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galenos yayınevi

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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.

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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.

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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.

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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/)

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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.

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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.

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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:

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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

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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.

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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.

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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.

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LETTER FROM THE PRESIDENT

Dear Colleagues,

The COVID-19 we familiarized within 2020 is still continues after welcoming 2021. The best news for all of us is with the great effort of scientists, vaccine is discovered and vaccination started in our country. Our hope is that the pandemic will come to an end with this promising endeavor.

During this difficult times, we, as physicians, were very tired and suffered many irreplaceable losses. In loving memory of our Board Member Faik Mümtaz Koyuncu, I wish my condolences to all doctors, nurses and healthcare professionals who lost their lives because of COVID-19 pandemic.

As always, with all healthcare workers, we will continue to be at the forefront of the fight against COVID-19 pandemic in 2021. My deepest gratitude to all the healthcare workers who are risking their lives to save others. We believe our community will turn to their daily life as soon as possible with the help of our hard-work, commitment and dedication.

Our biggest wish for this year is to end this pandemic and restart our face-to-face scientific activities for women's health with enthusiasm as before. I hope 2021 is still holding promise for everyone as healthy, safe, and productive year to come. I thank all the authors of articles published in 2020. They brought us interesting, useful and high-quality information that we can use in clinical practice. We are planning to continue to provide you with a broad spectrum of outstanding articles in our journal this year as well.

Ateş Karateke, Prof. MD
President of TJOD

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EDITORIAL

Dear Colleques,

Covid pandemic influenced every aspect of scientific publishing. The number of outpatient admissions decreased, all efforts and funds are focused on Covid patients in many areas. Despite these changes article submissions to our journal "Turkish Journal of Obstetrics and Gynecology" increased by 25%. Due to the limited number of pages we can publish our rejection rate became 68%.

The editorial board of our journal has reshuffled. After an Editorial Board meeting eight years ago, we decided to nominate experts of H index 10 or more to become Editorial board members after they served as reviewer for at least one year for the journal. Among the Editorial Board members we nominate those with H index 15 or more to be section Editors and those with h index 20 or more to be editors. If Editors have fewer time to spent for the journal they exchange with equally qualified Editorial Board members. I hope this healthy scientific competition lasts for many years.

Here are some figures about our journal and home page of the journal. Monthly web page visits are about 45 thousand. Monthly article download is more than 25 thousand. Top rated articles were cited 9 times in the preceding two years. Citations were from more than 10 different countries. The citations per year has increased 20 fold in the last four years and exceeded 260 per year. Our calculated unofficial Impact Factor became 1.1.

In 2021 an Education series will be recorded at the time of masterclasses endorsed by Turkish society of Obstetrics and Gynecology. The target population will be residents and specialists in the area of Obstetrics and Gynecology.

I would like to thank to all the contributors in this success story.

Best regards,

Eray Çalışkan
Editor in Chief

Clinical Investigation / Araştırma

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Turk J Obstet Gynecol 2021;18:1-6

Searching for radiologic and histologic evidence on live vaginal tissue: Does the G-spot exist?

Canlı vajinal dokuda radyolojik ve histolojik kanıt için araştırma: G-noktası var mı?

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Abstract

Objective: There is a growing debate on the existence of the G-spot. G-spot amplification by various surgical interventions has become mainstream for esthetic vaginal surgery despite a lack of conclusive proof of the G-spot. The aim of this study was to search for histologic evidence in regions of so-called hyperintense focus (HF) (considered as the G-spot) using magnetic resonance imaging (MRI) mapping and biopsied tissues.

Materials and Methods: Fifteen patients who had grade 2 or higher anterior compartment defects were enrolled in the study. All patients were subjected to MRI. When a HF was seen, its localization, dimensions, and distances to adjacent structures were measured in images. Dissections in the anterior vaginal wall were performed in accordance with the measurements derived from MRI and tissue measuring 0.5x0.5 cm was biopsied from the determined HF.

Results: An HF was determined in MRI of three (20%) patients. However, no significant neurovascular tissue density was observed histologically in any of the biopsy specimens obtained from the surgical dissections under the guidance of MRI mapping.

Conclusion: Our findings denote that there is no G-spot in the anterior vaginal wall.

Keywords: G-spot, hyperintense focus, MRI, neurovascular tissue

Öz

Amaç: G-noktasının varlığı konusunda büyüyen bir tartışma vardır. Öte yandan, G-noktasının kesin kanıtı olmamasına rağmen, çeşitli cerrahi müdahalelerle G-noktası amplifikasyonları estetik vajinal cerrahide ana akım haline gelmiştir. Bu çalışmanın amacı, hiperintens odak (HF) (G-noktası olarak kabul edilmiştir) denilen bölgelerde manyetik rezonans görüntüleme (MRG) ile haritalama ve biyopsi aracılığıyla histolojik kanıtları araştırmaktır.

Gereç ve Yöntemler: Grade 2 veya daha yüksek ön kompartman defekti olan on beş hasta çalışmaya alındı. Tüm hastalar MRG'ye tabi tutuldu. HF görüldüğünde; lokalizasyonu, boyutları, komşu yapılara olan mesafeleri görüntülerde ölçüldü ("vajinanın ön duvarının haritalanması"). Ön vajinal duvardaki diseksiyonlar MRG'den elde edilen ölçümlere uygun olarak gerçekleştirildi ve HF denilen dokudan 0,5x0,5 cm boyutlarında doku biyopsisi yapıldı.

Bulgular: Üç hastada (%20) HF belirlendi. Ancak MRG haritalaması kılavuzluğunda cerrahi diseksiyonlardan elde edilen biyopsi örneklerinin hiçbirinde histolojik olarak önemli bir nörovasküler doku yoğunluğu gözlenmedi.

Sonuç: Bulgularımız vajen ön duvarda G-noktasının bulunmadığını göstermektedir.

Anahtar Kelimeler: G-noktası, hiperintens odak, MRG, nörovasküler doku

PRECIS: The G-spot does not exist.

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Introduction

The existence of the G-spot is a debatable issue in sexual medicine. Despite a lack of definitive evidence for its existence, use of the term "G-spot" has become widely accepted both in the lay media and scientific research. Moreover, although the G-spot has not been definitely shown, G-spot amplification by various surgical interventions has become mainstream for esthetic vaginal surgery.

In their observational magnetic resonance imaging (MRI) study, Maratos et al.[1] claimed that the G-spot had been visualized as a hyperintense focus (HF). Hence, the main aim of this study was to shed light on this controversial issue using MRI mapping (MRIM) and to search for histologic evidence in tissues biopsied from the projection of HF.

Materials and Methods

The study is a prospective observational study. The ethics committee of the university approved the study (decision date and number: June 18th, 2020-06/V). Fifteen patients who had anterior vaginal compartment defects (Ba point ≥2 according to POP-Q) and were willing to undergo surgery between July 1st, 2020, and October 1st , 2020, were enrolled in the study. All patients were asked if they had any knowledge concerning the G-spot and whether they believed in its existence. All surfaces of the anterior vaginal wall were tactilely stimulated by starting at the urethrovesical junction and staying within the boundaries of the lateral fornix towards the anterior fornix, by making a beckoning gesture with the right-hand forefinger while wearing a sterile glove during a gynecologic examination in the lithotomy position. The patients were asked whether they had any increased sensitivity in any area during this examination.

Patients with the following were not included in the study: previous vaginal surgery, presence of concomitant apical prolapse and or paravaginal defect, history of estrogen and/or antidepressant use, postmenopausal status, a known malignancy and the patients whose coital frequency is <1/week. Informed consent regarding the MRI and surgery (biopsy + anterior compartment surgery) was given by the enrolled patients.

All patients were subjected to MRI with a 5-mm slice thickness. When a HF (putative G-spot) was seen, its localization, dimensions, distance to the hymenal ring (vaginal introitus), to the external urethral meatus and the depth from the vaginal lumen were measured on images so that the localization of the "putative G-spot" seen in the MRI was precisely determined and this procedure was named as "mapping of the anterior wall of the vagina." Subsequently, each patient was enlisted for anterior compartment defect surgery.

Before the main surgery, the HF was projected on the anterior vaginal wall in accordance with the measurements derived from MRI (under the strict guidance of the mapping of the anterior wall of the vagina) and a spot was marked with a sterile pen. A tissue measuring 0.5x0.5 cm was biopsied from this region (surgical pictures, Picture 1-3). This is a novel idea and we called it MRIM.

Statistical Analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences software, version 23 (SPSS, Inc., Chicago, IL). The data are expressed as the mean and range for continuous variables, and binary variables are reported as numbers and percentages.

Radiological Technique and Evaluation

All patients underwent pelvic MRI in the supine position using a 3T MR (Siemens Magnetom Skyra, Erlangen, Germany) before surgery. T1-weighted (W) images were obtained in axial and sagittal planes. T2-W images were acquired in axial, sagittal and transverse planes. The slice thickness of the sequences was 5 mm.

T2-W axial and sagittal images were scrutinized for the detection of a HF. If there was an HF in the images (Radiologic images, Image 5), it was recorded as a "putative G-spot" as described in the study of Maratos et al.[1]. The location of the HF (right or left side of the vagina), the distance between HF and vaginal introitus, the distance between the HF and the external urethral meatus, and the depth of HF's location with respect to the vaginal lumen were measured in appropriate planes (Radiologic images, Image 1, 2). In addition, the antero-posterior diameter and area of the HF were measured in axial T2-W planes (Radiologic images, Image 3, 4). The lower abdominal MRIs were interpreted by the same radiologist.

