed that the CA-125 serum concentration increase was related with the upsurge in extra-uterine disease incidence. Similarly, Chen et al.[14 revealed that elevated CA-125 concentrations were intensely correlated with lymph node metastasis, advanced surgical stage, and poor prognosis[13,14]. Elevated serum concentrations of CA-125 at initial diagnosis are linked with extrauterine tumor extension and lymph node metastases[15,16]. In the present study, patients with recurrent disease were observed to have a significantly higher CA-125 concentration at initial diagnosis.

Abnormal preoperative cytology was demonstrated to be associated with a number of poor prognostic features such as tumor grade, degree of myometrial invasion, and lymph node spread[17-19]. Abnormal cytology is correlated with advanced-stage for both endometrioid and papillary serous or clear cell histologic subtypes[18,19]. The aggressive performance of the papillary subtype was firstly defined by Hendrickson et al.[20] as a subtype of EC with precise pathologic and clinical features related with a high frequency of tumor recurrence. Papillary subtype represents 3-10% of all ECs but accounts for nearly 40% of EC deaths[20,21]. In a recent study of patients with EC, cervical cytology was revealed to be an individual predictor of lymph node spread[18]. Regardless of the strong connection between abnormal cytology and recurrence, the direct association between abnormal cytology and recurrence risk has not been comprehensively assessed. Fukuda et al.[19] stated that abnormal cytology was associated with disease-free survival in univariate analysis but was not an independent predictor of survival on multivariate analysis. In the present study, papillary growth pattern of the primary tumor was significantly associated with disease recurrence as compared with polypoid and infiltrative patterns. Our results yielded that omentum involvement, papillary-type tumor growth, and advanced age were linked with mortality risk.

There is an increasing awareness in observational reports of the need to assess the effects of treatment on results. In observational and nonrandomized studies, treatment choice

Table 5. The effect of various parameters on survival

Variable Mean		Survival time (week) 95% CI Lower	Upper		p Estimate	Hazard ratio 95% CI Lower	Upper	
Polyp	Negative*	359.54	273.53	445.55	0.268	1.411	0.764	2.605
	Positive	205.95	139.73	272.16				
Myoma	Negative	287.09	206.25	367.93	0.073	1.823	0.936	3.55
	Positive*	359.81	254.99	464.62				
Adenomyosis	Negative*	346.96	257.5	436.41	0.520	1.205	0.682	2.129
	Positive	229.18	158.61	299.77				
LVSI	Negative*	352.03	270.03	434.03	0.523	1.443	0.739	2.816
	Positive	211.79	133.45	310.12				
Differentiation	Poor*	340.363	243.32	437.40	0.305	1.349	0.759	2.399
	Moderate	208.57	147.12	270.02				
Omentum Involvement	Negative*	507.16	415.34	598.96	<0.001	5.712	2.251	14.493
	Positive	231.66	165.52	297.78				
Pathologic subtype	Papillary	289.89	217.95	361.85	0.019	4.668	1.128	19.317
	Others*	495.42	345.73	645.10				
CA-125	441*	352.59	256.89	448.29	0.336	1.614	0.897	2.904
	>441	273.84	195.03	352.64				
Age					<0.001	1.041	1.019	1.064

(*: Reference category) CI: Confidence intervals, LVSI: Lymphovascular space invasion

is frequently affected by patient individualities. Consequently, researchers have to account for for systematic alterations in baseline features amongst compared groups when assessing the result of treatment on outcomes. Lately, when using observational data, there has been growing concern regarding techniques established on the propensity score to decrease or eradicate the outcomes of confounding[22].

Some limitations of the present study should be mentioned. First, the retrospective nature of the study causes bias. Secondly, this was a single-institution study, and caution must be taken before generalizing the conclusions to further settings. Another limitation is the long study period because study group comprised patients who experienced distinct surgical treatment (i.e. systematic pelvic lymphadenectomy was only recommended for high-risk ECs from 2010) [23]. Thirdly, physical activity, diet, and presence of chronic diseases such as hypertension or diabetes were not taken into account, though numerous investigators have formerly shown that these features might normalize endometrial hormone-receptor expressions and definitely effect survival[24,25].

Conclusion

Higher CA-125 concentrations at initial diagnosis and a papillary growth pattern of the primary tumor were found to be associated with disease recurrence. Omental involvement, papillary tumor growth, and advanced age were found to increase mortality rates. Further randomized, prospective, controlled trials on larger series are necessary for making more precise interpretations.

Acknowledgments

The authors received no financial support for the research, authorship, and/or publication of this article.

Ethics

Ethics Committee Approval: The study was approved by the Akdeniz University Faculty of Medicine Ethics Committee (no: 15/03.01.2018)

Informed Consent: All patients gave written informed consent, which allowed the participating institution to use their clinical data.

Peer-review: External and internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: T.Ş., H.A.A., Concept: G.E., Design: H.A.A., Data Collection or Processing: H.A.A., Analysis or Interpretation: H.E.P., Literature Search: H.A.A., Writing: H.A.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. Ferlay J, Soerjomataram I, Ervik M, et al. GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC Cancer Base No. 11 [Internet]. Lyon: International Agency for Research on Cancer; 2013.
2. Ueda SM, Kapp DS, Cheung MK, et al. Trends in Demographic and Clinical Characteristics in Women Diagnosed with Corpus Cancer and Their Potential Impact on the Increasing Number of Deaths. Am J Obstet Gynecol 2008;198:218.e211-6.
3. Lukka H, Chambers A, Fyles A, Thephamongkhol K, Fung-Kee-Fung M, Elit L et al. Adjuvant radiotherapy in women with stage I endometrial cancer: a systematic review. Gynecologic Oncol 2006;102:361-8.
4. Smith CJ, Heeren M, Nicklin JL, et al. Efficacy of Routine Follow-up in Patients with Recurrent Uterine Cancer. Gynecol Oncol 2007;107:124-9.
5. Frandsen JE, Sause WT, Dodson MK, et al. Survival Analysis of Endometrial Cancer Patients with Cervical Stromal Involvement. J Gynecol Oncol 2014;25:105-10.
6. Creutzberg CL, van Putten WL, Koper PC, et al. Survival After Relapse in Patients with Endometrial Cancer: Results From a Randomized Trial. Gynecol Oncol 2003;89:201-9.
7. Yoshiba T, Takei Y, Machida S, et al. Prognosis of Endometrial Cancer Patients with and without Symptoms at Recurrence. J Obstet Gynaecol Res 2016;42:1814-21.
8. Ouldamer L, Bendifallah S, Body G, et al. Predicting Poor Prognosis Recurrence in Women with Endometrial Cancer: A Nomogram Developed by the FRANCOGYN Study Group. Br J Cancer 2016;115:1296-1303.
9. Sohaib SA, Houghton SL, Reznek RH et al. Recurrent Endometrial Cancer: Patterns of Recurrent Disease and Assessment of Prognosis. Clin Radiol 2007;62:28-34.
10. Fung-Kee-Fung M, Dodge J, Elit L, et al. Follow-up After Primary Therapy for Endometrial Cancer: A Systematic Review. Gynecol Oncol 2006;101:520-9.
11. Kotowicz B, Fuksiewicz M, Jonska-Gmyrek J, et al. Preoperative Serum Levels Of YKL 40 and CA125 as A Prognostic Indicators in Patients With Endometrial Cancer. Eur J Obstet Gynecol Reprod Biol 2017;215:141-7.
12. Rose PG, Sommers RM, Reale FR, et al. Serial Serum CA 125 Measurements for Evaluation of Recurrence in Patients with Endometrial Carcinoma. Obstet Gynecol 1994;84:12-6.
13. Yildiz A, Yetimalar H, Kasap B, et al. Preoperative Serum CA 125 Level in the Prediction of the Stage of Disease in Endometrial Carcinoma. Eur J Obstet Gynecol Reprod Biol 2012;164:191-5.
14. Chen YL, Huang CY, Chien TY, et al. Value Of Pre-Operative Serum CA125 Level for Prediction of Prognosis in Patients with Endometrial Cancer. Aust N Z J Obstet Gynaecol 2011;51:397-402.
15. Yamazawa K, Hirashiki K, Usui H, et al. Discordance Between Serum Level and Tissue Immunohistochemical Staining of CA125 in Endometrioid Adenocarcinoma of the Uterine Corpus. Int J Gynecol Pathol 2005;24:254-9.
16. Hsieh CH, ChangChien CC, Lin H, et al. Can a Preoperative CA 125 Level Be A Criterion for Full Pelvic Lymphadenectomy in Surgical Staging of Endometrial Cancer? Gynecol Oncol 2002;86:28-33.
17. DuBeshter B. Endometrial Cancer: Predictive Value of Cervical Cytology. Gynecol Oncol 1999;72:271-2.
18. DuBeshter B, Deuel C, Gillis S, et al. Endometrial Cancer: The Potential Role of Cervical Cytology in Current Surgical Staging. Obstet Gynec 2003;101:445-50.
19. Fukuda K, Mori M, Uchiyama M, et al. Preoperative Cervical Cytology in Endometrial Carcinoma and its Clinicopathologic Relevance. Gynecol Oncol 1999;72:273-7.
20. Hendrickson M, Ross J, Eifel P, et al. Uterine Papillary Serous Carcinoma: A Highly Malignant Form of Endometrial Adenocarcinoma. Am J Surg Pathol 1982;6:93-108.
21. O'Hanlan KA, Levine PA, Harbatkin D, et al. Virulence of Papillary Endometrial Carcinoma. Gynecol Oncol 1990;37:112-9.
22. Austin PC. An Introduction to Propensity Score Methods for Reducing the Effects of Confounding in Observational Studies. Multivariate Behav Res 2011;46:399-424.
23. Cancer de l'endomètre, Collection Recommandations & référentiels, Boulogne-Billancourt: INCa; Nov 2010.
24. Pekmezi DW, Demark-Wahnefried W. Updated Evidence in Support of Diet and Exercise Interventions in Cancer Survivors. Acta Oncol 2011;50:167-78.
25. Karaman E, Karaman Y, Numanoglu C, Ark HC. Evaluation of hemoglobin A1c Levels in Endometrial Cancer Patients: a Retrospective Study in Turkey. Asian Pac J Cancer Prev 2015;16:1817-20.

Clinical Investigation / Araştırma

DOI: 10.4274/tjod.galenos.2018.89663

Turk J Obstet Gynecol 2019;16:63-71

The seroprevalence of Rubella in pregnant women in Turkey: a meta-analysis research of 90988 Rubella IgM, 84398 Rubella IgG, and 522 avidity results

Türkiye'de gebelerdeki Rubella seroprevalansı: 90988 Rubella IgM, 84398 Rubella IgG ve 522 avidite sonucunun meta-analiz araştırması

Rıza Aytaç Çetinkaya, Ercan Yenilmez

University of Health Sciences, Sultan Abdülhamid Han Training and Research Hospital, Clinic of Infectious Diseases and Clinical Microbiology, İstanbul, Turkey

Abstract

Objective: Rubella infection prevalence in pregnant women can vary from country to country, or even across regions in the same country. In this meta-analysis, the seroprevalence Rubella among pregnant women in Turkey in the last decade was evaluated.

Materials and Methods: Studies conducted in Turkey between 2007 and 2017 were analyzed, and differences in seroprevalence between provinces were compared by evaluating Rubella immunoglobulin (Ig)-G, IgM, and IgG avidity results in pregnancy in this period. A data search was performed using the search terms Rubella, kızamıkçık, gebe, hamile, pregnancy, Türkiye, Turkey in Google Scholar, PubMed, Web of Science, Türk Medline, and the YÖK thesis database center.

Results: A total of 26 articles associated with the seroprevalence of Rubella among pregnant women in Turkey were enrolled in the meta-analysis. As a result of an analysis of 84398 Rubella IgG, and 90988 Rubella IgM serology tests among pregnant women in 26 studies; Rubella IgG and IgM seroprevalence rates in pregnant woman in Turkey were found as 93.47% (95% CI: 91.72 to 95.03) and 0.783% (95% CI: 0.505 to 1.120), respectively. Rubella IgG low, intermediate, and high avidity rates were 4.66% (95% CI: 0.969 to 10.906), 7.51% (95% CI: 5.101 to 10.345), and 93.55% (95% CI: 82.584 to 99.311), respectively.

Conclusion: The Rubella IgG seropositivity rate in Turkey among pregnant woman is high, whereas it is low for IgM. These rates may be considered as the result of successful immunization policies and practices. In a few provinces, it is necessary to revise the Rubella immunization procedures and adult vaccination strategies should be developed in order to control Rubella infections in adults, including pregnant women.

Keywords: Rubella, pregnant women, meta-analysis, Turkey

Öz

Giriş: Hamilelerde Rubella prevalansı ülkeler arasında, hatta aynı ülke içinde farklı bölgelerde değişiklik göstermektedir. Bu meta-analiz ile son on yılda Türkiye'deki gebelerde Rubella seroprevalansı araştırıldı.

Gereç ve Yöntemler: Türkiye'de 2007 ve 2017 yılları arasında gebelerde Rubella immunoglobulin (Ig)-G, IgM ve IgG avidite sonuçları araştırıldı. Bu işlem Google Scholar, PubMed, Web of Science, Türk Medline ve YÖK tez merkezi veri tabanlarında Rubella, kızamıkçık, gebe, hamile, pregnancy, Türkiye, Turkey anahtar kelimeleri kullanılarak gerçekleştirildi.