Anatomic Dissection

Patients underwent surgery in the lithotomy position. The surgical field was cleaned with 4% chlorhexidine and draped. The projection of HF was marked on the vaginal wall using a sterile pen according to the MRI mapping (Surgical pictures, Picture 1, 2). The vaginal mapping taken in the supine position sufficiently corresponded to the biopsies performed in the lithotomy position. Afterwards, surgical dissection was started. A full-thickness linear incision was performed starting from the urethrovesical junction and extending to the cervico-vesical junction. The bladder was dissected off the pubocervicovaginal fascia. A biopsy of 0.5x0.5 cm was taken from the projection spot of the region marked at the beginning of the procedure and was placed into 10% formaldehyde solution and sent to the pathology laboratory (Surgical pictures, Picture 3). Biopsies were taken from the pubocervicovaginal fascia (e.g. vaginal adventitia) underneath the bladder. The marking, biopsy, and anterior compartment surgery were performed by the same surgeon.

Histologic Evaluation

The slides were ready for evaluation after routine automated tissue processing, paraffin embedding, and hematoxylin&eosin (H&E) staining. In addition, for detailed microscopic evaluation, immunohistochemistry was performed on biopsied tissues. S100 and CD34 immunostaining were used to identify neuronal and vascular structures, respectively. Three-four-mm-thick

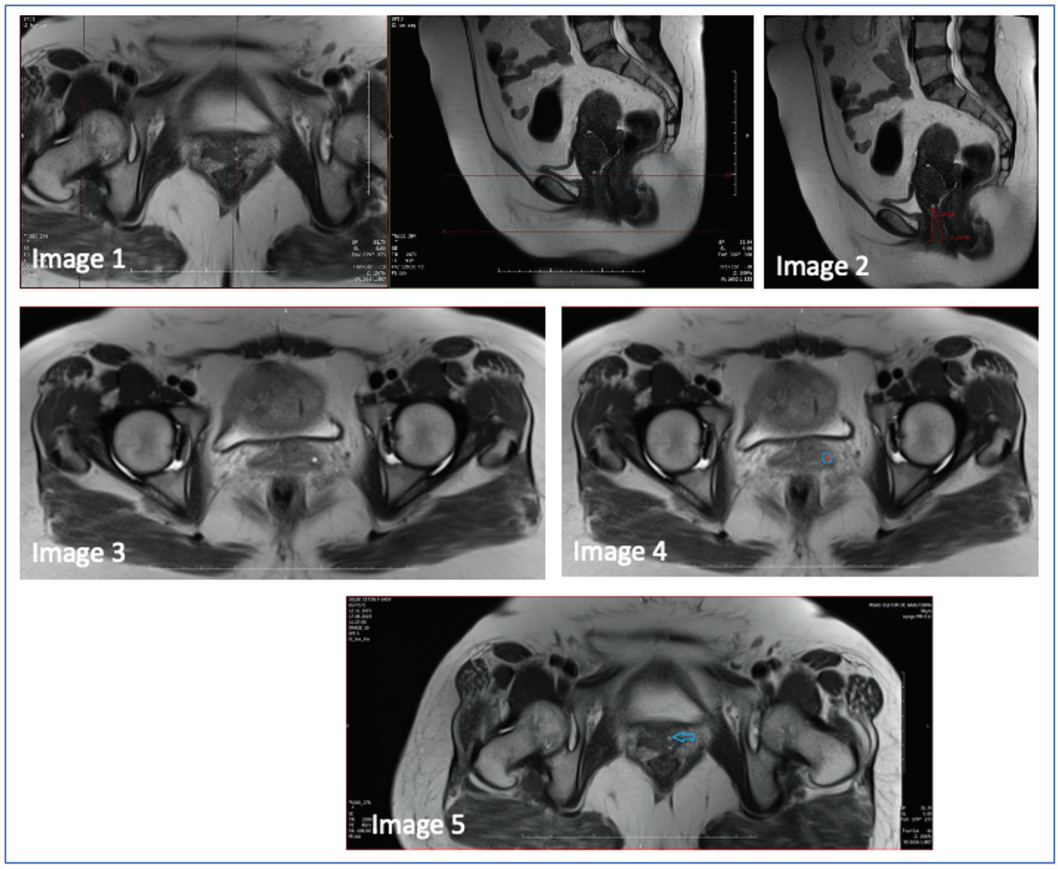


Image 1. Localization of hyperintense focus on axial and sagittal T2-weighted images of case number 13

Image 2. On sagittal T2-weighted image hyperintense focus and the distance between urethra-focus (first red line) and introitus-focus (second red line) of case 13

Image 3. Hyperintense focus on axial T2-weighted images marked with asterix the of case number 4

Image 4. Anteroposterior distance (red line) and area (blue line) of hyperintense focus on axial T2-weighted images of case number 4

Image 5. Hyperintense focus on axial T2-weighted image shown with blue arrow

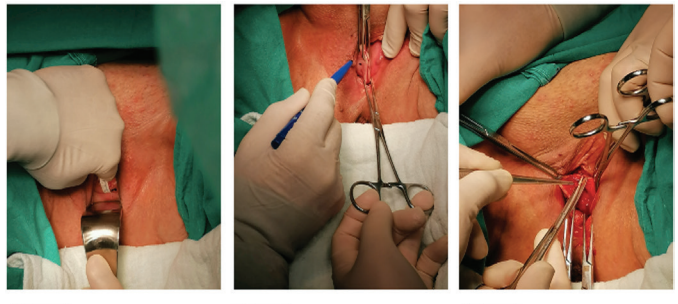
sections were cut from the paraffin blocks and immunostaining was performed automatically using a Leica Bond-Max with anti-S100 and anti-CD34 antibodies (Leica). The H&E and immunostained slides were examined for the presence and intensity of neural and vascular structures under a Nikon Ni-U light microscope (Histological images 1-6). The presence of neural bundles was verified using S100 immunohistochemistry and S100-stained neural structures were counted under the light microscope. The total count of neural structures were divided by the total microscopic area to calculate the number of neural bundles per mm^2.

The biopsy specimens were evaluated by the same pathologist.

Results

A total number of 15 patients were included in the study. The demographic data of the patients are given in Table 1. The mean age of the patients was 45±5.12 years.

Eleven of the 15 patients (73%) knew of the G-spot, and 4/15 (27%) did not. Interestingly, these 4 patients had heard about the G-spot, but they had no clear idea regarding its existence. On the other hand, only 1 patient (0.06%) answered positively when asked whether she had increased sensitivity during the



Picture 1, 2. The hyperintense focus marked on the vaginal wall using MRI mapping and a full-thickness linear incision from the urethrovesical junction and extending the cervico-vesical junction

Picture 3. Taken of a biopsy of 0.5x0.5 cm from the region marked by MRIM

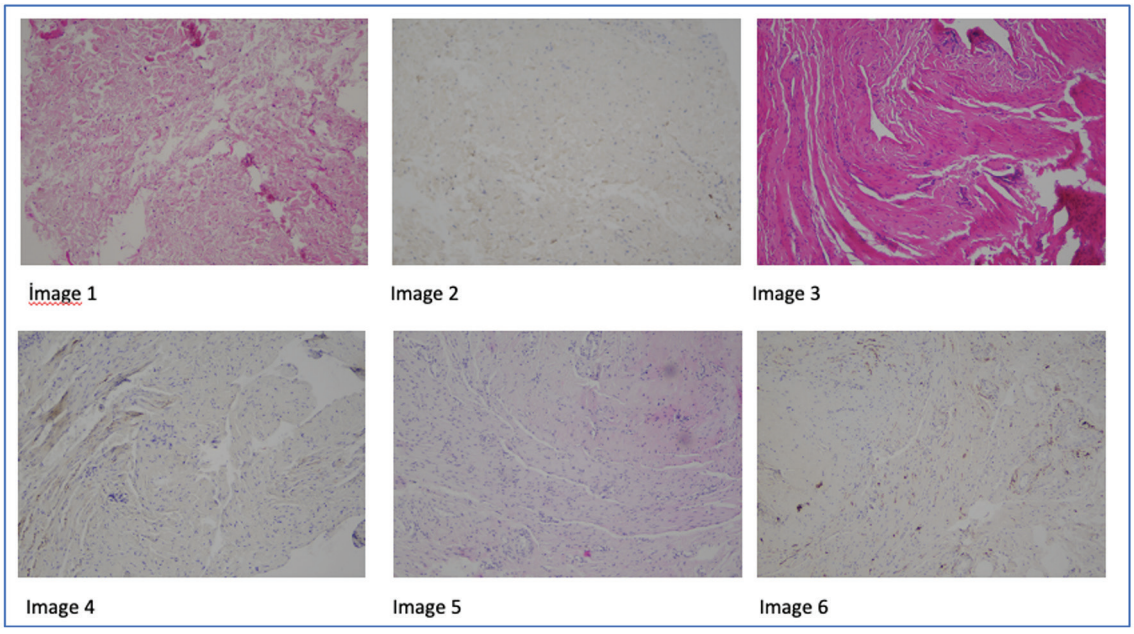


Image 1. Vascular lumina between the fascial bundles without neural structures, H&E x10, case number 4
Image 2. S100 (+) neural plexus between fascial bundles, DAB, x10, case number 4
Image 3. Vascular lumina in between fascial bundles without neural structures, H&E x10, case number 10
Image 4. No S100 (+) neural structures seen between fascial bundles DAB, x10, case number 10
Image 5. Vascular lumina between fascial bundles without neural structures, H&E x10, case number 13
Image 6. No S100 (+) neural structures between fascial bundles, DAB, x10, case 13
H&E: Hematoxylin&eosin