Bulgular: Ülkemizde gebe kadınlar arasında Rubella seroprevalansı ile ilişkili toplam 26 makale meta-analize dâhil edildi. 26 çalışmada gebe kadınlar arasında 84398 Rubella IgG ve 90988 Rubella IgM seroloji sonucuna göre; IgG ve IgM seroprevalans oranları sırasıyla %93,47 (%95 CI: 91,72-95,03) ve %0,783 (%95 CI: 0,505-1,120) saptandı. Rubella IgG düşük, orta ve yüksek avidite oranları sırasıyla %4,66 (%95 CI: 0,969 ila 10,906),%7,51 (%95 CI: 5,101 ila 10,345), %93,549 (%95 CI: 82,584 ila 99,311) saptandı.

PRECIS: In this meta-analysis, studies conducted in Turkey between 2007 and 2017 were analyzed, and differences in seroprevalence between provinces were revealed by evaluating Rubella IgG, IgM and IgG avidity results in pregnancy in this period.

Address for Correspondence/Yazışma Adresi: Rıza Aytaç Çetinkaya, MD,
University of Health Sciences, Sultan Abdülhamid Han Research and Training Hospital, Clinic of Infectious Diseases and Clinical Microbiology, İstanbul, Turkey
Phone: +90 505 438 28 14 **E-mail:** aytaccetinkaya@yahoo.com **ORCID ID:** orcid.org/0000-0002-5676-9527
Received/Geliş Tarihi: 14.11.2018 **Accepted/Kabul Tarihi:** 12.02.2019

Sonuç: Türkiye'deki gebelerde Rubella IgG seropozitiflik oranı yüksek, IgM ise düşüktür. Bu oranlar ülkemizdeki başarılı bağışıklama politikaları ve uygulamalarının bir sonucudur. Fakat birkaç ilde çocukluk çağı ve yetişkin Rubella aşılama prosedürlerinin yeniden gözden geçirilmesinin faydalı olabileceği değerlendirilmektedir.
Anahtar Kelimeler: Rubella, gebe, meta-analiz, Türkiye

Introduction

Rubella is a vaccine-preventable disease and is one of the most infectious viral diseases known in humans. Congenital Rubella syndrome (CRS), consisting of cardiac disorders, cataract, deafness, cleft palate, autism, and fetal death can occur when the Rubella virus vertically infects the fetus during pregnancy[1]. Owing to the vaccination practices in childhood and adults, the prevalence of Rubella in pregnant women decreased and Rubella has become a rare infection in many developed and some developing countries. Some countries in the Western hemisphere and Europe have eliminated Rubella and CRS[2].

Immune status can be evaluated by enzyme-linked immunoreactive techniques (enzyme immunoassay, ELISA). From these tests, immunoglobulin (Ig)-G antibody shows a previous infection or immunization by vaccines. The Rubella serum IgM test indicates an acute Rubella infection and must be confirmed by at least one of these tests: Rubella-RNA polymerase chain reaction or Rubella IgG-avidity or western-blot[2].

The prevalence of Rubella infection in pregnant women can vary from country to country, and even across regions in the same country. In this meta-analysis, Rubella antibody tests in the last 11 years in pregnant women in Turkey were examined, and the differences in prevalence between provinces in our country, and between our country and other countries were compared.

Materials and Methods

In this meta-analysis, studies conducted in Turkey between 2007 and 2017 were analyzed, and differences in seroprevalences between provinces were compared by evaluating Rubella IgG, IgM, and IgG avidity results in pregnancy in this period. Data in these studies were screened and evaluated using the preferred reporting items for systematic reviews and meta-analyses (PRISMA) flow-chart according to the inclusion criteria (Figure 1).

Source of data

A data search was performed using the search terms Rubella, kızamıkçık, gebe, hamile, pregnancy, Türkiye, Turkey in Google Scholar, PubMed, Web of Science, Türk Medline, and the YÖK thesis database center by two independent researcher in 2018.

Inclusion and exclusion criteria

Studies with Rubella IgM, IgG, and IgG avidity test results in pregnant women in Turkey between 2007 and 2017 were included in the study. Original articles with antibody test results of at least 150 pregnant women with full text in Turkish or English were recorded. Studies were excluded if they included the results of the antibodies in non-pregnant women and male or child patients, and if the studies did not include the study period or were published after 2007 but the data were collected before 2007 (Figure 1).

Data search and collection of data

Considering the criteria, data were screened and evaluated by two different researchers (RAC, EY) in order to prevent publication bias. Study data: author's surname, date of publication, years of tests performed, numbers (n) and rates (%) of Rubella IgG, IgM, and IgG-avidity tests, and province of the study performed were recorded in the Microsoft Office 2016 Professional Plus Excel program. Before the meta-analysis, all data were listed in alphabetical order according to the author's surname in the extended format. Disputes between researchers were resolved by mutual discussion.

Statistical Analysis

Medcalc© software version 17.9.7 program was used for meta-analysis. Data were transferred from the Excel program. A funnel plot was used to evaluate possible bias and the results were interpreted.

A statistical test for heterogeneity was performed to measure the data heterogeneity. According to this; $I^2 \leq 25\%$ heterogeneity was assumed to be insignificant and a fixed effect was used. $I^2 > 25\%$ heterogeneity was assumed to be insignificant; the study data were considered as nonhomogeneous and the random effect value was used. $P<0.01$ was considered to be no need to add more studies.

Results

In this meta-analysis, 884 articles were found in accordance with the research criteria (Figure 1). A total of 681 articles were excluded from the study because of repetition in two or more different databases. After the removal, we had 203 studies, 201 of which we could screen. After evaluation of the study title and summary, another 31 were excluded from the study. After full text evaluations, 144 of the 170 studies were excluded from the study according to the determined criteria. As a result, a total of 26 articles associated with Rubella seroprevalence in pregnant women in Turkey were enrolled in the meta-analysis.

The studies included in the meta-analysis were from Afyon (n=2), Artvin (n=1), Bingöl (n=1), Denizli (n=1), Edirne (n=1), Isparta (n=1), Istanbul (n=4), Kahramanmaraş (n=1), Konya (n=2), Manisa (n=1), Muğla (n=1), Middle Black Sea (n=1), Rize (n=1), Uşak (n=1), Van (n=2), Yozgat (n=1), Zonguldak (n=1).

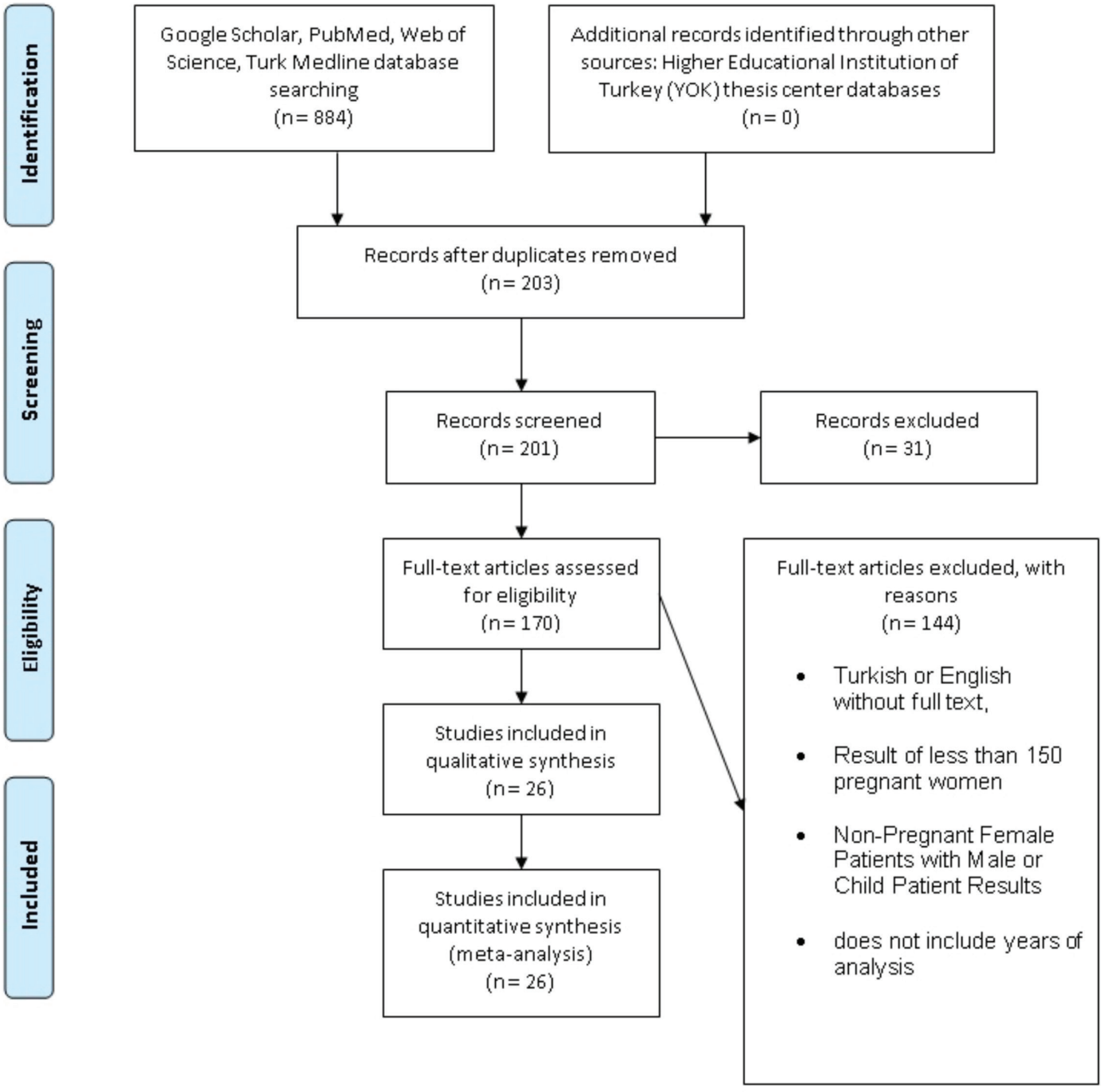


Figure 1. Flow chart for study selection and literature review. Summary of the literature search and study selection on Rubella antibodies in pregnant women

The study of Sargın and Saygan[3] from Ankara had the maximum number of cases with 31385 pregnant women, and the study of Bakacak et al.[4] from Kahramanmaraş had high number of cases with 11823 pregnant women.

According to the meta-analysis of 84398 serologic tests of pregnant women in the 26 studies, the seroprevalence rate of Rubella IgG in pregnant woman in Turkey was 93.47% (95% CI: 91.72 to 95.03). The Cochrane Q test was 2032,5378; I^2=98.77% and p<0.0001, respectively (Table 1). In funnel plot analysis, minimal asymmetry was found in the studies of Varol et al.,[5] Nazik et al.,[6] Başkesen et al.,[7] and Çeltek et al.[8] (Figure 2,3). Overall, the asymmetry test showed no bias.

According to a meta-analysis of 90,988 serologic tests of pregnant women in the 26 studies, the seroprevalence rate of Rubella IgM in pregnant woman in Turkey was 0.783%

Table 1. Meta-analysis of anti-Rubella IgG among pregnant women in Turkey

Study name, year, reference	Province	Sample size	Proportion (%)	95% CI	Weight (%)	
					Fixed	Random
Akpınar (2017)[9]	Isparta	805	97.52	96.19 to 98.48	0.95	3.84
Aşık (2013)[10]	Afyon	505	92.08	89.37 to 94.28	0.60	3.75
Aynıoğlu (2015)[22]	Zonguldak	910	93.85	92.08 to 95.32	1.08	3.86
Bakacak (2014)[4]	Kahramanmaraş	11.823	93.20	92.73 to 93.65	14.01	3.99
Başkesen (2010)[7]	Manisa	1202	83.69	81.48 to 85.74	1.42	3,89
Çeltek (2014)[8]	Middle Black Sea	3162	99.68	99.42 to 99.85	3.75	3.96
Doğan (2014)[23]	İstanbul	1641	95.73	94.64 to 96.66	1.94	3.92
Efe (2009)[12]	Van	613	99.51	98.58 to 99.90	0.73	3.79
Gündem (2014)[24]	Konya	419	92.84	89.94 to 95.12	0.50	3.70
İnci (2014)[25]	Artvin	1292	95.20	93.89 to 96.30	1.53	3.90
Karabulut (2011)[26]	Denizli	1268	95.11	93.77 to 96.23	1.50	3.90
Karacan (2014)[27]	İstanbul	1258	95.55	94.26 to 96.62	1.49	3.90
Kasap (2017)[28]	Mugla	189	89.95	84.75 to 93.84	0.23	3.39
Keskin (2013)[29]	İstanbul	1926	95.74	94.74 to 96.60	2.28	3.93
Nazik (2017)[6]	Bingöl	10.178	84.31	83.59 to 85.01	12.06	3.99
Numan (2015)[30]	İstanbul	1101	94.19	92.64 to 95.50	1.31	3.88
Özdemir (2011) [31]	Konya	249	95.98	92.74 to 98.06	0.30	3.52
Parlak (2015)[14]	Van	416	86.54	82.88 to 89.67	0.49	3.70
Satılmış (2014)[32]	Yozgat	804	94.03	92.16 to 95.57	0.95	3.84
Şentürk (2016)[33]	Rize	424	93.87	91.14 to 95.96	0.50	3.71
Şevki (2013)[3]	Ankara	31.385	93.92	93.65 to 94.19	37.18	4.00
Şimşek (2016)[11]	Afyon	1076	94.52	92.98 to 95.80	1.28	3.88
Şirin (2017)[34]	İzmir	7189	93.49	92.89 to 94.05	8.52	3.99
Toklu (2013)[35]	Uşak	1465	92.15	90.65 to 93.48	1.74	3.91
Varıcı-Balcı (2014) [13]	İzmir	1871	93.21	91.98 to 94.31	2.22	3.93
Varol (2011)[5]	Edirne	1227	76.61	74.14 to 78.95	1.45	3.90
Total (fixed effects)	TURKEY	84.398	93.06	92.89 to 93.23	100.00	100.00
Total (random effects)			93.48	91.727 to 95.033	100.00	100.00

Test for heterogeneity	
Q	2032.5378
DF	25
Significance level	p<0.0001
I^2 (inconsistency)	98.77%
95% CI for I^2	98.57 to 98.94

DF: Dickey-Fuller test

(95% CI: 0.505 to 1.120). The Cochrane Q test was 583,6836; I^2=95,72% and p<0.0001 (Table 2). A negligible asymmetry was found in the funnel plot analysis, and the asymmetry test showed no bias (Figure 4). The results of Başkesen et al.[7] and Akpınar et al.[9] were most distant from the average value of Turkey.

Additionally, Rubella IgG-avidity rates were analyzed in the present meta-analysis (Table 3). Accordingly, the low avidity rate was 4.66% (95% CI: 0.969 to 10.906) and the Cochrane Q test was 10,3230; I^2=70.94% (p<0.0001) in 427 pregnant in four studies[10-13], intermediate avidity rate was 7.51% (95% CI: 5.101 to 10.345), and the Cochrane Q test was 1,8145; I^2=0.00 (p=0.404) in 384 pregnant in three studies[11-13], the high avidity rate was 93.55% (95% CI: 82.584 to 99.311), and the Cochrane Q test was 46,4845, I^2=91.39% (p<0.0001) in 522 pregnant women in five studies[3,10-13].

Discussion

Rubella infection is usually subclinical in childhood, but may be more severe at older ages in life. It can also lead to

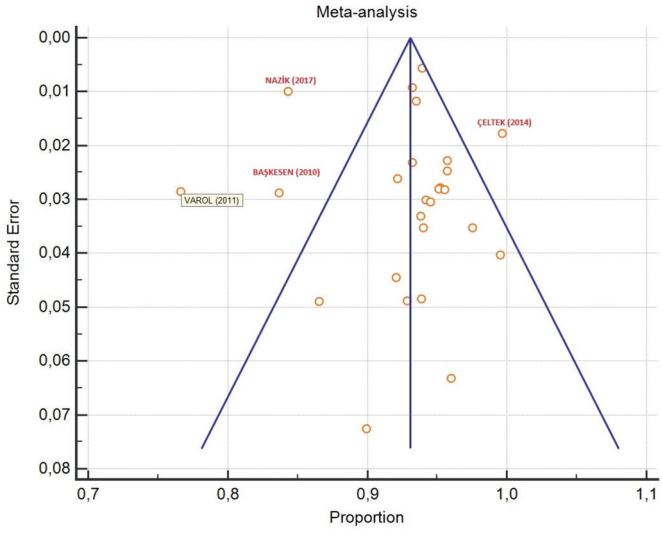


Figure 2. Funnel plot analysis graph of anti-Rubella immunoglobulin-G in Turkey

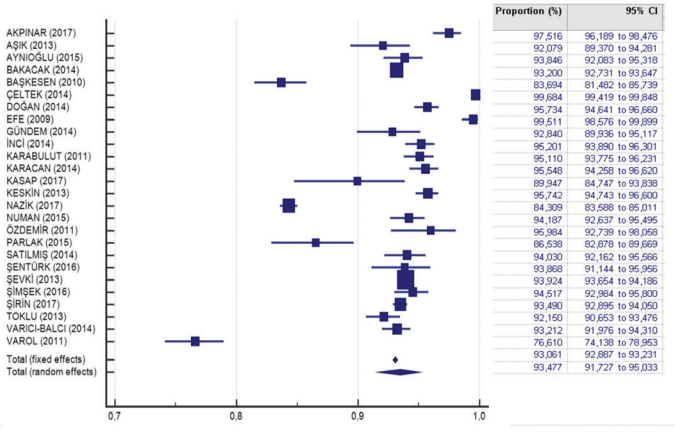


Figure 3. Meta-analysis graph of anti-Rubella immunoglobulin-G

severe anomalies or death in the fetus in the first trimester of pregnancy[1,14].

The serologic test showing previous Rubella infection or immunization status alone is the Rubella IgG antibody. Due to the increase in the awareness of pregnant women and socioeconomic developments in our country, there has been an increase in prenatal screening tests in recent years. According to the meta-analysis of 26 studies analyzed, the Rubella IgG seropositivity rate in Turkey was 93.4%. This rate was higher than 44% of the studies, and lower than 56% of the studies included in the meta-analysis. The study of Şevki et al.[3] in Ankara with 31,385 cases represents the Turkey's average best; the seropositivity rate of 93.9% and 37.1% weight in the meta-analysis[3].

In a general perspective, it is considered that the vaccination rates in the west part of Turkey is higher, and in the east part of Turkey, immunity is gained after acquiring infections against infections that can be prevented by vaccination. The study of Varol et al.[5] conducted in the Thrace region

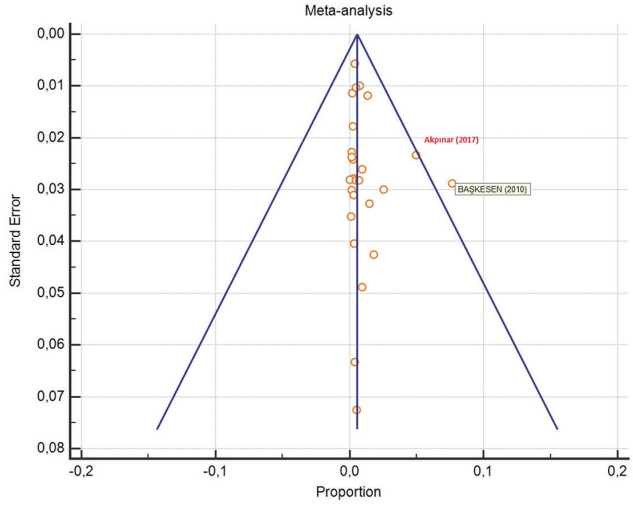


Figure 4. Funnel plot analysis graph of anti-Rubella immunoglobulin-M

of Turkey revealed the lowest IgG ratio (76.6%), and also Baskesen et al.[7] from Manisa revealed a lower rate (83.6%) than the average of Turkey. In these two studies, it was not possible to make an inference because the age status of the pregnant women was not given in cross-sectional intervals. However, these rates raise doubts about effective vaccination strategies in the regions where both studies were conducted. More comprehensive randomized controlled prospective research is needed for these two regions.

Rubella IgG seropositivity rate was 84.3% in the study conducted by Nazik et al.[6] in the Bingöl province in 10,178 pregnant women. Similarly, the study of Parlak et al.[14] in the Van province in 416 pregnant women, the rate was 86.5% for IgG seropositivity. These rates could be considered to be due to the low level of vaccinations of the people living in the region, but it is not possible to form a definite opinion on this issue.

In the study of Çeltek et al.[8] with 3162 pregnant women representing the Middle Black Sea region, Rubella IgG seropositivity rate was found as 99%. Çeltek et al.[8] interpreted their result as close to the average of Turkey. However, the ratio in their study was found to be higher than the average of Turkey according to our meta-analysis. The high rate in this region indicates that the number of pregnant women who could be infected with Rubella during pregnancy was low. In order to use the results across the country, the characteristics of the cases should be evaluated further.

The study of Özdemir et al.[15] was not included in the meta-analysis because the test and technique used in the multicenter study conducted in seven provinces were not explicitly written. In this multicenter study, Rubella IgG positivity was between 76-96.4% and the results varied significantly in different provinces. Our meta-analysis, or similarly, this multicenter study shows that different results can be obtained in different regions. This difference can be

Table 2. Meta-analysis of anti-Rubella immunoglobulin-M among pregnant women in Turkey

Study Name, Year, References	Province	Sample size	Proportion (%)	95% CI	Weight (%)	
					Fixed	Random
Akpınar (2017)[9]	Isparta	1829	4.97	4.02 to 6.07	2.01	4.04
Aşık (2013)[10]	Afyon	552	1.81	0.87 to 3.31	0.61	3.48
Aynıoğlu (2015)[22]	Zonguldak	933	1.50	0.82 to 2.50	1.03	3.79
Bakacak (2014)[4]	Kahramanmaras	7733	0.19	0.11 to 0.32	8.50	4.27
Başkesen (2010)[7]	Manisa	1202	7.65	6.21 to 9.30	1.32	3.90
Çeltek (2014)[8]	Middle Black Sea	3162	0.25	0.09 to 0.50	3.48	4.16
Doğan (2014)[23]	İstanbul	1714	0.23	0.064 to 0.60	1.88	4.02
Efe (2009)[12]	Van	613	0.33	0.039 to 1.17	0.67	3.55
Gündem (2014)[24]	Konya	419	0.95	0.26 to 2.43	0.46	3.28
İnci (2014)[25]	Artvin	1292	0.31	0.084 to 0.79	1.42	3.93
Karabulut (2011)[26]	Denizli	1268	0.00	0.00 to 0.29	1.39	3.92
Karacan (2014)[27]	Istanbul	1258	0.48	0.17 to 1.03	1.38	3.92
Kasap (2017)[28]	Muğla	189	0.53	0.013 to 2.91	0.21	2.52
Keskin (2013)[29]	Istanbul	1926	0.16	0.032 to 0.45	2.12	4.06
Nazik (2017) [6]	Bingol	10.178	0.78	0.61 to 0.97	11.18	4.29
Numan (2015)[30]	İstanbul	1101	0.18	0.02 to 0.65	1.21	3.86
Özdemir (2011)[31]	Konya	249	0.40	0.010 to 2.22	0.27	2.81
Parlak (2015)[14]	Van	9340	0.47	0.34 to 0.63	10.26	4.28
Satılmış (2014)[32]	Yozgat	804	0.12	0.003 to 0.69	0.88	3.71
Şentürk (2016)[33]	Rize	1037	0.29	0.06 to 0.84	1.14	3.84
Şevki (2013)[3]	Ankara	31.385	0.39	0.33 to 0.47	34.48	4.32
Şimşek (2016)[11]	Afyon	1112	2.52	1.68 to 3.62	1.22	3.87
Şirin (2017)[34]	İzmir	7189	1.33	1.08 to 1.63	7.90	4.26
Toklu (2013)[35]	Uşak	1465	0.96	0.52 to 1.60	1.61	3.97
Varıcı-Balcı (2014)[13]	İzmir	1784	0.17	0.035 to 0.49	1.96	4.03
Varol (2011)[5]	Edirne	1254	0.72	0.33 to 1.36	1.38	3.92
Total (fixed effects)	**TURKEY**	**90.988**	**0.58**	**0.53 to 0.63**	**100.0**	**100.0**
Total (random effects)			**0.78**	**0.50 to 1.12**	**100.0**	**100.0**

explained by the wide geographic structure of Turkey and by the sociocultural differences that exist between the regions. Therefore, we believe that accurate rates throughout the country can only be obtained by meta-analysis studies.

The age and dose of the Rubella vaccination may vary depending on a country's vaccination policy. In general, an 84.7% seropositivity rate is achieved by single-dose vaccination, and this rate reaches 90% with two doses of vaccine (ages 1 and 5)[16]. In our country, Rubella vaccines are administered as two doses in children at 12 months and 7 years. Turkey, with a rate of 93.4% Rubella IgG seropositivity, has a rate just above that in the general literature. Turkey's neighboring Iran has a seropositivity rate of 94%,[17] and Greece, which is a member of the European Union, has a seropositivity rate of 97% in women with childbearing age[18].

Test for heterogeneity	
Q	583.6836
DF	25
Significance level	p<0.0001
I^2 (inconsistency)	95.72%
95% CI for I^2	94.62 to 96.59

DF: Dickey-Fuller test

This ratio is 89.3% in Brazil and 99.3% in the United States of America[16]. In India, which is a Far East country, it is 68.3%. The reason for this low rate is that Rubella vaccine is currently not included in the national immunization program[19]. In

Table 3. Meta-analysis of Rubella immunoglobulin-G-avidity test results

Study name and year	Rubella IgG-avidity positive								
	Low avidity			Intermediate avidity			High avidity		
	Sample size	Pro-portion (%)	95% CI	Sample size	Proportion (%)	95% CI	Sample size	Proportion (%)	95% CI
ASIK (2013)[10]	43	2.33	0.060 to 12.29	N/A	N/A	N/A	43	97.67	87.71 to 99.94
SEVKI (2013)[3]	N/A	N/A	N/A	N/A	N/A	N/A	95	100.00	96.19 to 100.00
ŞIMSEK (2016)[11]	16	12.50	1.55 to 38.35	16	12.50	1.55 to 38.35	16	75.00	47.62 to 92.73
SIRIN (2017)[34]	54	0.00	0.00 to 6.60	54	3.70	0.45 to 12.75	54	98.15	90.11 to 99.95
VARICI-BALCI (2014)[13]	314	7.32	4.70 to 10.79	314	7.64	4.96 to 11.16	314	85.03	80.60 to 88.79
Total (fixed effects)	427	5.88	3.85 to 8.54	384	7.51	5.09 to 10.61	522	91.41	88.68 to 93.66
Total (random effects)	427	4.66	0.97 to 10.91	384	7.51	5.10 to 10.34	522	93.55	82.58 to 99.31
Q	10.3230			1.8145					
DF	3			2					
Significance level	p=0.0160			p=0.4036					
I^2 (inconsistency)	70.94%			0.00%					
95% CI for I^2	16.99 to 89.83			0.00 to 96.30					

NA: Not applicable

China, although this rate varies according to age in pregnant women, this rate is approximately 84.0%[20]. The high level of IgG antibody seropositivity in our country reveals that we have a successful vaccination policy compared with other developing countries.