Table 1. Demographic features of the patients

	Age (years)	Gravida	Hyperintense focus
Case 1	42	2	-
Case 2	35	2	-
Case 3	39	3	-
Case 4	40	1	+
Case 5	45	2	-
Case 6	46	2	-
Case 7	49	3	-
Case 8	51	3	-
Case 9	44	1	-
Case 10	50	1	+
Case 11	43	2	-
Case 12	40	2	-
Case 13	52	2	+
Case 14	50	3	-
Case 15	49	1	-

-: Absent, +: Present

gynecologic examination of the anterior vaginal wall (case number 6); however, this woman had no structure compatibility (HF) with the G-spot complex in the lower abdominal MRI.
Three of 15 patients (20%) had putative G-spots (HFs) in the lower abdominal MRI (case number 4, case number 10, and case number 13). The data related to putative G-spots are given in Table 2.
All putative G-spots were detected on the left side of the vagina. The mean distance to the external urethral meatus was calculated as 38.53±6.74 (range 31-44) mm. Neurovascular tissue density was not observed histologically in any of the biopsied tissue mapped using MRI. Histologic examination of tissue samples showed only a few neural structures both in the sections stained with H&E and S100 (Histopathologic images 1-6).

Discussion

This is the first study on live tissues searching for the G-spot both histologically and radiologically. The G-spot is defined as "a sensitive area inside a woman's vagina that is thought to give great sexual pleasure when touched"[2]. Hence, it would be prudent to scrutinize the anatomy of the anterior vaginal wall. The vagina is essentially a tube that connects the uterus to the perineum. The vagina is composed of four histologic layers (internal to external): (1) Stratified non-keratinized squamous epithelium - this layer provides protection and is lubricated by

Table 2. Data related to hyperintense focus (putative G-spot)

Case	Side	Anteroposterior diameter (mm)	Area (mm^2)	Distance to urethra (mm)	Distance to introitus (mm)	Distance to vaginal lumen (mm)
Case 4	L	6	39	44	41	3.33
Case 10	L	4.05	16	40.6	38.5	2.9
Case 13	L	3	12	31	32.8	2.6
Average ± SD		4.35±1.52	22.33±14.57	38.53±6.74	37.43±4.20	2.94±0.36

SD: Standard deviation

cervical mucus, the vagina itself does not contain any glands, besides, this layer has no nerve fibers. (2) Elastic lamina propria - a dense connective tissue layer that projects papillae into the overlying epithelium. The larger thin-walled veins and nerve fibers are located here. (3) Fibromuscular layer - comprising two layers of smooth muscle (an inner circular and an outer longitudinal layer) and some nerve fibers. (4) Adventitia - a fibrous layer, which provides additional strength to the vagina. This layer also binds the vagina to surrounding structures.

The lower part of the vagina is innervated by the pudendal nerve, and the upper part is mainly innervated by hypogastric plexuses and splanchnic nerves. The nerve fibers of the vagina are mostly parasympathetic and arise vasodilatory effects on the erectile tissue of the vestibular bulbs and clitoris. The distal third of the vaginal wall possibly has a richer innervation and blood supply compared with the proximal third. The distal anterior vaginal wall is a highly sensitive area.

In 1950, Ernst Gräfenberg described an area along the anterior vaginal wall, close to the bladder, and noted it to be sensitive to stimulation[3]. Addiego described an area approximately 1.5-2 cm anterior to the urethra, associated with pleasurable sensation and enlargement by 50% during stimulation in a multiparous female patient[4].

Although the first definition of the G-spot was made in the 1950s, scientific studies about its existence started to appear in the literature after the 2000s. First, in 2012, Ostrezenski defined the G-spot as fibroconnective erectile tissue on the dorsal perineal membrane in an 83-year-old fresh cadaver, approximately 16.5 mm away from the urethral meatus[5]. Later, in the dissection study of Ostrezenski in 2014, the author stated that a macroscopically grape-like structure in the anterior wall of the vagina was rich in neurovascular tissue and had its own ganglionic nerve[6]. Ostrezenki stated that 72% of G-spots were located on the left and the distance from the urethral meatus was 55 mm[7]. In our study, we also located hyperintense foci (e.g corresponding to the putative G-spot) and the distance from the urethral meatus was measured as an average of 39.43 mm in MRI but we could not prove its existence histologically (neural and vascular components were not observed in either H&E or S100 staining).

Some authors reacted to Ostrezenski's assertive study about the presence of the G-spot. Hoag found that there was no microscopic structure other than the urethra and vaginal wall epithelium in the location of the putative G-spot in a study on 13 fresh cadavers with an age range of 32 to 97 years[8]. Hoag also emphasized that the lateral vaginal veins observed in anatomic dissection were not erectile tissue and the veins were responsible for the venous flow of the urethra, vaginal wall, and clitoris with dense vascular structure[9]. Our findings are in accordance with Hoag's findings. Moreover, Puppo revised the female sexual anatomic terminology and noted that there was no G-spot and this may be scientific fraud[10].

As the world became more open to sexuality in recent years, female sexuality has taken its place in the centre of sexual medicine. In this context, claiming the G-spot as a hypererogenic erectile area and amplification procedures (such as G-shot, hyaluronic acid injections, autologous adipose tissue injections) started to generate great marketing and interest. It is noteworthy that the presence of the G-spot is contradictory and the scientific background is weak regarding the benefits of amplification procedures applied to this spot. In addition, amplification interventions to the G-spot are even said to be female genital mutilation type 4. The belief that the presence of the G-spot both creates motivation for women to achieve sexual satisfaction and opportunities for those who benefit from this market.

Although the presence of a hypererogenic region in the anterior vaginal wall has been claimed to be the G-spot[1], the histologic structure of the anterior wall should not be forgotten. The anterior wall of the vagina is thinner and richer in neural tissue than the posterior wall of the vagina[11]. Keeping in mind this anatomic information, the amplification procedures performed to the region defined as the "G-spot" in the anterior wall of the vagina may in fact cause ballooning of the anterior wall of the vagina so that penile contact of this region results in increased sexual pleasure.

In our study, only one patient (0.06%, case number 6) reported a hypererogenous region in the anterior wall of the vagina; however, we could not find a HF in the MRI of that patient.

In this study, no neurovascular element was found microscopically in biopsy specimens taken from the so-called HF (putative G spot). In the literature, cadaveric dissection studies related to the existence of the G-spot have been performed[5,6,7,9]. However, this study is more advanced because imaging and histologic examination were performed sequentially in live tissues in the search for the G-spot.

Conclusion

Previous studies were performed on cadavers of elderly women, whereas ours is the first to be performed in both a younger (premenopausal) population and in living tissue. So-called HFs were seen in three patients but we could not identify any neuronal element in the biopsied tissues of these women. Our findings denote that there is no G-spot in the anterior vaginal wall. However, more imaging and histologic studies are needed to form a solid conclusion.

Ethics

Ethics Committee Approval: The ethics committee of the university approved the study (decision date and number: June 18th, 2020-06/V).

Informed Consent: Informed consent regarding the MRI and surgery (biopsy + anterior compartment surgery) was given by the enrolled patients.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

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

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

Financial Disclosure: Authors have no financial interests about the research.

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Cervical dysplasia after renal transplantation: A retrospective cohort study

Renal transplantasyon sonrası servikal displazi: Retrospektif bir kohort çalışması

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Abstract

Objective: Since the first days of organ transplantation, it has been accepted that solid transplant recipients have a high risk of developing cancer. Chronic immunosuppression and environmental factors play a role in cancer development in recipients. In the present study, we tried to evaluate the cumulative incidence of cervical dysplasia after renal transplantation, risk factors for disease development, and the time until high-grade dysplasia occurred.
Materials and Methods: A total of 50 patients with renal transplantation who presented for gynecologic follow-up was included in the study. The medical records of the patients were reviewed until the last clinical visit, their demographic characteristics, transplant history, gynecologic history, and gynecologic examination results (cervical cytology and histology reports) were reviewed.
Results: Of the 50 women in the study population, 29 (58%; 95% confidence interval: 8.8-15.9) developed cervical dysplasia after the first transplant at a median follow-up of 7.8 (range: 4.6-12.9) years. Twenty-one women with benign cervical cytology before transplantation had evidence of low-grade intraepithelial lesions + after transplant (47% of these were within 2 years after transplant). During the follow-up, 8 women (18.2%) were diagnosed as having high-grade intraepithelial lesions + (within 5 years after transplantation).
Conclusion: Renal transplant patients were found to have higher abnormal cervical cytology and histology rates than the normal population.
Keywords: Immunosuppression, cervical dysplasia, renal transplantation