It is reported that if immunity against Rubella is below 90%, the risk of acute infection and CRS might increase in childbearing age[9]. Rubella IgM antibody is the first-step test in determining acute infection. In our meta-analysis, the IgM seroprevalence rate in Turkey was about 0.8%. The study of Başkesen et al.,[7] which included 1202 pregnant women in the Manisa region, is one of the most important studies on this subject with 7.6% IgM seropesitivity rate and 1.3% weight in meta-analysis. In patients in Manisa, clinical evaluations should be made, IgM results, cross-reactions or primary/re-infection should be confirmed, and it should be kept in mind that antibody positivity may persist for a year following vaccination or asymptomatic infection. There is a need for a prospective randomized controlled trial in the Manisa region to identify these patients.

As a part of the "Elimination of Rubella and CRS Prevention Program", Rubella vaccine has administered in Turkey, including Isparta, since 2006[21]. The Rubella IgM seropositivity rate was found as 4.9% in the retrospective study of Akpınar et al.[9] in Isparta, which comprised 1829 pregnant women. There is not enough information about whether pregnant women are vaccinated because of the fact that the study was retrospective; however, it can still be concluded that the routine vaccination program is not adequately successful in the Isparta province.

In pregnant woman with both IgG and IgM positivity, an IgG avidity test should be performed to estimate the time of infection. According to our analysis, Rubella IgG avidity was studied in only 5 studies in Turkey, and the low avidity rate was 4.6% in a total of 427 pregnant women. One of the reasons for this high rate was the study of Şimsek et al.[11] conducted in Afyon. In this study, there were 28 patients with IgM positivity and 44 patients with gray-zone IgM positivity, but avidity was studied in only 12 of them. Two patients with low avidity in this group increased the rate of high avidity in the analysis.

It is not possible to make a detailed and direct comparison through avidity results because publications about CRS are generally case studies in our country. There is a need for a multicenter prospective study for our country including CRS patient series with validation tests, advanced diagnostic tests, and large-scale IgG-avidity results.

There are some restrictions for meta-analyses; there may be

studies that have not completed the publication procedure in the analysis period and also there may be publications for which the full text cannot be reached. There may also be changes in the interpretation of the study results due to differences in the kit and devices used in the studies analyzed. In summary, our study cannot show the whole the Rubella seroprevalence in Turkey. The high seroprevalence of IgG antibodies in Turkey may be considered as the result of successful immunization policies and practices. In a few provinces, it is necessary to revise the Rubella immunization procedures and adult vaccination strategies should be developed in order to control Rubella infections in adults including pregnant woman.

Acknowledgement: The authors are very thankful to Prof. Dr. Arzu Kanık for her contribution for the statistical analysis.

Ethics Committee Approval: Meta-analysis studies are accepted as the original article. However, ethics committee approval document has not been submitted since it is not required to obtain an ethics committee approval in the meta-analysis studies.

Informed Consent: Meta-analysis study.

Peer-review: External and internal peer-reviewed.

Authorship Contributions

Concept: R.A.C., E.Y., Design R.A.C., E.Y., Data Collection or Processing: R.A.C., E.Y., Analysis or Interpretation: R.A.C., E.Y., Literature Search: R.A.C., E.Y., Writing: R.A.C., E.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. Orenstein WA, Cairns L, Hinman A, Nkowane B, Olive JM, Reingold AL. Measles and Rubella Global Strategic Plan 2012-2020 midterm review report: Background and summary. Vaccine 2018;36(Suppl1):A35-A42.
2. Berger A, Doerr HW. Preventing vertical virus infections: the role of serologic screening of pregnant women. Med Microbiol Immunol 2018;207:249-53.
3. Sargın A., Saygın S. Seroprevalence of Toxoplasma gondii and rubella among pregnant women in central Turkey. African Journal of Microbiology Research 2013;7:2524-9.
4. Bakacak M, Bostancı MS, Köstü B, Ercan Ö, Serin S, Avcı F. Gebelerde Toxoplasma gondii, rubella ve sitomegalovirüs seroprevalansı. Dicle Med J 2014;41:326-31.
5. Varol FG, Sayin NC, Soysuren S. Seroprevalance of toxoplasma gondii antibodies in antenatal population of Trakya region. J Turk Soc Obstet Gynecol 2011;8:93-9.
6. Nazik S, Duran İ, Nazik H, Duran Ş. Gebelikte Toksoplazma ve Rubella seropozitifliğinin değerlendirilmesi. Balıkesir Medical Journal 2017;1:22-5.
7. Başkesen T, Ecemiş T, Şanlidağ T. Gebelerde rubella bağışıklığının değerlendirilmesi. Kocatepe Tıp Dergisi 2010;11:19-21.
8. Çeltek NY, Tetikçok R, Günal Ö, Demirtürk F, Duygu F, Barut HŞ, et al. Türkiye'nin Orta Karadeniz Bölgesi'nde Gebelerde Rubella, CMV ve Toksoplazmozis Seroprevalansı Gaziosmanpaşa Üniversitesi Tıp Fakültesi Dergisi 2014;6:54-62.
9. Akpınar O, Akpınar H. Gebe Kadınlarda Rubella ve Sitomegalovirus Seroprevelansının ELISA yöntemi ile Araştırılması. Balıkesir Sağlık Bilimleri Dergisi 2017;6:11-5.
10. Aşık G, Ünlü BS, Er H, Yoldaş Ö, Köken G, Çufalı D, et al. Afyon bölgesinde gebelerde Toksoplazma ve Rubella seroprevelansı. Pamukkale Tıp Dergisi 2013;6:128-32.
11. Şimşek M, Keşli R, Demir C, Çetinkaya Ö, Ariöz DT. Afyon Kocatepe Üniversitesi, Araştırma ve Uygulama Hastanesinde takip edilen gebelerde Toksoplazma, Rubella, Sitomegalovirus ve Herpes Simpleks Virus Tip 2 seroprevalansının incelenmesi. Ortadogu Medical Journal/Ortadogu Tip Dergisi 2016;8:1-6.
12. Efe Ş, Kurdoğlu Z, Korkmaz G. Van yöresindeki gebelerde Sitomegalovirüs, Rubella ve Toksoplazma antikorlarının seroprevalansı. Van Tıp Derg 2009;16:6-9.
13. Varıcı BF, Arslan A, Sertöz R, Altuğlu İ. Rubella and cytomegalovirus seroprevalance among pregnant women admitted to Ege University Hospital. Ege Tıp Dergisi 2014;53:179-83.
14. Parlak M, Çim N, Erdin BN, Güven A, Bayram Y, Yıldızhan R. Seroprevalence of Toxoplasma, Rubella, and Cytomegalovirus among pregnant women in Van. Turkish journal of obstetrics and gynecology 2015;12:79.
15. Özdemir M, Taşbent FE, Terzi HA, Çetinkol Y, Uysal EB, Aşık G, et al. Seroprevalence of major viral pathogens during pregnancy: A multicenter study in Turkey. Adv ClinMed Microbiol 2016;1:1-5.
16. Mongua-Rodriguez N, Díaz-Ortega JL, García-García L, Pina-Pozas M, Ferreira-Guerrero E, Delgado-Sánchez G, et al. A systematic review of rubella vaccination strategies implemented in the Americas: impact on the incidence and seroprevalence rates of rubella and congenital rubella syndrome. Vaccine 2013;31:2145-51.
17. Ghaderi R, Ghaderi F. Rubella Immunity among Pregnant Women in Iran. MOJ Immunol 2016;4:00118:1-4. 18.
18. Gioula G, Exindari M, Melidou A, Minti F, Sidiropoulou E, Dionisopoulou S, et al. Seroprevalence of measles in Northern Greece 2017;62:145-50.
19. Jayakrishnan Thayyil VK, Moorkoth AP, Rao B, Selvam P. Prevalence of rubella-specific IgG antibodies in unimmunized young female population. Journal of family medicine and primary care 2016;5:658-62.
20. Meng Q, Luo J, Li L, Shi W, Yu J, Shen Y, et al. Rubella seroprevalence among pregnant women in Beijing, China. BMC infectious diseases 2018;18:130.
21. Genişletilmiş Bağışıklama Programı Genelgesi, Sağlık Bakanlığı Temel Sağlık Hizmetleri Genel Müdürlüğü 2006/120 sayılı genelge. Available from: https://dosyasb.saglik.gov.tr/Eklenti/1117,gbpgenelge2008pdf.pdf Available date: 15.01.2019.
22. Aynioglu A, Aynioglu O, Altunok ES. Seroprevalence of Toxoplasma gondii, rubella and Cytomegalovirus among pregnant females in north-western Turkey. Acta Clinica Belgica 2015;70:321-4.
23. Dogan K, Güraslan H, Özel G, Aydan Z, Yasar L. Seroprevalence Rates of Toxoplasma gondii, Rubella, Cytomegalovirus, Syphilis, and Hepatitis B, Seroprevalences Rate in The Pregnant Population in Istanbul. Türkiye Parazitolojii Dergisi 2014;38:228.
24. Gündem NS, Ağir MÇ. Investigation of seroprevalences of Rubella and Toxoplasma gondii among pregnant women. Anatolian Journal of Clinical Investigation 2014;8:152-4.
25. İnci A, Yener C, Güven D. Bir devlet hastanesinde gebe kadınlarda toksoplazma, rubella ve sitomegalovirüs seroprevalansının araştırılması. Pamukkale Tıp Dergisi 2014:143-6.

26. Karabulut A, Polat Y, Türk M, Balci YI. Evaluation of rubella, Toxoplasma gondii, and cytomegalovirus seroprevalences among pregnant women in Denizli province. Turkish Journal of Medical Sciences 2011;41:159-64.
27. Karacan M, Batukan M, Çebi Z, Berberoglugil M, Levent S, Kır M, et al. Screening cytomegalovirus, rubella and toxoplasma infections in pregnant women with unknown pre-pregnancy serological status. Archives of gynecology and obstetrics 2014;290:1115-20.
28. Kasap B, Öner G, Küçük M, Öztürk Turhan N, Akın MN, Arıkan S, et al. Muğla'daki gebelerin Toksoplazma, Rubella, Sitomegalovirüs ve Hepatit prevalansının değerlendirilmesi. İzmir Tepecik Eğitim ve Araştırma Hastanesi Dergisi 2017;27:31-6.
29. Deha K. İlk Trimester Gebelerde Toksoplazma, Rubella, CMV, HBV, AntiHBs, HCV, HİV Seroprevelansları. Selçuk Tıp Derg 2013;3:123-6.
30. Numan O, Vural F, Aka N, Alpay M, Ertürk Coşkun AD. İstanbul Anadolu Kuzey Kamu Hastaneleri Birliği Haydarpaşa Numune Eğitim ve Araştırma Hastanesi Gebe Polikliniği'ne Başvuran Hastalarda TORCH Seroprevalansının Araştırılması 2015;2:203-9.
31. Özdemir M, Kalem F, Feyzioğlu B, Baysal B. Investigation of viral pathogens during pregnancy in a city region in Turkey. AJCI 2011;5:78-81.
32. Kiriş Satılmış Ö, Yapça ÖE, Yapça D, Çatma T. Seroprevalance of Rubella, Cytomegalovirus and Toxoplasma Antibodies Among Pregnant Women that Referred to Sorgun State Hospital. Istanbul Kanuni Sultan Süleyman Tip Dergisi (IKSST) 2014:6:90-6.
33. Şentürk Ş, Kağıtçı M, Balik G, Şahin K, Şahin FK,. Doğu Karadeniz Bölgesi'ndeki Gebe Kadınlarda Rubella Virus Seroprevalansı. Van Tıp Derg 2016;23:242-5.
34. Sirin MC, Agus N, Yilmaz N, Bayram A, Derici YK, Samlioglu P, et al. Seroprevalence of Toxoplasma gondii, Rubella virus and Cytomegalovirus among pregnant women and the importance of avidity assays. Saudi Medical Journal 2017;38:727.
35. Toklu GD. Antibodies Frequency Against Toxoplasmosis, Rubella Virus and Cytomegalovirus in Pregnant Women Gebelerde Toksoplazma, Rubella Virus ve Sitomegalovirus'a Karşı Oluşan Antikorların Sıklığı. J Clin Anal Med 2013;4:38-40.

Review / Derleme

DOI: 10.4274/tjod.galenos.2018.15931

Turk J Obstet Gynecol 2019;16:72-5

Cadaveric anatomy and dissection in surgical training

Cerrahi eğitimde kadavrada anatomi ve disseksiyon

İlker Selcuk[1], Ilkan Tatar[2], Emre Huri[3]

[1]*University of Health Sciences, Zekai Tahir Burak Woman's Health Training and Research Hospital, Clinic of Gynecologic Oncology, Ankara, Turkey*

[2]*Hacettepe University Faculty of Medicine, Department of Anatomy, Ankara, Turkey*

[3]*Hacettepe University Faculty of Medicine, Department of Urology, Ankara, Turkey*

Abstract

Detailed knowledge of anatomy is an essential part of surgical practice. However, there are many drawbacks in anatomy education that make many residents feel inadequate when they start performing surgeries. Cadaveric dissection courses aim to close the gap between the anatomic knowledge and surgical practice. This review focuses on the role of cadaveric dissection on surgical education, and additionally states the panel decision of the Surgical Anatomy and Technologies Association on the proper use of cadavers

Keywords: Anatomy, surgery, education, cadaver, gynecology

Öz

Cerrahi pratiğin en önemli parçası detaylı bir anatomi bilgisidir. Ancak anatomi eğitiminde birçok engel ve eksiklik bulunmaktadır, ve birçok cerrahi asistanı cerrahi prosedürleri uygularken yetersizlik hissetmektedir. Diğer bir açıdan, kadavra kursları anatomik bilgi ve cerrahi pratik arasında oluşmuş olan bu boşluğu kapatmayı hedeflemektedir. Bu derleme, kadavra disseksiyonunun cerrahi eğitimdeki rolüne odaklanmakla beraber; Cerrahi Anatomi ve Teknolojileri Derneği'nin kadavraların uygun kullanımı ile ilgili panel kararını da içermektedir.

Anahtar Kelimeler: Anatomi, cerrahi, eğitim, kadavra, jinekoloji

Introduction

This review covers the concept of cadaveric dissection in terms of teaching anatomy and post-graduate surgical training.

The role of cadaveric dissection in teaching anatomy

Cadaveric dissection has been the main teaching modality in anatomy education since the ancient times. In the 3rd century before Christ, the first human cadaveric dissections were performed in Greece by Herophilus of Chalcedon and Erasistratus of Chios to understand the whole body from the viewpoint of anatomy and physiology. However, religious and moral attitudes and taboos towards physicians and medical schools had many detrimental effects on the scientific value of cadaver-based education[1].

Cadaveric dissection is the traditional way of teaching anatomy after theoretical lessons and discussions on the atlas images[2]. Medical students gain knowledge and strengthen theoretical data through visualization of real anatomic structures. Additionally, by practicing on cadavers they touch and feel the anatomic relations more efficiently[3]. Owing to the role of cadaveric dissection in generating a three-dimensional (3D) perspective, and providing an easy way of understanding and recalling anatomic structures, the literature indicates that cadaveric dissection is one of the most powerful ways of teaching topographic and regional anatomy[1,4].

Recently, there has been a trend shift in modern anatomy education, and many novel options are used in teaching anatomy. Cadaveric dissection and didactic lessons with atlas images constitute the traditional methods; however, 3D simulation technologies, virtual/augmented reality, 3D printed materials, simulation/training models, and radiology-based comparative illustrations form the new methodologies in current anatomy education[5].

Address for Correspondence/Yazışma Adresi: İlker Selcuk, MD,
University of Health Sciences, Zekai Tahir Burak Woman's Health Training and Research Hospital, Clinic of Gynecologic Oncology, Ankara, Turkey
Phone: +90 530 201 05 46 **E-mail:** ilkerselcukmd@hotmail.com **ORCID ID:** orcid.org/0000-0003-0499-5722
Received/Geliş Tarihi: 16.10.2018 **Accepted/Kabul Tarihi:** 24.12.2018

Cadaveric dissection and post-graduate surgical education

Post-graduate anatomy education is the core praxis in improving surgical and technical knowledge for surgery residents. Simulation-based education with hands-on courses and cadaveric dissections focus on a detailed practice of surgical procedures prior to live patient operations, consequently an increase in confidence levels and surgical skills of residents will be noticed[6]. Nevertheless, cadaveric dissection is not the sole method of teaching anatomy and it should be complemented by other innovative educational tools. In this way, medical students and residents will be better equipped and well prepared for future medical activities[7].

The problem beyond the inadequacy in anatomy education for surgery residents gave rise to the wide use of cadaveric courses in many surgical disciplines. Giving the opportunity to residents to attend these cadaveric courses will improve the educational quality during the first steps of learning surgical procedures by raising self-confidence and better surgical skills[8].

Cadaveric courses in obstetrics-gynecology and other surgical disciplines

Cadaveric dissection under the supervision of senior surgeons and anatomists will develop a practical competency in the field of minimally invasive surgery, gynecology, gynecologic oncology, and urogynecology, even if in obstetrics, and also for the management of complications[9].

Sharma et al.[10] reported the positive effect of procedure-oriented cadaveric courses, which target teaching specific operations to improve operative confidence and surgical technique. Some advanced surgical procedures could be observed or performed for the first time at the cadaveric courses, which deeply enhance anatomic knowledge and may also provide a higher level of autonomy to perform procedures independently[6].

Post-course surveys showed that improvements in surgical skills, which are transferrable to the operating room, were essentially maintained by cadaveric dissections[11] and Lim et al.[8] showed that the best improvements were observed in hysterectomy and salpingo-oophorectomy procedures for gynecology practice. Both basic and advanced surgical procedures can be performed on cadavers and the use of fresh frozen cadavers instead of traditional embalmed ones make it easy to learn the general objectives of a surgical operation in which a special and tailored practice is needed[12]. Simulation of surgical scenarios, identifying the steps of new techniques, and teaching the tips and tricks of advanced surgical procedures are effectively performed by using fresh frozen cadavers for postgraduate surgical education.

Previously, a newer surgical method used in urogynecology practice, transobturator tape procedure, was widely discussed in cadaveric illustration studies[13,14]. Safe implementation of surgical procedures with regard to vascular and neural structures was also studied for transvaginal tape and sacrospinous ligament fixation operations in urogynecology literature[15,16].

Additionally, the complex structure of pelvic anatomy and the pathway of the ureter were also demonstrated in cadaveric studies for surgical education[17-19]. Soft-preserved cadavers, which are prepared using phenol, alcohol, and glycerol, were used by Barton et al.[20] during a gynecologic oncology cadaveric course, and the need of such a cadaveric anatomy education during gynecologic oncology fellowship was the objective result of the end-course evaluation.

In addition, management of complications is a major requirement that a resident needs to learn during the surgical training period; however, it is not easy to observe these kinds of cases all the time. Cadaveric courses can mimic complications through simulations and this yields a higher practice level and competency for less common procedures and real-life conditions[21].

In practical obstetrics, cadaveric education increased the ability to manage surgical cases where a serious episiotomy tear or a massive peripartum bleeding occurred[22-24].

Minimally invasive gynecology, with the advantage of video recording, especially in the laparoscopic technique, is a well-known surgical practice to popularize the surgical approach and maneuvers to a widespread population, and this is also valid for teaching and demonstrating anatomic structures. Cundiff et al.[25] evaluated the effectiveness of laparoscopic cadaveric dissection after starting the residency program of obstetrics and gynecology to overcome the deficiencies in anatomy education; the course produced satisfactory results for the participants and also allowed video recording, which could spread the educational materials to non-active participants.

Other surgical branches also benefit from cadaveric courses; residents, fellows, junior surgeons, and new consultants gain experience and improve skills with confidence. Cadaveric courses are also the approved method of surgical education to share knowledge in brain surgery, plastic surgery, orthopedics, general surgery, urology, vascular surgery, and other branches. Cadaveric courses will lead to improvement of surgical outcomes by means of identifying proper anatomical landmarks, practicing more without any stress of the operation room in a comfortable environment, and teaching the basis of a new modification in a surgical approach[26-30].

Panel decision on the "Ethical and Proper Use of Cadavers in Surgical Education"

Surgical Anatomy and Technologies Association (SATA) organized a panel titled "Ethical and Proper Use of Cadavers in Surgical Education" during the First National Anatomy and Cadaveric Dissection Symposium of SATA, which was held at Kars, Turkey, in March 2018. More than 50 participants

attended this interactive session, which was conducted by three mentors (EH, IT, IS) who were proficient in surgery and anatomy. The role of cadaveric dissection in surgical education was discussed in many aspects.

The final decisions on the Ethical and Proper Use of Cadavers in Surgical Education:

-The scientific committee of cadaveric courses should be consisted of phyicians and anatomists, a multidisciplinary/ advisory board.

-Cadaveric courses without the integration of anatomy departments and anatomists will give harm to an effective education.

-Dissection subgroups will improve the surgical education both for mentors and residents.

-A certification program should be formed to train the teachers of cadaveric surgical education ("teach the teachers").

-Number of participants per cadaver during the cadaveric dissection courses should be defined according to the aim and tasks of the course to maintain a cost-effective and educational activity.

-Criteria of a well-designed cadaveric course should be established with respect to the training aim, ethics, and health conditions of the environment.

-Cadaveric dissection laboratories should be high quality to preserve the health conditions of the participants and laboratory personnel.

-A rotation program to anatomy departments should be implemented to surgical residency education to improve anatomic knowledge.

Despite the higher costs of cadavers, if used effectively and properly, by conducting small but meticulous dissections, cadaveric courses will produce successful educational results in a cost-effective manner. However, measurements of the validity and effectiveness of these gains are lacking during surgical operations, as such there is a need for further studies in this area[31].

Conclusion

In conclusion, to gain surgical skills, experience, and confidence before performing a procedure on a live patient, hands-on cadaveric courses should be an integrated part of surgical education during residency and the post-graduate period. Fundamentally, these courses should be planned under the supervision of an advisory board consisting of both surgeons and anatomists with limited numbers of participants to allow each attendee achieve the final goals of the course.

Acknowledgement

Special thanks to the board committee of Surgical Anatomy and Technologies Association.

Ethics

Peer-review: Internally peer-reviewed.

Authorship Contributions

Concept: İ.S., Design: İ.S., Data Collection or Processing: İ.S., İ.T., Analysis or Interpretation: İ.S., E.H., Literature Search: İ.S., Writing: İ.S., İ.T.

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. Ghosh SK. Human cadaveric dissection: a historical account from ancient Greece to the modern era. Anat Cell Biol 2015;48:153-69.
2. Ahmed K, Rowland S, Patel V, Khan RS, Ashrafian H, Davies DC, et al. Is the structure of anatomy curriculum adequate for safe medical practice? Surgeon 2010;8:318-24.
3. Nwachukwu C, Lachman N, Pawlina W. Evaluating dissection in the gross anatomy course: Correlation between quality of laboratory dissection and students outcomes. Anat Sci Educ 2015;8:45-52.
4. Winkelmann A. Anatomical dissection as a teaching method in medical school: a review of the evidence. Med Educ 2007;41:15-22.
5. Hu M, Wattchow D, de Fontgalland D. From ancient to avant-garde: a review of traditional and modern multimodal approaches to surgical anatomy education. ANZ J Surg 2018;88:146-51.
6. Kim SC, Fisher JG, Delman KA, Hinman JM, Srinivasan JK. Cadaver-Based Simulation Increases Resident Confidence, Initial Exposure to Fundamental Techniques, and May Augment Operative Autonomy. J Surg Educ 2016;73:e33-e41.
7. Ghosh SK. Cadaveric dissection as an educational tool for anatomical sciences in the 21st century. Anat Sci Educ 2017;10:286-99.
8. Lim CP, Roberts M, Chalhoub T, Waugh J, Delegate L. Cadaveric surgery in core gynaecology training: a feasibility study. Gynecol Surg 2018;15:4.
9. Heisler CA. Importance of adequate gross anatomy education: the impact of a structured pelvic anatomy course during gynecology fellowship. Anat Sci Educ 2011;4:302-4.
10. Sharma G, Aycart MA, Najjar PA, van Houten T, Smink DS, Askari R, et al. A cadaveric procedural anatomy course enhances operative competence. J Surg Res 2016;201:22-8.
11. Ahmed K, Aydin A, Dasgupta P, Khan MS, McCabe JE. A novel cadaveric simulation program in urology. J Surg Educ 2015;72:556-65.
12. Jansen S, Cowie M, Linehan J, Hamdorf JM. Fresh frozen cadaver workshops for advanced vascular surgical training. ANZ J Surg 2014;84:877-80.
13. Bonnet P, Waltregny D, Reul O, de Leval J. Transobturator vaginal tape inside out for the surgical treatment of female stress urinary incontinence: anatomical considerations. J Urol 2005;173:1223-8.
14. Zahn CM, Siddique S, Hernandez S, Lockrow EG. Anatomic comparison of two transobturator tape procedures. Obstet Gynecol 2007;109:701-6.
15. Neuman M, Masata J, Hubka P, Bornstein J, Martan A. Sacrospinous ligaments anterior apical anchoring for needle-guided mesh is a safe option: a cadaveric study. Urology 2012;79:1020-2.
16. Masata J, Hubka P, Martan A. Pudendal neuralgia following transobturator inside-out tape procedure (TVT-O)--case report and anatomical study. Int Urogynecol J 2012;23:505-7.
17. Nakamura M, Fujii T, Imanishi N, Jinzaki M, Yamada M, Kuribayashi S, et al. Surgical anatomy imaging associated with cervical cancer treatment: A cadaveric study. Clin Anat 2014;27:503-10.