Öz

Amaç: Organ naklinin ilk günlerinden bu yana, nakil alıcılarının kansere yakalanma riskinin yüksek olduğu kabul edilmiştir. Kronik immünosüpresyon ve çevresel faktörler, alıcılarda kanser gelişiminde rol oynar. Sunulan çalışmada, renal transplantasyon sonrası kümülatif servikal displazi insidansını, hastalık gelişimi için risk faktörlerini ve yüksek dereceli displazinin ortaya çıkmasına kadar geçen süreyi değerlendirmeye çalıştık.
Gereç ve Yöntemler: Çalışmaya jinekolojik takip için başvuran toplam 50 renal transplantasyon hastası dahil edildi. Hastaların tıbbi kayıtları son klinik ziyarete kadar gözden geçirildi, demografik özellikleri, nakil öyküleri, jinekolojik öyküleri ve jinekolojik muayene sonuçları (servikal sitoloji ve histoloji raporları) gözden geçirildi.
Bulgular: Çalışma popülasyonundaki 50 kadından 29'u (%58; %95 güven aralığı: 8,8-15,9) ortalama 7,8 yıllık bir takip süresinde (4,6-12,9) ilk nakilden sonra servikal displazi geliştirdi. Transplantasyondan önce benign servikal sitolojisi olan 21 kadında, transplantasyondan sonra düşük derecede intraepitelyal lezyonlar + kanıtı vardı (bunların %47'si transplantasyondan sonraki 2 yıl içinde idi). Takip sırasında 8 kadına (%18,2) yüksek dereceli intraepitelyal lezyonlar + (transplantasyondan sonraki 5 yıl içinde) tanısı kondu.
Sonuç: Böbrek nakli hastalarının normal popülasyona göre daha yüksek anormal servikal sitoloji ve histoloji oranlarına sahip olduğu bulunmuştur.
Anahtar Kelimeler: İmmünosüpresyon, servikal displazi, renal transplantasyon

PRECIS: Incidence of cervical dysplasia increased in patients with renal transplant.

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Introduction

Primary (*de novo*) malignancy that develops after renal transplant is seen as an unfortunate complication of a successful surgery. The development of such malignancies may be caused by various factors such as individual and regional susceptibility, pre-transplant disease states, viral status of the recipient, and high doses of various immunosuppressive drugs to protect the graft. Lymphoproliferative disease, skin cancer, Kaposi's sarcoma, and cervical dysplasia have been reported with high incidence in these patient groups after transplantation[1]. The persistence of human papillomavirus (HPV) is the most important factor in the development of dysplasia. Therefore, if immunologic control of the virus is interrupted, this adverse event poses a higher risk to patients. Immunocompromised women are at high risk for a variety of premalignant and malignant conditions, including cervical dysplasia[2]. There are many factors in the development of cervical malignant lesions but immunosuppression on high-risk HPV clearance is the most important[2,3]. The long-term consequences of the suppressed immune system are becoming increasingly important for improving life expectancy and quality. Cervical cancer screening guidelines are constantly updated due to the increase in knowledge about the etiopathogenesis of the disease; however, the ideal method and frequency for cervical cancer screening after organ transplantation is still uncertain. Therefore, in this study, we aimed to evaluate the abnormal findings resulting from cervical cancer screening and histology in women with renal transplantation. We tried to evaluate the cumulative incidence of cervical dysplasia after renal transplantation, to determine risk factors for disease development, and to evaluate the time to high-grade dysplasia. In addition, we aimed to determine whether the risk of abnormal cervical testing approached the general population risk. The point we wanted to draw attention to in our study was to emphasize the factors that might be cofactors of cervical dysplasia and to stress the importance of multidisciplinary evaluation of patients with organ transplants who are difficult to follow-up.

Materials and Methods

We retrospectively reviewed the patient records. The study was approved by the ethics committee of our university (no: 99166796-050.06.04, approval no: 20-8.1T/8). Patients with renal transplant who presented for gynecologic follow-up were determined from our hospital's database. Written informed consent was obtained from participants to use their medical records for research. As the inclusion criteria, it was decided to have documented gynecologic follow-up for at least 1 year after renal transplant and to have at least one cervical pathology sample before or after transplant. Patients who had a hysterectomy prior to transplantation or within 1 year were excluded. After applying the inclusion and exclusion criteria, a total of 50 patients were included in the study. The medical records of the patients were reviewed until the last clinical visit, and demographic characteristics, transplant history, gynecologic history and results of gynecologic exams (cervical cytology and histology reports) were reviewed.

We created three categories according to the cytologic and histologic features:

1. Cervicitis, inflammation, atypical squamous cells of undetermined significance (similar lesions were classified as benign)
2. Low-grade intraepithelial lesions (LSIL), cervical intraepithelial neoplasia (CIN) I were classified as LSIL+
3. High-grade intraepithelial lesions (HSIL), CIN II, and CIN III were classified as HSIL+

Descriptive statistics were reported as means and standard deviations (SD), and interquartile medians, frequencies, and percentages as indicated. The cumulative incidence of cervical dysplasia in the general population was calculated based on the gold standard tissue diagnosis. Findings of abnormal screening tests (based on clinical documentation or pathologic diagnosis) have also been reported. Patient demographics such as age, transplant age, body mass index (BMI) (weight in kilograms divided by height in square meters), parity, immunosuppression agent, and dialysis type and time status are tabulated.

Statistical Analysis

Data are presented as the number of observations (n, %), mean ± SD, range. The results of homogeneity (Levene's test) and normality (Shapiro-Wilk test) were used to decide the statistical methods for comparing the study groups. Among normally distributed groups with homogeneous variances, dependent groups were compared using Student's t-test. According to the test results, parametric test assumptions were not available for some variables; therefore, the independent groups were compared using the Mann-Whitney U test. Categorical data were analyzed using Fisher's Exact test and the chi-square test. In cases in which the expected counts for inclusion were not met in less than 20% of the cells, Monte Carlo simulation was used and the values were determined. Logistic regression analysis was performed to determine whether cervical dysplasia was positive or negative. Statistical analyses were performed using the IBM SPSS Statistics for Windows Version 25.0 software package (Armonk, NY: IBM Corp). P-values <0.05 were considered statistically significant. Nonparametric cumulative incidence analyses were performed using the Stata 11.0/MP for Linux package (StataCorp, College Station, Tex). Non-parametric cumulative incidence estimates were produced using the stcompet command, and multivariate comparisons of cumulative incidence functions were completed using stcrreg.

Results

A total of 50 women with renal transplantation were evaluated for gynecologic follow-up and cervical screening. Transplantation was performed when the patients were aged 41.6-14.2 years on average, and gynecologic follow-up was started at least 1

year before transplantation. The mean follow-up period was 5.07 (4.98) years in patients with cervical dysplasia and 6.76 (4.72) years in patients without dysplasia. The most common renal diseases were diabetes mellitus (66%), hypertension (14%), and lupus nephritis (10%). For 11 (22%) patients, the diagnosis was other renal diseases. Table 1 summarizes the demographic and basic characteristics of the patients. The median age of these 50 women was 44.55 in the group that developed cervical dysplasia and 44.67 in the group that did not develop cervical dysplasia [interquartile range (IQR): 29-53, 24-36, respectively] (Table 1). Of the 50 women in the study population, 29 [58%; 95% confidence interval (CI): 8.8-15.9] developed cervical dysplasia after the first transplant at a median follow-up of 7.8 years (IQR: 4.6-12.9). Forty-four women (88%) had at least one documented benign cervical pathology prior to renal transplant. All patients had at least one documented cervical screening report after renal transplant.

Twenty-one women with benign cervical cytology before transplantation had evidence of LSIL+ after transplant (47% of these were within 2 years after transplant). During the follow-up, eight women (18.2%) were diagnosed as having HSIL+ (within 5 years after transplantation). Table 2 and Figure 1 show the cumulative incidence rates for LSIL+ cytology-histology, HSIL+ cytology-histology for the cohort.

Factors associated with an increased risk of developing cervical dysplasia in univariate and multivariate analysis were: age [Odds ratio (OR)=1.22, 95% CI: 0.395-3.770], time after transplant (OR=1.007, 95% CI: 0.929-1.091), the use of cyclosporine-A (OR=1.381 95% CI: 0.554-2.336), the use of tacrolimus (OR=1.731 95% CI: 0.224-2.382), the use of azathioprine (OR=1.893, 95% CI: 0.268-2.971), the use of mycophenolate mofetil (OR=2.184, 95% CI: 0.101-47.266), hemodialysis (OR=1.25, 95% CI: 0.292-5.348), peritoneal dialysis (OR=1.5, 95% CI: 0.255-8.817), and hemodialysis + peritoneal dialysis (OR=1.851, 95% CI: 0.218-9.695). Among the factors that reduce the risk of the development of cervical dysplasia were the following parameters: age at transplantation (OR=0.817, 95% CI: 0.261-2.557), years of follow-up (OR=0.689, 95% CI: 0.340-1.398), current smoker or quit within the past 1 year (OR=0.214, 95% CI: 0.045-1.032), and BMI (OR=0.847, 95% CI: 0.712-1.006). Table 3 shows the univariate and multivariate analyses of factors associated with cervical dysplasia.