18. Barksdale PA, Brody SP, Garely AD, Elkins TE, Nolan TE, Gasser RF. Surgical landmarks of the ureter in the cadaveric female pelvis. Clin Anat 1997;10:324-7.
19. Kraima AC, Derks M, Smit NN, van de Velde CJ, Kenter GG, DeRuiter MC. Careful Dissection of the Distal Ureter Is Highly Important in Nerve-sparing Radical Pelvic Surgery: A 3D Reconstruction and Immunohistochemical Characterization of the Vesical Plexus. Int J Gynecol Cancer 2016;26:959-66.
20. Barton DP, Davies DC, Mahadevan V, Dennis L, Adib T, Mudan S, et al. Dissection of soft-preserved cadavers in the training of gynaecological oncologists: report of the first UK workshop. Gynecol Oncol 2009;113:352-6.
21. Hammond I, Taylor J, Obermair A, McMenamin P. The anatomy of complications workshop: an educational strategy to improve the training and performance of fellows in gynecologic oncology. Gynecol Oncol 2004;94:769-73.
22. Kerbage Y, Debarge V, Lucot JP, Clouqueur E, Rubod C. Simulation training to teach postpartum hemorrhage surgery to residents. Eur J Obstet Gynecol Reprod Biol 2016;201:27-30.
23. Selcuk I, Yassa M, Tatar I, Huri E. Anatomic structure of the internal iliac artery and its educative dissection for peripartum and pelvic hemorrhage. Turk J Obstet Gynecol 2018;15:126-9.
24. Emmanuelli V, Lucot JP, Closset E, Cosson M, Deruelle P. [Development and assessment of a workshop on repair of third and fourth degree obstetric tears]. J Gynecol Obstet Biol Reprod (Paris) 2013;42:184-90.
25. Cundiff GW, Weidner AC, Visco AG. Effectiveness of laparoscopic cadaveric dissection in enhancing resident comprehension of pelvic anatomy. J Am Coll Surg 2001;192:492-7.
26. Martin KD, Patterson DP, Cameron KL. Arthroscopic Training Courses Improve Trainee Arthroscopy Skills: A Simulation-Based Prospective Trial. Arthroscopy 2016;32:2228-32.
27. Matsuo S, Baydin S, Gungor A, Middlebrooks EH, Komune N, Iihara K, et al. Prevention of postoperative visual field defect after the occipital transtentorial approach: anatomical study. J Neurosurg 2018;129:188-97.
28. Ali S, Ibrahim A. Course Review: Birmingham and Coventry Hand and Forearm Cadaveric Dissection Course. Ann Plast Surg 2018;80:596-7.
29. Acar HI, Kuzu MA. Perineal and pelvic anatomy of extralevator abdominoperineal excision for rectal cancer: cadaveric dissection. Dis Colon Rectum 2011;54:1179-83.
30. Ozcan S, Huri E, Tatar I, Sargon M, Karakan T, Yagli OF, et al. Impact of cadaveric surgical anatomy training on urology residents knowledge: a preliminary study. Turk J Urol 2015;41:83-7.
31. Anastakis DJ, Regehr G, Reznick RK, Cusimano M, Murnaghan J, Brown M, et al. Assessment of technical skills transfer from the bench training model to the human model. Am J Surg 1999;177:167-70.

Case Report / Olgu Sunumu

DOI: 10.4274/tjod.galenos.2018.31855

Turk J Obstet Gynecol 2019;16:76-9

Uterine giant cell carcinoma: a case report and review of the literature

Uterin dev hücreli karsinom: olgu sunumu ve literatür sunumu

Hülya Ayık Aydın[1], Hasan Aykut Tuncer[1], Gülgün Erdoğan[2], Tayup Şimşek[1]

[1]*Akdeniz University Faculty of Medicine, Department of Obstetrics and Gynecology, Division of Gynecologic Oncological Surgery, Antalya, Turkey*

[2]*Akdeniz University Faculty of Medicine, Department of Pathology, Division of Gynecopathology, Antalya, Turkey*

Abstract

Endometrial carcinoma is the most common genital malignancy in women. Endometrioid type is the most common variant of endometrial carcinoma described in literature. Giant cell carcinoma is a rare, and infrequently reported variant of endometrial carcinoma. We present a 75-year-old patient admitted with vaginal bleeding. Transvaginal ultrasound revealed a 26x28 mm hypodense lesion without any adnexal pathology. The patient underwent total abdominal hysterectomy, bilateral salpingo-oophorectomy, and bilateral pelvic, and paraaortic lymph node dissection. The final histopathology report indicated a 3.8x2x9 cm giant cell carcinoma variant of endometrial carcinoma and one positive external iliac lymph node metastasis. Administration of adjuvant carboplatin and paclitaxel chemotherapy was given. As far as we know, this is the fifteenth case reported in the English literature.
Keywords: Endometrial carcinoma, giant cell tumor, uterus

Öz

Endometrial karsinom kadınlarda en sık genital malignitedir. Endometrioid tip literatürdeki en sık varyanttır. Dev hücreli karsinom nadir ve az rapor edilen bir varyanttır. Yetmiş beş yaşında vajinal kanama ile başvuran bir hastayı sunduk. Transvajinal ultrasonda 26x28 mm hipodens lezyon mevcuttu adneksiel patoloji yoktu. Hastaya total abdominal histerektomi bilateral salpingooferektomi ve bilateral pelvik ve paraaortik lenfadenektomi yapıldı. Final patolojide 3.8x2x9 cm dev hücreli uterin karsinom saptandı ve bir pozitif external iliak lenf nodu metastazı saptandı. Karboplatin ve paklitaksel kemoterapi planı yapıldı. İngilizce literatürde şimdiye kadar tanımlanmış 15. olgu olduğunu düşünmekteyiz
Anahtar Kelimeler: Endometrial karsinom, dev hücreli tümör, uterus

Introduction

Abnormal vaginal bleeding is the most common cause of referrals to gynecology outpatient clinics[1]. In women age 40-50 years, the endometrial cancer (EC) incidence was 13.6-24 in 100,000 women, and 87.3 in 100,000 women in the 70-74 years age group[2]. EC is the 4th most common genital cancer in women, and endometrioid type accounts for 80% of all ECs[3]. Rare, and infrequently reported variants of EC include hepatoid carcinoma, glassy cell carcinoma, lymphoepithelioma-like carcinoma, adenocarcinoma with trophoblastic differentiation, and giant cell carcinoma (GCC) [3]. However, infrequent variants are under-reported in the English literature.

Nash and Stout[4] described GCC in 1958 to define an aggressive cancer of the lung. GCC is a recently defined variant of EC. It is a unique and rarely described entity with only 14 cases reported in the literature to date[5-9]. Consequently, even though this tumor appears to have aggressive behavior in particular cases, the prognosis of GCC remains uncertain. Herein, we aimed to present a rare case of uterine GCC in a 75-year-old female.

Case Report

A 75-year-old G5P5 patient who had been postmenopausal for 23 years was admitted with symptoms of vaginal bleeding. The patient additionally had type 2 DM and hypertension. A gynecologic examination revealed normal external genitalia, atrophic collum, intact adnexa, and free parametrium. Laboratory test results were as follows: CA125: 82 U/mL, CA19-9: 42 U/mL, and glycated hemoglobin (HbA1c): 11%.

Address for Correspondence/Yazışma Adresi: Hülya Ayık Aydın
Akdeniz University Faculty of Medicine, Department of Obstetrics and Gynecology, Division of Gynecologic Oncological Surgery, Antalya, Turkey
Phone: +90 507 708 02 45 **E-mail:** hulya_ayik@hotmail.com **ORCID ID:** orcid.org/0000-0002-3028-7247
Received/Geliş Tarihi: 09.08.2018 **Accepted/Kabul Tarihi:** 24.11.2018

Transvaginal ultrasound revealed linear endometrium, minimal intracavitary fluid, and a 26x28 mm hypodense lesion extending to the serosa with no adnexal pathology. Abdominal computed tomography revealed no pathology in the liver, spleen, kidney, small and large bowels, and ovarian loge. Endometrial cavity had a heterogeneous appearance, and no intra- and retro- peritoneal pathologic lymph node was detected (Figure 1).

Endometrial biopsy established the diagnosis of mixed EC [GCC (structural grade 3, and nuclear grade 3), and EC (structural grade 2, nuclear grade 2)]. Immunohistochemically, vimentin, and EMA produced widespread staining in the lesion (Figure 2). The histologic feature is bizarre multinucleated giant cells admixed with mononucleate tumor cells (Figures 3 and 4). Both tumors were stained with P53 focally, and ER dye stained areas of the EC. The tumor did not stain with P16, CEA, beta HCG and P63, desmin, MyoD1, CD10, caldesmon, and cyclinD1.

The results of cytokeratin staining were as follows: microscopic examination revealed large geographic tumor necrosis, multinuclear and mononuclear giant cells, and atypical mitosis. Therefore, endometrial neoplasms involving giant cells were considered and differential diagnosis included carcinoma, carcinosarcoma, leiomyosarcoma with osteoclast-like giant cells, undifferentiated sarcoma and choriocarcinoma with osteoclast-like giant cells. B-HCG was administered immunohistochemically and a negative reaction was observed. AE1/AE3 also showed a positive reaction in giant cells.

The patient underwent laparotomy, total abdominal hysterectomy, bilateral salpingo-oophorectomy, omental biopsy, and bilateral pelvic, and paraaortic lymph node dissection. The intraoperative frozen section result was reported as a tumor with a size of 3.8 cm, and more than half of the myometrium was invaded. Postoperative follow-up of the patient was uneventful, so she was discharged. The final histopathology report indicated a 3.8x2x9 cm GCC variant of EC and one positive external iliac lymph node metastasis. Cytology of intraabdominal specimens was unremarkable. Administration of adjuvant carboplatin and paclitaxel chemotherapy was planned upon the decision of the multidisciplinary council.

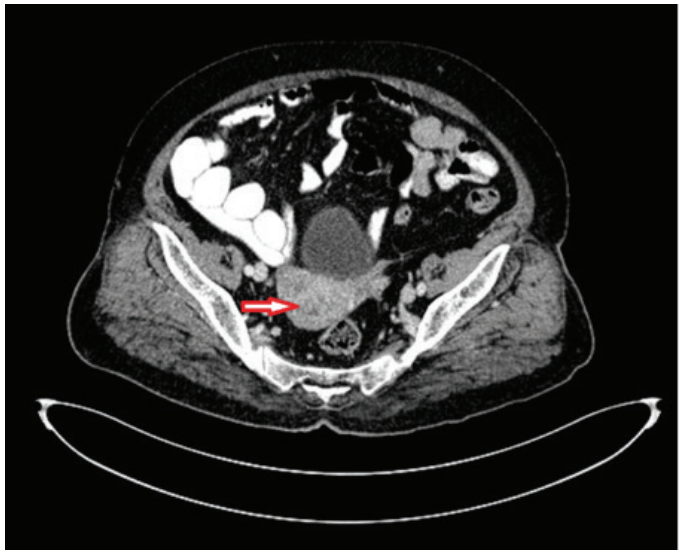


Figure 1. Abdominal computed tomography showing heterogeneous appearance in the endometrial cavity

Discussion

This is a unique case presenting a GCC of the endometrium. GCC is a rare and aggressive endometrial variant that was first described in 1991 by Jones et al.[5]. As far as we know, this is the 15th case reported in the English literature (Table 1).

Endometrial sampling should be performed on all women aged over 45 years who are suspected of having anovulatory uterine bleeding[6]. Postmenopausal bleeding is the most common symptom of EC, which is detected in 10-15% of cases[7]. Therefore, patients with postmenopausal bleeding

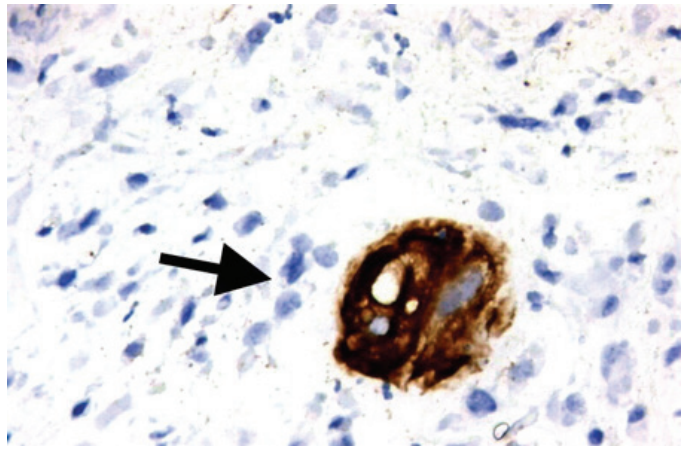


Figure 2. Immunohistochemistry PanCk positive staining of the tumor giant cells (x400)

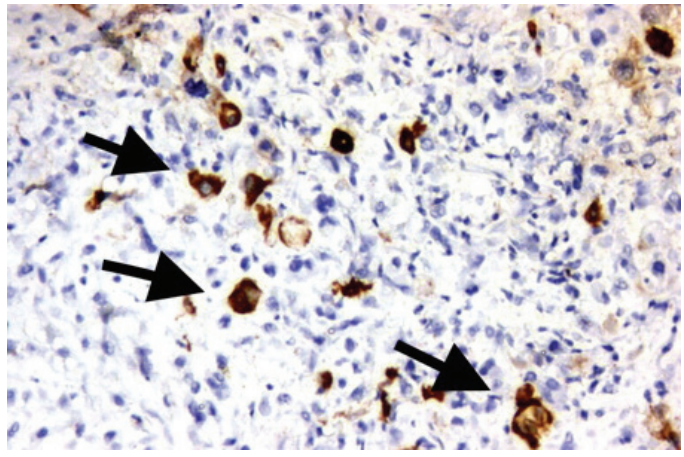


Figure 3. Immunohistochemistry PanCk positive staining of the tumor giant cells (x200)

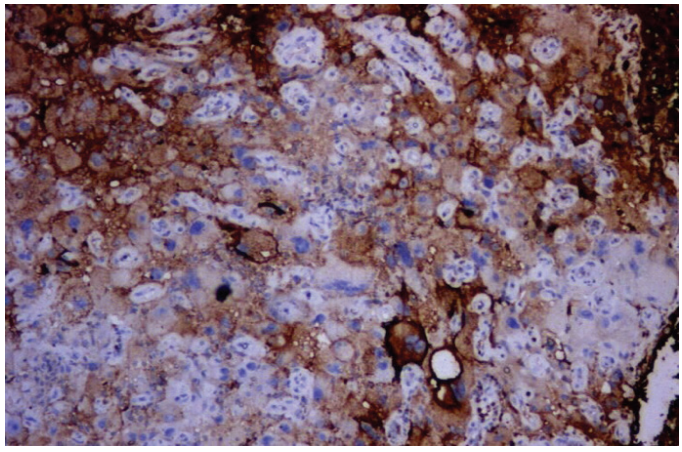


Figure 4. Immunohistochemistry vimentin positive staining of the tumor (x200)

should be evaluated in detail. The ages of all patients reported in the literature so far ranged from 43 to 85 years. Jones et al[5]. reported six patients with uterine GCC. All patients presented with vaginal bleeding. Occasional giant cells were positive for CK and EMA, whereas desmin and SMA were negative in all cases. Of the first six patients reported in the literature, four developed recurrence and three died in 3 years. Mulligan et al[8]. reported five patients, three of whom admitted with vaginal bleeding, one with anemia, and one with a pelvic mass. Of these five patients, one was disease-free after 14 years, three patients showed no symptoms or signs related to the disease during the 15 to 32-month follow-up period, and one patient had metastasis to the lung four years after diagnosis. Bhattacharyya et al. reported a 70-year-old postmenopausal patient with symptoms of vaginal bleeding[9]. Johannesen et al.[10] reported a 70-year-old woman with postmenopausal bleeding. Sharma et al.[11] presented a 60-year-old patient with vaginal bleeding. In the present case, a 75-year-old G5P5 postmenopausal patient admitted with symptoms of vaginal bleeding.

Endometrial carcinoma diagnosis should be verified by curettage and histopathologic examination of the tissue, as performed in the present case, also, when the final diagnosis can only be achieved in the surgical specimen[7]. Precise classification is mandated because the histologic type complemented by staging is crucial in the selection of treatment of choice[8,9]. The present case was categorized as International Federation of Gynecologists and Obstetricians stage 3C1. Accurate classification should be accomplished through histopathological examination, which is the gold standard.

The surgical treatment of EC is panhysterectomy with or without pelvic and paraaortic lymphadenectomy depending upon the grade of the tumor. Hormone therapy in EC is a well-established treatment modality for primary, metastatic, and recurrent cases. However, the role of hormone therapy in this rare and aggressive subtype of EC remains unstudied. To date, there are no data on the receptor (ER and PR) status of this tumor, positivity of which in our study could have a therapeutic implication.

Bhattacharyya et al.[9], Johannesen et al.[10], and Sharma et al.[11] all reported total hysterectomy and bilateral salpingo oophorectomy as the treatment of choice in their patients who were reported to have been followed up in the outpatient clinic with no complaints. The present patient underwent total abdominal hysterectomy, bilateral salpingo oophorectomy, and bilateral pelvic and para-aortic lymphadenectomy.

Conclusion

In conclusion, GCC is a rare, and infrequently reported variant of EC diagnosed through histopathologic examinations of resected specimens. Awareness of this subtype of EC is essential to avoid misclassification of these cases due to a wide variety of differential diagnoses and poor prognosis.

Ethics

Ethics Committee Approval: The study was approved by the Institutional Review Board (No:562/08.08.2018). The study was performed in accordance with the ethical standards described in an appropriate version of the 1975 Declaration of Helsinki, as revised in 2000.

Informed Consent: The patient's consent was obtained.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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

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

Uterine giant cell carcinoma: a case report and review of the literature

We present a 75-year-old patient was diagnosed as having giant cell uterine carcinoma.

The patient's consent was obtained. The study was approved by the Institutional Review Board (no:562 / date: 08.08.2018). The study was performed in accordance with the ethical standards described in an appropriate version of the 1975 Declaration of Helsinki, as revised in 2000.

References

1. Coulter A, Noone A, Goldacre M. General practitioners' referrals to specialist outpatient clinics. I. Why general practitioners refer patients to specialist outpatient clinics. BMJ 1989;299:304-306.
2. Brenner PF. Differential diagnosis of abnormal uterine bleeding. Am J Obstet Gynecol 1996;175:766-9.
3. Rutgers JK. Update on pathology, staging and molecular pathology of endometrial (uterine corpus) adenocarcinoma. Future Oncol 2015;11:3207-18.
4. Nash AD, Stout AP. Giant cell carcinoma of the lung; report of 5 cases. Cancer 1958;11:369-376.

5. Jones MA, Young RH, Scully RE. Endometrial adenocarcinoma with a component of giant cell carcinoma. Int J Gynecol Pathol 1991;10:260-70.
6. ACOG Committee on Practice Bulletins-Gynecology. Practice bulletin no. 136: management of abnormal uterine bleeding associated with ovulatory dysfunction. Obstet Gynecol 2013;122:176-85.
7. Epstein E, Skoog L, Valentin L. Comparison of Endorette and dilatation and curettage for sampling of the endometrium in women with postmenopausal bleeding. Acta Obstet Gynecol Scand 2001;80:959-64.
8. Mulligan AM, Plotkin A, Rouzbahman M, Soslow RA, Gilks CB, Clarke BA. Endometrial giant cell carcinoma: A case series and review of the spectrum of endometrial neoplasms containing giant cells. Am J Surg Pathol 2010;34:1132-38.
9. Bhattacharyya A, Gon S, Bandyopadhyay G, Majumdar B, Gayen P. Giant cell carcinoma of endometrium: A rare clinical entity. Iran J Pathol 2012;7:197-202.
10. Johannesen E, Nguyen V. Malignant mixed mullerian tumor with an endometrioid adenocarcinoma with a component of giant cell carcinoma: a case report and literature review. J Clin Exp Pathol 2015;5:212.
11. Sharma P, Ahluwalia C, Kolte S, Mandal AK. Giant cell carcinoma of endometrium: A rare case report and review of literature. Clin Cancer Investig J 2016;5:79-82.

Case Report / Olgu Sunumu

DOI: 10.4274/tjod.galenos.2018.66743

Turk J Obstet Gynecol 2019;16:80-3

Palliative pelvic exenteration using iliofemoral bypass with synthetic grafts for advanced cervical carcinoma

İleri evre serviks kanseri için yapılan palyatif pelvik egzenterasyonda sentetik greftli ilyofemoral bypass kullanılması

Burak Tatar[1], Yakup Yalçın[2], Evrim Erdemoğlu[3]

[1]*University of Health Sciences, Samsun Training and Research Hospital, Clinic of Gynecologic Oncology, Samsun, Turkey*

[2]*Isparta State Hospital, Clinic of Gynecologic Oncology, Isparta, Turkey*

[3]*Suleyman Demirel University Faculty of Medicine, Department of Gynecologic Oncology, Isparta, Turkey*

Abstract

Objective: Recurrent cervical cancer can cause severe morbidity. Despite the severe morbidity after surgery, pelvic exenteration is still used today for mainly curative intent. This intention is neither based on randomized controlled trials (RCTs) nor high quality non-RCTs with adequate patient numbers comparing medical management with surgery. The same is true for exenteration for palliative intent, so the patient selection for either curative or palliative intent must be considered on a patient-by-patient basis.

Materials and Methods: A 35-year-old patient who had undergone primary chemo-radiotherapy for advanced cervical cancer presented with intractable pain on the swollen left leg and pelvis 8 months later. Left lower extremity Doppler ultrasound revealed echogenic thrombus in the external iliac, femoral, and popliteal veins, consistent with acute deep vein thrombus. She underwent total exenteration, end colostomy, ileal urinary conduit, pelvic lymphadenectomy, paraortic lymph node sampling, and ilio-femoral arterial and venous bypass.

Results: The procedure relieved her pain, the leg diameter dramatically decreased from 75 cm to 44 cm, and circulation of the leg was reestablished. The procedure deferred leg amputation for about five months.

Conclusion: To the best of our knowledge, this is the first report of a palliative pelvic exenteration for cervical cancer with combined iliofemoral arterial and venous bypasses. These procedures, with high morbidity and mortality, are also more controversial when undertaken for just palliation of symptoms. They must be considered in the basis of each patient, and the benefits and risks must be discussed thoroughly in a realistic perspective with the patient.

Keywords: Cervical cancer, iliofemoral bypass, palliation, pelvic exenteration

Öz

Amaç: Nüks serviks kanseri ciddi morbiditeye neden olabilir. Cerrahi sonrası yüksek morbiditeye rağmen, pelvik egzenterasyon günümüzde temel olarak küratif amaçlı kullanılır. Bu yaklaşım, medikal tedavi ve cerrahiyi karşılaştıran randomize kontrollü çalışmalar (RCT) ya da yüksek kaliteli yeterli sayıda hastayı içeren RCT dışı çalışma temelli değildir. Aynı durum palyasyon amaçlı yapılan egzenterasyon için geçerlidir. Bu nedenle palyatif ya da küratif amaçlı yapılan egzenterasyonun hasta seçimi hasta bazlı olmalıdır.

Gereç ve Yöntemler: İleri evre serviks kanseri için daha önce primer kemo-radyoterapi almış olan 35 yaşındaki hasta, tedaviden 8 ay sonra sol bacakta şişlik ve anti enflamatuvar ilaçlarla dinmeyen ağrı şikayeti ile başvurdu. Sol alt ekstremite Doppler incelemesinde akut derin ven trombozu ile uyumlu; eksternal ilyak, femoral ve popliteal venlerde ekojenik trombüs saptandı. Hastaya total pelvik egzenterasyon, uç kolostomi, ileal üriner konduit, pelvik-paraaortik lenfadenektomi ve ilio-femoral arteriyel ve venöz bypass yapıldı.

Bulgular: Operasyon hastanın ağrısını dindirdi, bacak çapı 75 cm'den 44 cm'ye dramatik olarak geriledi ve bacaktaki dolaşım yeniden sağlandı. Bu operasyon, bacak ampütasyonunu 5 ay öteledi.

Sonuç: Bildiğimiz kadarı ile bu olgu sunumu, kombine ilio-femoral arteryel ve venöz bypass içeren ilk palyatif egzenterasyon sunumudur. Bu tip bir cerrahi işlemin yüksek morbidite ve mortalitesi vardır. Özellikle sadece palyasyon amaçlı kullanımı tartışmalıdır ve hasta bazlı tartışılmalıdır. İşlemin risk ve faydaları gerçekçi bir perspektifte hasta ile paylaşılmalıdır.

Anahtar Kelimeler: Serviks kanseri, iliofemoral bypass, palyasyon, pelvik egzenterasyon

PRECIS: Report of a pelvic exenteration for cervical cancer with combined ilio-femoral arterial and venous bypasses.

Address for Correspondence/Yazışma Adresi: Burak Tatar
University of Health Sciences, Samsun Training and Research Hospital, Clinic of Gynecologic Oncology, Samsun, Turkey
Phone: +90 533 347 95 71 **E-mail:** buraktatar@yahoo.com **ORCID ID:** orcid.org/0000-0002-6495-0174
Received/Geliş Tarihi: 22.09.2018 **Accepted/Kabul Tarihi:** 24.11.2018

Introduction

It is estimated that there are 527.600 new cervical cancer cases every year, nearly 265.700 deaths attributable to this malignancy, and most of the cases are seen in developing countries[1]. Most patients present at advanced stages and more than half of all patients with cervical cancer receive radiotherapy during the course of their treatment. Nearly one-third of patients who receive radiotherapy at any stage (stage I to stage IV) will have local or distant failure[2]. Recurrence after radiotherapy is maybe one of the most challenging situations in gynecologic oncology for patients with cervical cancer.

Recurrent or advanced cervical cancer can cause severe morbidity including intractable pain, continuous foul-smelling discharge, fecal and urinary incontinence due to fistula formation, vaginal bleeding, intestinal or ureteric obstruction related symptoms, and sepsis. In 1948, Brunshwig[3] defined pelvic exenteration as, "a one-stage abdominoperineal operation with end colostomy and bilateral ureteral implantation into the colon above the colostomy," to alleviate these symptoms for 22 patients. The perioperative mortality rate was 23%. Despite it having palliative intent when it was first defined, with the improvements in the surgical technique, especially with the use of modern urinary conduit technics, it has rather become a surgery for curative intent with much lower mortality rates[4,5].

Case Report

A 35-year-old patient previously underwent primary chemo-radiotherapy for a bulky [magnetic resonance imaging (MRI) revealed a mass of 70x65x35 mm] non-keratinizing squamous cell cervical carcinoma with invasion to the proximal one-third of the vagina and parametria, and a 50x30 mm lymph node chain, probably metastatic, on the left iliac chain according to MRI. She presented with intractable pain in the left leg and pelvis 8 months later.

Her left leg was 75 cm in diameter, whereas its right counterpart was 40 cm at its maximum (Figure 1). Left lower extremity Doppler ultrasound revealed echogenic thrombus in the external iliac, femoral, and popliteal veins, consistent with acute deep vein thrombus.