Table 1. Demographic characteristics of patients

Characteristic	Cervical dysplasia negative (n=21)	Cervical dysplasia positive (n=29)
Age		
Mean (SD)	44.67 (11.77)	44.55 (12.83)
Range	38.00	50.00
Age at transplant		
Mean (SD)	38.14 (14.10)	39.38 (14.75)
Range	48.00	50.00
Time after transplant (month)	75.90 (57.10)	59.59 (72.97)
Years of follow-up	6.76 (4.72)	5.07 (4.98)
Smoking no. (%)		
Never smoked	9 (33.3)	18 (66.7)
Previous smoker (quit all use >1 year previously)	5 (38.5)	8 (61.5)
Current smoker (or quit within past 1 year)	7 (70.0)	3 (30.0)
BMI (kg/m^2)	29.90 (4.21)	7.24 (4.09)
Gravidity no. (%)		
0	3 (37.5)	5 (62.5)
1 or more	18 (42.9)	24 (57.1)
Parity no. (%)		
0	5 (31.3)	11 (68.8)
1 or more	16 (47.1)	18 (52.9)
Indication for kidney transplant		
Diabetes mellitus	13 (39.4)	20 (60.6)
Hypertension	3 (42.9)	4 (57.1)
Lupus nephritis Kidney disease - other	3 (60.0) 2 (40.0)	2 (40.0) 3 (60.0)
Immunosuppressive regimen		
Cyclosporine-A	8 (47.1)	9 (52.9)
Tacrolimus	13 (41.9)	18 (58.1)
Azathioprine	5 (31.3)	11 (68.8)
Mycophenolate mofetil	15 (46.9)	17 (53,1)
Prednisolone	21(42.0)	29 (58.0)
Dialysis type		
None	5 (50)	5 (50)
Hemodialysis	15 (44.4)	12 (55.6)
Peritoneal dialysis	4 (40.0)	6 (60.0)
Hemodialysis+Peritoneal dialysis	0 (0.0)	3 (100.0)

Discussion

After the spread of organ transplantation worldwide, lymphoid and non-lymphoid tissue malignancies, especially skin cancers, have started to be seen in organ transplant recipients with a high incidence. Organ transplant recipients are at a 3- to 4-fold risk of malignancy due to chronic immunosuppression. However, compared with the general population, the relative risk for certain cancers increases 100-fold[4]. The risk of malignancy is estimated as 20% after 10 years of chronic immunosuppression[5]. Possible mechanisms for malignancy

Table 1 continued

Dialysis time (month)		
Hemodialysis	57.60 (84.98)	47.25 (74.39)
Peritoneal dialysis	5.45 (15.22)	15.93 (31.36)
Benign cervical cytology before transplantation		
Yes	17 (38.6)	27 (61.4)
No	4 (66.7)	2 (33.3)
Histologic outcomes after transplantation		
Benign	21	0
LSIL+	0	21
HSIL+	0	8

SD: Standard deviation, BMI: Body mass index, LSIL: Low-grade intraepithelial lesion, HSIL: High-grade intraepithelial lesion

development include replication of oncogenic viruses (HPV, herpes simplex virus, Epstein-Barr virus, cytomegalovirus), immunity disorders (suppression of natural killer cell activity, impairment of immune regulation, use of blood products, decrease in interferon levels), and direct carcinogenic effects of immunosuppressives[6]. The most important risk for cervical cancer is infection with high-risk HPV types[7]. The most common sexually transmitted disease seen in the general population and patients with renal transplantation is HPV infection. Patients with renal transplants have a higher rate of permanent disease and disease burden compared with the general population[8]. It was reported that the risk of developing cervical neoplasia (usually ***in situ***) was 14 times higher in female patients who had renal and liver transplantation compared with controls[9]. Chapman and Webster[10] reported that 46 of 13,077 patients (6.6%) who had a renal transplant were diagnosed as having cervical cancer. It has been reported that the incidence

Table 2. Histologic outcomes after transplantation

Cumulative incidence of each outcome at t years after transplantation, % (95% CI)					
Full cohort (n=50)	t=1	t=2	t=5	t=10	t >10
Benign	-	-	-	-	-
LSIL+	0.29 (0.09-0.48)	0.43 (0.22-0.64)	0.71 (0.52-0.91)	0.95 (0.86-1.00)	100
HSIL+	0.20 (0.04-0.55)	0.60 (0.17-0.83)	0.33 (0.09-0.87)	-	100
Benign cervical cytology before transplantation (n=44)					
Benign	-	-	-	-	-
LSIL+	0.32 (0.11-0.52)	0.47 (0.25-0.70)	-	-	0.95 (0.85-1.00)
HSIL+	0.20 (0.03-0.55)	0.60 (0.17-0.85)	0.67 (0.13-0.98)	-	0.87 (0.43-0.98)
	Cumulative incidence of each outcome by immunosuppressive use, % (95% CI)				
Full cohort (n=50)	Prednisolone	Cyclosporine-A	Tacrolimus	Azathioprine	Mycophenolate mofetil
Benign	-	-	-	-	-
LSIL+	0.95 (0.86-1.00)	0.83 (0.54-0.98)	100	0.89 (0.79-0.99)	100
HSIL+	100	100	100	100	100
Benign cervical cytology before transplantation (n=44)					
Benign	-	-	-	-	-
LSIL+	0.95 (0.85-1.00)	0.83 (0.69-0.98)	100	0.88 (0.76-0.99)	100
HSIL+	100	100	100	100	100
Cumulative incidence of each result by dialysis method, % (95% CI)					
Full cohort (n=50)	Hemodialysis				Peritoneal dialysis
Benign	-				-
LSIL+	0.91 (0.83-0.99)				100
HSIL+	100				100
Benign cervical cytology before transplantation (n=44)					
Benign	-				-
LSIL+	0.90 (0.81-0.99)				100
HSIL+	100				100

LSIL: Low-grade intraepithelial lesion, HSIL: High-grade intraepithelial lesion, CI: Confidence interval

Table 3. Univariate and multivariable analysis: associations with positive cervical dysplasia

Characteristic	Univariate positive cervical dysplasia			Multivariate (OR- %95 CI)	
	Cervical dysplasia negative	Cervical dysplasia positive	p	OR (95% CI)	p
Age					
Mean (SD)	44.67 (11.77)	44.55 (12.83)	0.974	1.221 (0.395-3.770)	0.729
Range	38.00	50.00			
Age at transplant					
Mean (SD)	38.14 (14.10)	39.38 (14.75)	0.767	0.817 (0.261-2.557)	0.729
Range	48.00	50.00			
Time after transplant (month)	75.90 (57.10)	59.59 (72.97)	0.398	1.007 (0.929-1.091)	0.868
Years of follow-up	6.76 (4.72)	5.07 (4.98)	0.231	0.689 (0.340-1.398)	0.302
Smoking no. (%)					
Never smoked	9 (33.3)	18 (66.7)	0.132	Indicator	0.153
Previous smoker (quit all use >1 year previously)	5 (38.5)	8 (61.5)		0.800 (0.202-3.162)	0.750
Current smoker (or quit within past 1 year)	7 (70.0)	3 (30.0)		0.214 (0.045-1.032)	0.045
BMI (kg/m^2)	29.90 (4.21)	7.24 (4.09)	0.029	0.847 (0.712-1.006)	0.049
Gravidity no. (%)					
0	3 (37.5)	5 (62.5)	0.778	1.537 (0.169-13.997)	0.703
1 or more	18 (42.9)	24 (57.1)			
Parity no. (%)					
0	5 (31.3)	11 (68.8)	0.291	0.409 (0.069-2.420)	0.324
1 or more	16 (47.1)	18 (52.9)			
Indication for kidney transplant					
Diabetes mellitus	13 (39.4)	20 (60.6)	0.925	Indicator	0.864
Hypertension	3 (42.9)	4 (57.1)		0.867 (0.166-4.521)	0.865
Lupus nephritis	3 (60.0)	2 (40.0)		0.433 (0.063-2.958)	0.393
Kidney disease-other	2 (40.0)	3 (60.0)		0.975 (0.143-6.655)	0.979
Immunosuppressive regimen					
Cyclosporine-A	8 (47.1)	9 (52.9)	0.603	1.381 (0.554-2.336)	0.260
Tacrolimus	13 (41.9)	18 (58.1)	0.991	1.731 (0.224-2.382)	0.603
Azothiopurine	5 (31.3)	11 (68.8)	0.291	1.893 (0.268-2.971)	0.853
Mycophenolate mofetil	15 (46.9)	17 (53.1)	0.352	2.184 (0.101-47.266)	0.619
Prednisolone	21(42.0)	29 (58.0)	0.994	1.381 (0.228-2.997)	0.260
Dialysis type					
None	5 (50)	5 (50)	0.546	Indicator	0.977
Hemodialysis	15 (44.4)	12 (55.6)		1.250 (0.292-5.348)	0.764
Peritoneal dialysis	4 (40.0)	6 (60.0)		1.500 (0.255-8.817)	0.654
Hemodialysis + peritoneal dialysis	0 (0.0)	3 (100.0)		1.851 (0.218-9.695)	0.999
Dialysis time (month)					
Hemodialysis	57.60 (84.98)	47.25 (74.39)	0.656	0.996 (0.987-1.006)	0.465