She was discussed in an oncology round, consulted by the cardiovascular surgery department, and the risks of the operation were discussed thoroughly, explaining no possible survival benefit, extreme risk of morbidity and mortality, and that the procedure would performed only for the possible alleviation of symptoms. She was fully cooperating and demanded the surgical intervention. The surgery was undertaken as total exenteration, end colostomy, ileal urinary conduit, pelvic lymphadenectomy, paraaortic lymph node sampling, and iliofemoral arterial and iliofemoral venous bypass. Intraoperatively, the tumor was visualized infiltrating the left external and internal iliac artery, left external and internal iliac vein, recto-sigmoid, bladder, and left ureter. The left ureter was seen as hydropic. In order to remove the tumor, the external and internal iliac artery, vein, and ureter were cut (Figure 2). With the help of an inguinal incision, the femoral artery and vein were identified, dissected, and cut. The backflow from the common femoral artery was confirmed and ilio-femoral arterial bypass was completed using an 8 mm ringed polytetrafluoroethylene (PTFE) graft. The thrombi in the femoral vein were cleared, and after the blood flow from the common femoral vein was confirmed, the femoro-iliac venous bypass was completed using a 10-mm PTFE graft (Figure 3). The flow from the distal part of the artery and back from the vein was confirmed. The right internal iliac artery was ligated and cut. The left presacral area was dissected and the sciatic nerve was preserved. The tumor was removed by stripping the pubis and ilium. After completing the exenteration and conduit, a prolene mesh was used to reconstruct the cut inguinal ligament.

The patient was discharged from hospital three weeks after the procedure, as her leg diameter dramatically decreased from 75 cm to 44 cm. The circulation of the leg was re-established

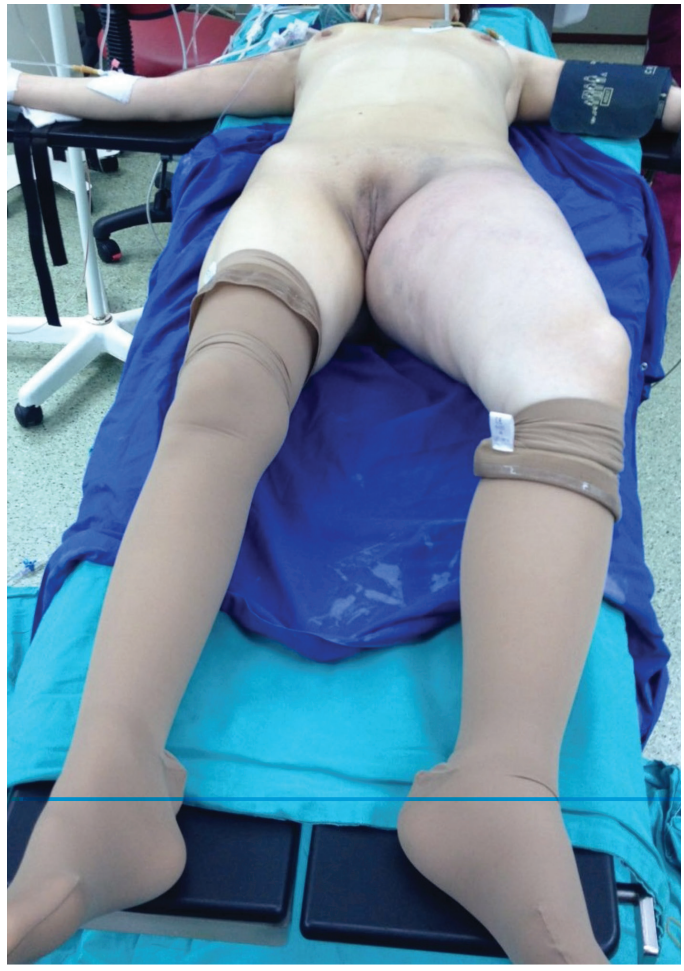


Figure 1. The image of the patient just before the procedure. Swollen left lower extremity is clearly seen

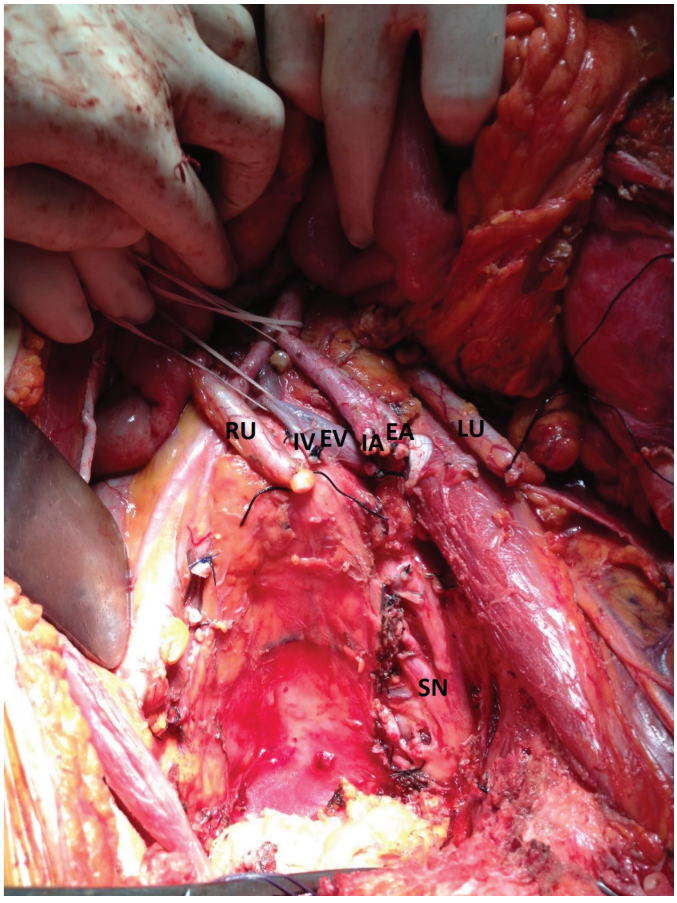


Figure 2. Before the reconstruction phase; operative field after total pelvic exenteration and left external iliac vessels ligated

RU: Right ureter, IV: Internal iliac vein, EV: External iliac vein, IA: Internal iliac artery, EA: External iliac artery, LU: Left ureter, SN: Sacral nerve roots

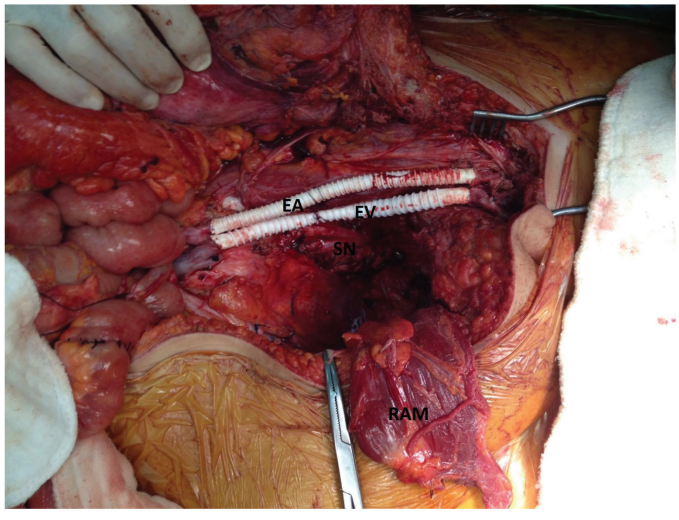


Figure 3. Operative field after iliofemoral arterial bypass and femoro-iliac venous bypass with polytetrafluoroethylene grafts

EA: External iliac artery, EV: External iliac vein, SN: Sacral nerve roots, RAM: Rectus abdominis muscle before flap reconstruction

and amputation was delayed until five months later when she was admitted to hospital for ischemic changes in her left foot and pain. The patient died of sepsis, approximately eight months after the palliative surgery.

Discussion

After the initial description of pelvic exenteration by Brunshwig in 1948, there has been much debate about the surgery despite the refinement of the technique, especially for urinary conduits. The debate is about its indications, patient selection criteria, the technique, its aim, and its necessity.

According to some articles after the 2000's, survival after palliative pelvic exenteration is between 10.5% for 2 years to 27% for 5 years, whereas the reports from same authors indicate a 5-year survival between 50% and 60% if the exenteration is performed with curative intent[4,6].

There is a more recent palliative pelvic exenteration series of 13 patients from Brazil, 9 of which were performed for recurrent cervical cancer. The 2-year overall survival was 15.4% and only 6 of 13 patients survived more than 5 months[7].There is also a controversy about the definition of palliative pelvic exenteration. An early publication of Deckers et al. [8] defined pelvic exenteration as an efficient way to alleviate symptoms such as pain, fistulas, pelvic sepsis, hemorrhage, and malodorous discharge. Nevertheless, there is controversy about the definition of palliative pelvic exenteration. From the above-mentioned authors, Marnitz et al. [4] explained that the difference between palliative and curative exenterations could be discriminated by the resection margin status. Finlayson and Eisenberg emphasized three definitions of palliative exenteration in their review[9]. First, based on the intent that the objective is just for palliation of symptoms. Second, for patients who undergo surgery for curative intent but intraoperatively macroscopic tumor is left behind because of the non-resectability of the tumor. The third definition they found in the literature is that all surgical effort for failed primary curative effort including radiation, surgery or chemotherapy, which may be combined with each other. In the review of Hope and Pothuri[10], they mentioned that palliative exenteration surgery was accomplished to alleviate discomfort and not necessarily in an attempt to prolong life. They also stressed that the literature for palliative pelvic exenteration was not homogenous in the tumor, patient, and surgical intervention basis, making it difficult to compare. This problem was also documented in a recent Cochrane review for all exenteration procedures[11].

In our case, because the tumor was in close proximity of the sacral plexus, the treatment was planned with palliative intent, not curative. Intraoperatively, the tumor was stripped from the nerve plexus. This region is the boundary between curative intent of laterally extended endopelvic resection as described by Höckel[12] and palliative surgery.

There is a controversy over all kinds of pelvic exenterations for gynecologic malignancies; their indications are not clear, the surgical procedures are not uniform, and most importantly, their efficacy over non-surgical treatments are not proven. Chemotherapy may be an alternative for palliation of symptoms to surgery, but there are no randomized controlled trials comparing one with the other in the literature. Full recovery, if possible, from a palliative exenteration may take about 4-5 months, survival may not be much more and quality of life during this period is poor.

There is a case series from Memorial Sloan Kettering Cancer Center; 11 patients with recurrent uterine cancer and 3 with recurrent cervical cancer underwent pelvic exenteration for curative intent. Two of the patients had femoral-femoral arterial bypass procedures. The specific survival and prognosis of these two patients is not mentioned[13].

A recent report from Romania described palliative posterior pelvic exenteration with partial cystectomy for a tumor invading the sciatic foramen for fistula after a previous radical hysterectomy[14]. No detail was included regarding the prognosis or survival of the patient in the article.

To the best of our knowledge, this is the first report of a palliative pelvic exenteration for cervical cancer with combined iliofemoral arterial and venous bypasses.

Under these circumstances, such procedures with high morbidity and mortality are also more controversial when undertaken simply for palliation of symptoms. They must be considered on a patient-by-patient basis, and the benefits and risks must be discussed thoroughly in a realistic perspective, taking into account the physical and emotional aspects of the patient before planning the procedure.

Ethics

Informed Consent: It was obtained.

Peer-review: External and internal peer-reviewed.

Authorship Contributions

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

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. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA: a cancer journal for clinicians 2015;65:87-108.
2. Hong JH, Tsai CS, Lai CH, Chang TC, Wang CC, Chou HH, et al. Recurrent squamous cell carcinoma of cervix after definitive radiotherapy. International journal of radiation oncology, biology, physics 2004;60:249-57.
3. Brunschwig A. Complete excision of pelvic viscera for advanced carcinoma; a one-stage abdominoperineal operation with end colostomy and bilateral ureteral implantation into the colon above the colostomy. Cancer 1948;1:177-83.
4. Marnitz S, Kohler C, Muller M, Behrens K, Hasenbein K, Schneider A. Indications for primary and secondary exenterations in patients with cervical cancer. Gynecologic oncology 2006;103:1023-30.
5. Berek JS, Howe C, Lagasse LD, Hacker NF. Pelvic exenteration for recurrent gynecologic malignancy: survival and morbidity analysis of the 45-year experience at UCLA. Gynecologic oncology 2005;99:153-9.
6. Lambrou NC, Pearson JM, Averette HE. Pelvic exenteration of gynecologic malignancy: indications, and technical and reconstructive considerations. Surgical oncology clinics of North America 2005;14:289-300.
7. Guimaraes GC, Baiocchi G, Ferreira FO, Kumagai LY, Fallopa CC, Aguiar S, et al. Palliative pelvic exenteration for patients with gynecological malignancies. Archives of gynecology and obstetrics 2011;283:1107-12.
8. Deckers PJ, Olsson C, Williams LA, Mozden PJ. Pelvic exenteration as palliation of malignant disease. American journal of surgery 1976;131:509-15.
9. Finlayson CA, Eisenberg BL. Palliative pelvic exenteration: patient selection and results. Oncology (Williston Park) 1996;10:479-84; discussion 84-6, 90, 93.
10. Hope JM, Pothuri B. The role of palliative surgery in gynecologic cancer cases. The oncologist 2013;18:73-9.
11. Ang C, Bryant A, Barton DP, Pomel C, Naik R. Exenterative surgery for recurrent gynecological malignancies. The Cochrane database of systematic reviews 2014;2:CD010449.
12. Hockel M. Laterally extended endopelvic resection. Novel surgical treatment of locally recurrent cervical carcinoma involving the pelvic side wall. Gynecologic oncology 2003;91:369-77.
13. Caceres A, Mourton SM, Bochner BH, Gerst SR, Liu L, Alektiar KM, et al. Extended pelvic resections for recurrent uterine and cervical cancer: out-of-the-box surgery. International journal of gynecological cancer : official journal of the International Gynecological Cancer Society 2008;18:1139-44.
14. Bacalbasa N, Balescu I. Palliative Pelvic Exenteration for Pelvic Recurrence Invading the Sciatic Foramen with Chronic Cutaneous Perineal Fistula after Radical Surgery for Cervical Cancer: A Case Report. Anticancer research 2015;35:4877-80.