Table 3 continued

Peritoneal dialysis	5.45 (15.22)	15.93 (31.36)	0.133	1.014 (0.977-1.053)	0.465
Benign cervical cytology before transplantation					
Yes	17 (38.6)	27 (61.4)	0.192	1.315 (0.052-1.909)	0.209
No	4 (66.7)	2 (33.3)			
Histologic outcomes after transplantation					
Benign	21 (100.0)	0 (0.0)		Indicator	0.559
LSIL+	0 (0.0)	21 (100.0)	0.999	2.400 (0.638-9.028)	0.195
HSIL+	0 (0.0)	8 (100.0)		3.800 (1.558-12.669)	0.228

SD: Standard deviation, CI: Confidence interval, OR: Odds ratio, BMI: Body mass index, LSIL: Low grade intraepithelial lesion, HSIL: High grade intraepithelial lesion

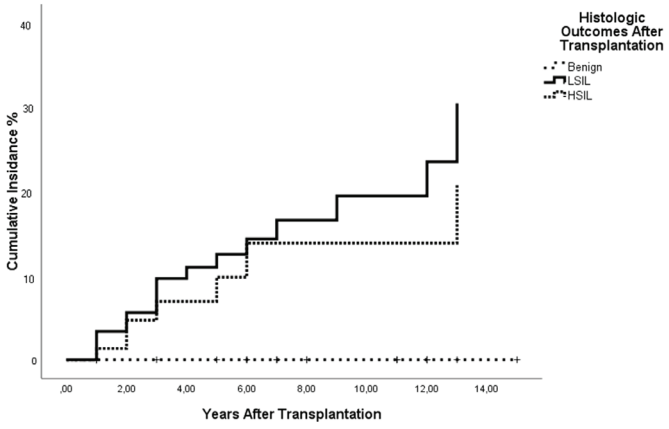

Figure 1. Cumulative incidence rates for LSIL+ and HSIL+ cytology-histology for the cohort

LSIL: Low-grade intraepithelial lesion, HSIL: High-grade intraepithelial lesion

of primary (***de novo***) malignancy after organ transplantation is between 2.92% and 3.36% in renal transplant recipients in Turkey[11]. Haberal et al.[12] reported that the incidence of malignancy among renal transplant recipients was 3.7%, a 47-year-old female patient was diagnosed as having cervical cancer 4 months after renal transplant, and the incidence of gynecologic malignancy was 2% among renal transplant recipients[12]. Akgun et al.[13] reported that of 347 renal and 24 liver transplants performed in Organ Transplantation Centers, malignancy developed in 15 renal transplant patients (3.36%) and one liver transplant patient (3.84%) during 13 years of follow-up, and one in situ cervical carcinoma developed 4 months after renal transplantation[13]. It has been reported that the HPV prevalence can be as low as 5% and as high as 63% in studies of female kidney transplant patients[14]. The risk of persistent infection with HPV type 16-18 genotypes is higher in immunocompromised patients than in the general population[8]. Similarly, in another study conducted in patients with kidney transplantation, although the incidence of HPV-related malignancy was found to be increased after transplantation, the same increase was not observed in patients who developed end-stage renal failure but who are not currently transplanted[15]. These findings support the role of immunosuppressive agents in increasing the risk of HPV-related diseases in patients with renal transplantation.

In our clinic, a total of 50 female patients underwent renal transplantation between 2016-2017 and received immunosuppressive therapy. Of the 50 women in the study population, 29 (58%; 95%: CI 8.8-15.9) developed cervical dysplasia after the first transplant at a median follow-up of 7.8 years. Twenty-one women with benign cervical cytology before transplantation had evidence of LSIL + after transplant (47% of these were within 2 years of transplantation). During the follow-up, eight women (18.2%) were diagnosed as HSIL + (within 5 years after transplantation). Cervical cancer was not detected in any patients in our study.

There are also studies reporting that there is no increase in the risk of developing gynecologic malignancy after organ transplantation, on the contrary, the relative frequency of gynecologic tumors decreased compared with the general population. Fung et al.[16] reported that gynecologic malignancies (breast, ovary, uterus and cervix) in women who underwent organ transplantation were 1.9 times less frequent than in the normal population and concluded that this was due to the active mammographic and gynecologic examination policy before and after liver transplantation. In a study conducted among 1,778 patients who underwent organ transplantation in the United Kingdom, it was reported that cervical cancer was detected in one of 78 women who developed primary (***de novo***) non-lymphoid tissue malignancy, the expected incidence of cancer in terms of cervical cancer was 0.79%, and no increased risk of cervical and breast cancer was observed[17]. However, the role and extent of immune dysfunction in the development of cervical dysplasia are not clear in this patient population. In a study evaluating the incidence of cervical cancer after transplantation, a similar incidence was found in groups with and without systemic lupus erythematosus (SLE); however, specific immunosuppressive drugs or the severity of SLE have not been evaluated[18].

In our study, 0.71% of patients developed LSIL+, and 0.33% developed HSIL or worse lesions within the first 5 years after transplantation. In a study examining the relationship between organ transplantation and invasive cervical cancer, an average

interval of 3.8 years was found between transplant and cancer[19]. In our study, we saw that a significant number of LSIL+ or worse lesions occurred within 5 years after transplantation; this increases the importance of annual screening especially in this patient group in the first 5 years. The 5-year cumulative HSIL+ incidence in our study was 0.33%, similar to the 0.3% reference cohort rate[20]. Our cohort consisted of women with lower levels of abnormal cytology, LSIL+ and HSIL+ histology, compared with a cohort of about one million women[21].

The treatment of primary (*de novo*) malignancies in renal transplant patients is the same as in normal non-transplant patients. In patients with immunosuppressive therapy, especially with solid organ transplantation, Papanicolaou (PAP) tests can be obtained at the first examination, if there is a positive PAP test, examination with colposcopy and biopsy should be taken from these lesions in the presence of suspicious lesions[22,23]. However, in these patients, the dose of immunosuppression is reduced to the lowest possible level immediately after tumor diagnosis. It is very important to diagnose and stage malignancies in organ transplant recipients as soon as possible. When these lesions are detected as either ***in situ*** or low-grade malignancies, oncologic results are undoubtedly better.

Study Limitations

Our study has some limitations. First, it has a retrospective design and the second is that it is conducted in a single institution. Our HPV data, which are the most important limitation of our study, are limited in this analysis, so we could conclude about how the HPV test might affect the screening range in this population.

Conclusion

Renal transplant patients have been found to have higher abnormal cervical cytology and histology rates than the normal population. Female patients undergoing organ transplantation should be screened for cervical cancer with annual PAP-smear tests and pelvic examinations. Organ recipients at high risk for malignancy (those with a history of cancer or an underlying disease predisposing to malignancy) should be followed up closely, including colposcopy.

Ethics

Ethics Committee Approval: The study was approved by the ethics committee and was conducted in accordance with the ethical standards described in an appropriate version of the 1975 Helsinki Declaration, revised in 2000 (no: 99166796-050.06.04, approval no: 20-8.1T/8).

Informed Consent: Written informed consent was obtained from participants to use their medical records for research.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: A.B., Design: A.B., O.İ., S.A.K., Data Collection or Processing: O.İ., M.C.T., Analysis or Interpretation: A.B., Literature Search: Ş.G.G., H.T., Writing: Ş.G.G., M.K., S.A.K.

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Obstetrician-gynecologists' practice patterns regarding HPV testing in cervical cancer screening in Turkey

Türkiye'de kadın hastalıkları ve doğum uzmanlarının serviks kanseri taramasında HPV testi ile ilgili uygulama paternleri

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Abstract

Objective: To determine obstetrician-gynecologists' (OBGYNs) practice patterns regarding human papillomavirus (HPV) testing in cervical cancer screening. Secondly, we aimed to examine OBGYNs' adherence to guidelines in the management of women with HPV-positive test results.

Materials and Methods: The study was a cross-sectional survey conducted in Antalya and Istanbul provinces in Turkey using a self-reported questionnaire. A 12-item questionnaire form was administered to the participants in face-to-face interviews. Of the targeted participants, 343 OBGYNs completed the questionnaire.

Results: The majority of participants, (81.0%) stated that they offered/used HPV testing in cervical cancer screening. Of those, most OBGYNs (89.9%) preferred to use HPV testing concomitant with cervical cytology (co-testing) whereas only 10.1% preferred to use HPV testing alone (primary HPV testing). The most preferred screening intervals for women with HPV-negative results were 5 years (53.4%) and 3 years (19.9%), respectively. In compliance with the guidelines, the rate of participants who recommended "referral directly to colposcopy" for women who were HPV16/18-positive and cytology-negative; and "co-testing at 12 months" for women who were positive for HPV genotypes other than HPV16/18 and cytology-negative was 53.1%. Multivariate analysis revealed that the "professional working setting" was the sole independent determinant of the adherence to the guidelines. OBGYNs working in private settings had the worst adherence rate (42.4%).

Conclusion: Primary HPV testing is not yet widespread among Turkish OBGYNs. Moreover, adherence to practice guidelines in the management of HPV-positive test results is relatively low. There is a need for continuing medical education regarding screening programs and the management of women with positive screening results.

Keywords: Cervical cancer, human papillomavirus, HPV test

Öz

Amaç: Birincil olarak, kadın hastalıkları ve doğum uzmanlarının servikal kanser taramasındaki uygulama paternlerini saptamayı; ikincil olarak ise kadın hastalıkları ve doğum uzmanlarının insan papilloma virüsü (HPV)-pozitif test sonucu olan kadınların yönetiminde kılavuzlara bağlılıklarını incelemeyi amaçladık.

Gereç ve Yöntemler: Bu çalışma, bir öz-bildirim anketi kullanılarak, Türkiye'de Antalya ve İstanbul illerinde yapılan çapraz-kesitsel bir araştırmadır. On iki maddelik anket formu, yüz yüze görüşme yöntemi ile katılımcılara uygulandı. Hedeflenen katılımcılardan 343 kadın hastalıkları ve doğum uzmanı anketi tamamladı.

Bulgular: Katılımcıların çoğunluğu (%81,0) servikal kanser taramasında HPV testini kullandıklarını/önerdiklerini belirtti. Bu hekimlerin büyük çoğunluğu (%89,9) HPV testini servikal sitoloji ile eş zamanlı (cotest) olarak kullanmayı tercih etmekteyken sadece %10,1'i HPV testini tek başına (primer HPV testi) kullanmayı tercih etmektedir. HPV-negatif sonucu olan kadınlarda en sık tercih edilen tarama aralığı sırasıyla 5 (%53,4) ve 3 yıl (%19,9) idi. Kılavuzlarla uyumlu olarak, HPV 16/18 pozitif ve sitoloji sonucu negatif olan kadınlarda "doğrudan kolposkopi", HPV16/18 haricindeki HPV genotipleri pozitif iken sitoloji sonucu negatif olan kadınlarda ise "12 ay sonra cotest" öneren katılımcıların oranı %53,1 idi. Çok değişkenli analiz, "profesyonel çalışma ortamı"nın

PRECIS: Using a 12-item self-reported questionnaire, we have evaluated obstetrician-gynecologists' practice patterns regarding HPV testing in cervical cancer screening in Turkey.

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kılavuzlara bağlılığın tek bağımsız belirleyicisi olduğunu gösterdi. Özel kurumlarda çalışan kadın hastalıkları ve doğum uzmanları kılavuzlara en kötü uyum oranına (%42,4) sahipti.
Sonuç: Primer HPV testi kullanımı Türk kadın hastalıkları ve doğum uzmanları arasında henüz yaygınlaşmamıştır. Üstelik, HPV-pozitif test sonuçlarının yönetiminde uygulama kılavuzlarına bağlılık görece düşüktür. Tarama programları ve pozitif tarama sonuçları olan kadınların yönetimi ile ilgili sürekli tıp eğitimine ihtiyaç bulunmaktadır.
Anahtar Kelimeler: Serviks kanseri, human papilloma virüs, HPV testi

Introduction

Cervical cancer is the third most common cancer in women worldwide. Approximately 85% of cases occur in developing countries where cervical cancer is a public health problem[1]. Persistent high-risk human papillomavirus (HPV) infection plays a key role in cervical carcinogenesis. The estimated absolute risk for cervical intraepithelial neoplasia of grade 3 (CIN3) or cancer within 12 years following a persistent HPV16 infection has been estimated as high as 47%[2]. Turkey, though it is a developing country, has a relatively low incidence (4.5/100000) of cervical cancer in line with its low prevalence (3.5%) of high-risk HPV infection[3,4].

Cervical cancer can be prevented, primarily with HPV vaccines, and secondarily with screening programs. Population-based screening programs using cervical cytology has successfully decreased cervical cancer incidence and mortality[5]. However, the false-negative rate of cytology (>50%) is still very high, particularly in endocervical adenocancers and postmenopausal women[6,7]. Studies on the detection of high-risk HPV nucleic acid in cervical epithelial cells (HPV testing) have revealed that HPV testing had a sensitivity of 90% and a specificity of 90% in the detection of CIN2 or worse (CIN2+) lesions, including glandular lesions[8]. As a result of studies demonstrating the high diagnostic performance of HPV testing, there have been changes in the joint American Cancer Society (ACS), American Society for Colposcopy and Cervical Pathology (ASCCP), and American Society for Clinical Pathology (ASCP) screening guidelines in 2012, and the use of HPV testing together with cytology (co-testing) every five years started to be recommended as the screening method for cervical cancer in women over the age of 30 years[9].

In the most recent studies, however, it has been shown that the 3-year risk following HPV screening alone (primary HPV testing) was identical to that following co-testing every three year[10], and lower than that following co-testing every five years[11]. Subsequent to these results, many national and international societies made changes in their guidelines[12-16]. In 2015, the Society of Gynecologic Oncology and ASCCP issued interim clinical guidance recommending primary HPV testing every 3 years for women aged ≥25 years[12]. One year later, the American College of Obstetricians and Gynecologists and the United Kingdom National Screening Committee recommended this strategy as an effective screening option[13,14]. At the same year, the American Society of Clinical Oncology endorsed primary HPV testing every 5 years for women aged ≥25 years as one of several screening strategies[15].

The Turkish Ministry of Health implemented a population-based screening program in 2014, which included primary HPV testing as the screening method, with a nationwide centralized diagnostics laboratory and a well-defined screening algorithm[16]. According to this program, women aged between 30 and 65 years are invited for screening by primary care physicians every 5 years. Women who are HPV16/18-positive are referred to centers specialized in colposcopy. In women who are positive for HPV genotypes other than HPV16/18, reflex cytology is performed. Women with negative cytology are invited for repeat HPV testing after 12 months, and women with cytologic abnormalities are immediately referred to colposcopy centers.

In the current study, we primarily aimed to determine obstetrician-gynecologists' (OBGYNs) knowledge, attitudes, and practice patterns regarding HPV testing in cervical cancer screening in Turkey. Secondly, we aimed to examine OBGYNs' adherence to the national and/or international guidelines in the management of women with HPV-positive test results.

Materials and Methods

The study was a cross-sectional survey conducted in Antalya and Istanbul provinces in Western Turkey between May and September 2018 using a structured self-reported questionnaire. The sample size was calculated using a random sample calculator with 5% margin of error and 95% confidential intervals (CI) [17]. According to the most recent report on health education and health manpower in Turkey, which is prepared jointly by the Turkish Ministry of Health and Turkish Council of Higher Education, there are 5,227 actively working OBGYNs in Turkey[18]. Based on these data, the optimal sample size required for the study was calculated as 358. The study was approved by the local ethics committee and it 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 was obtained from all participants.

From a list of all members of the Turkish Association of Obstetricians and Gynecologists stratified by region, 500 representative OBGYNs were selected at random for participation. All participants were given verbal instructions and written information about the study, and all were informed about confidentiality measures and their rights to withdraw. A 12-item questionnaire form was administered to the participants through face-to-face interviews. Of the targeted participants, 343 OBGYNs completed the questionnaire, yielding a response rate of 68.6%. The margin of error at 95% CI was calculated as 5.1%.

The survey questionnaire had two sections. The first section included six questions on the participants' demographic characteristics such as sex, age, the number of years in specialty practice, whether they had a subspecialty, professional working setting, and the type of practice. The second section included six questions that assessed the OBGYNs' knowledge, attitudes, and practice patterns regarding HPV testing in cervical cancer screening. These questions were as follows:

1. Do you offer/use HPV testing in cervical cancer screening? (No/Yes)
2. If your response to the first question was "yes", how do you prefer to use HPV testing in cervical cancer screening? Primary HPV testing (high risk-HPV testing alone) vs Co-testing (high risk-HPV testing concomitant with cytology)
3. If your response to the first question was "yes", do you prefer an age threshold for beginning HPV testing? (No vs ≥21 vs ≥25 vs ≥30 vs others)
4. If your response to the first question was "yes", what is your preferred screening interval for women with an HPV-negative test result? (Less than 1 year/annually/2 years/3 years/4 years/5 years/other)
5. What is your recommendation for women who are HPV16/18-positive and cytology-negative (negative for intraepithelial lesion or malignancy - NILM)?
6. What is your recommendation for women who are positive for HPV genotypes other than HPV16/18 and cytology-negative?

Statistical Analysis

Two separate binary logistic regression models were developed to investigate the determinants of OBGYNs' use of the HPV testing in cervical screening and their adherence to the guidelines in the management of women with HPV-positive test results. In univariate analyses, Pearson's chi-square test was used because all the variables were categorical. Categorization of the age and years in specialty practice was performed according to the median value. Validities of median values were tested using receiver operating characteristic curve analysis. Variables with a p-value <0.20 in univariate analyses were included in the multivariate analyses. The effects of variables on the use of HPV testing and adherence to the guidelines were reported as adjusted odds ratios (OR) and 95% CI.

Results

The mean age and the years in specialty practice of the participants were 43.3±9.2 years and 11.2±8.0 years, respectively. The rate of women (53.4%) was slightly higher in sex distribution. The majority of the participants were general OBGYNs (84.5%), working at secondary-care (public/private) settings (70%), and had no academic position (88.9%) (Table 1).

The practice behaviors of OBGYNs regarding HPV testing are shown in Table 2. The majority of participants (81.0%) stated that they offered/used HPV testing in cervical cancer screening. Of those, most OBGYNs (89.9%) preferred to use HPV testing concomitant with cytology, whereas only 10.1% preferred to use HPV testing alone. The two most frequent answers to the question of "Do you prefer an age threshold for beginning HPV testing?" were "no" (43.2%), and "from the age of 30 years" (41.4%), respectively. The most preferred screening intervals for women with an HPV-negative result were 5 years (53.4%) and 3 years (19.9%), respectively.

The determinants of OBGYNs' use of HPV testing in cervical cancer screening are presented in Table 3. In univariate analysis, only the "professional working setting" was found to be significantly associated with the use of HPV testing. OBGYNs

Table 1. Characteristics of participating OBGYNs (N=343)

Variables	Median (range)	Mean (SD)	n	%
Sex				
Female			183	53.4
Male			160	46.6
Age, years	41 (28-73)	43.36 (9.20)		
>40			133	38.8
40-49			121	35.3
50-59			63	18.4
≥60			26	7.6
Years in specialty practice	10 (1-42)	11.27 (8.05)		
<5			67	19.5
5-14			141	41.1
15-24			92	26.8
≥25			43	12.5
Subspecialty				
General OBGYN			290	84.5
Reproductive endocrinologist			21	6.1
Perinatologist			12	3.5
Gynecologic oncologist			20	5.8
Professional working setting				
Secondary-care healthcare			240	70.0
Public hospital			122	35.6
Private hospital			93	27.1
Private outpatient clinic			25	7.3
Tertiary-care healthcare			103	30.0
Type of practice				
Specialist			305	88.9
Academician			38	11.1

OBGYN: Obstetrician-gynecologist, SD: Standard deviation

Table 2. Practice behaviors of OBGYNs related to HPV testing

Variables	Frequency, n (%)
Do you offer/use HPV testing in cervical cancer screening?	
No	65 (19.0)
Yes	278 (81.0)
If your response to the first question was "yes", **how do you prefer to use HPV testing in cervical cancer screening?**	
Primary HPV testing (high risk-HPV testing alone)	28 (10.1)
Co-testing (high risk-HPV testing concomitant with cytology)	246 (89.9)
If your response to the first question was "yes", **do you prefer an age threshold for beginning HPV testing?**	
No	120 (43.2)
≥21	14 (5.0)
≥25	17 (6.1)
≥30	115 (41.4)
Others (including ages greater than ≥35)	12 (4.3)
If your response to the first question was "yes", **what is your preferred screening interval for women with an HPV-negative test result?**	
Less than 1-year	6 (2.2)
Annually	40 (14.4)
Two years	26 (9.4)
Three years	55 (19.9)
Four years	2 (0.7)
Five years	148 (53.4)
Missing data	1 (0.4)

OBGYN: Obstetrician-gynecologist, HPV: Human papillomavirus

working at secondary-care public hospitals used HPV testing at the lowest rate with 70.5%, this rate was 82.2% for those working at private hospitals/outpatient clinics and 92.2% for those working at tertiary-care hospitals. Three variables ("years in specialty practice", "professional working setting" and "type of practice") with a p-value <0.20 in univariate analyses were included in the multivariate analysis. Multivariate analysis revealed that "years in specialty practice" and "professional working setting" are independent determinants of the use of HPV testing in cervical cancer screening. OBGYNs working at secondary-care public hospitals and those with >10 years of practice experience (OR: 0.511; 95% CI: 0.280-0.933, p=0.029) use HPV testing significantly less often.

Practice behaviors of OBGYNs regarding the management of women with HPV-positive test results are summarized in Table 4. The majority of the participants (78.7%) stated that they recommended "referral directly to colposcopy" for women with HPV16/18 and concurrent NILM cytology. On the other hand, the most preferred recommendation for women with HPV genotypes other than HPV16/18 and concurrent NILM cytology was "co-testing at 12 months" (65.9%). In compliance with the joint ACS, ASCCP, ASCP guidelines (2012) and Turkish Ministry of Health practice guidelines, the rate of participants who recommend "referral directly to colposcopy" for women who are HPV16/18-positive and cytology-negative; and "co-testing at 12 months" for women who are positive for HPV genotypes other than HPV16/18 and cytology-negative was 53.1%.

The determinants of OBGYNs' adherence to the practice guidelines in the management of women with HPV-positive test result are presented in Table 5. Univariate analysis revealed that "age", "years in specialty practice" and "professional working setting" were significantly associated with adherence to the guidelines. Adherence to the guidelines decreased significantly as the age and the years in specialty practice increased. Also, OBGYNs working at private settings had significantly poorer adherence rates (42.9%) than their counterparts working at secondary-care public hospitals (63.9%) or tertiary-care hospitals (53.4). In multivariate analysis, however, only the "professional working setting" among these variables remained as an independent determinant of the adherence to the guidelines (OR: 0.490; 95% CI: 0.285-0.842; p=0.010 for OBGYNs working at private healthcare as compared with those working at secondary-care public hospitals).

Discussion

The current study investigated the OBGYNs' practice patterns regarding HPV testing in cervical cancer screening in Turkey. The study demonstrated that the majority of OBGYNs (81%) in Turkey used/offered HPV testing in cervical cancer screening; most (89.9%) preferred to use HPV testing as part of co-testing, a significant proportion (43.2%) used no age threshold for beginning HPV testing, and OBGYNs working at secondary-care public hospitals and those with >10 years of practice experience used HPV testing less often. The study also implied that the "professional working setting" was the sole independent determinant of the adherence to the guidelines in the management of HPV-positive test results. OBGYNs working at private settings had the worst adherence rate.

Accumulating evidence in the literature indicates that cervical screening with primary HPV testing is superior to screening with cytology alone, and is as effective as co-testing in the detection of CIN3+ lesions[10,11]. Wright et al.[10] compared the 3-year results of primary HPV testing, co-testing, and cytology, and found that the sensitivity for CIN3+ of cytology alone was 47% compared with 61% for co-testing and 76% for primary HPV testing. On the other hand, the specificity for CIN3+ was 97%, 94%, and 93% for cytology, co-testing, and primary HPV testing, respectively. The authors also noted that 3-year incidence rate for CIN3+ was lower in HPV-negative women (0.3%) than in cytology-negative women (0.8%), but was identical to that in co-testing-negative women. Gage et al.[11]

Table 3. Determinants of OBGYNs' use of the HPV testing in cervical cancer screening

Variables	Univariate			Multivariate		
	Use of HPV testing, n (%)	Test value	p	OR	95% CI	p
Sex						
Female	152/183 (83.1)	1.032	0.310[a]	-	-	-
Male	126/160 (78.8)			-	-	-
Age, years						
≤41	145/174 (83.3)	1.199	0.273[a]	-	-	-
>41	133/169 (78.7)			-	-	-
Years in specialty practice						
≤10[1]	144/170 (84.7)	2.934	0.087[a]	1.00		
>10	134/173 (77.5)			0.511	0.280 - 0.933	0.029
Subspecialty						
General OBGYN	231/290 (79.7)	3.782	0.286[a]	-	-	-
Reproductive endocrinologist	17/21 (81.0)			-	-	-
Perinatologist	11/12 (91.7)			-	-	-
Gynecologic oncologist	19/20 (95.0)			-	-	-
Professional working setting						
Secondary-care public hospital[1]	86/122 (70.5)	17.343	<0.001[a]	1.00		
Secondary-care private hospital/outpatient clinic	97/118 (82.2)			2.438	1.266-4.695	0.008
Tertiary-care hospital	95/103 (92.2)			4.906	1.932-12.458	0.001
Type of practice						
Specialist[1]	243/305 (79.7)	3.401	0.065[a]	1.00		
Academic	35/38 (92.1)			1.266	0.317-5.058	0.738

OBGYN: Obstetrician-gynecologist, HPV: Human papillomavirus, OR: Odd's ratio, CI: Confidential interval
[a]Pearson chi-square test
[1]Reference category
Variables with a p-value <0.20 were included in the multivariate logistic regression analysis

compared the risk of CIN3+ for the three strategies among approximately one million women and reported that the 3-year risk of CIN3+ following an HPV-negative result (0.069%) was lower than the 3-year risk following a cytology-negative result (0.19%) and 5-year risk following a negative co-test result (0.11%).

In a survey study conducted in the United States (US) in 2013, Darwish-Yassine et al.[19] reported that almost all (95%) of OBGYNs offered HPV testing in cervical screening, mostly as part of co-testing, and in women aged over 30 years. The rate of participants who recommended HPV testing in women younger than 30 years was only 14%. In another study from the US, which was conducted in 2015, Cooper and Saraiya[20] reported that co-testing was recommended by 95% of OBGYNs; however, the rate of participants who recommended HPV testing for women aged under 30 years was 22%. Caglioti et al.[21] investigated the practice behaviors of Italian OBGYNs in terms of HPV testing in 2015. The authors reported that the vast majority (94%) recommended HPV testing in women aged ≥30 years, but 42% stated that it is always preferable to perform HPV testing as part of co-testing. In women with an HPV-negative test, 44% recommended subsequent HPV testing at 5-year intervals, and 33% preferred a shorter interval, mainly 3 years.

Although the tendencies in our study, to some degree, are similar to the tendencies reported from the US and Italy, the frequency of using HPV testing in cervical screening (81% vs 95% vs 94%; Turkey, US and Italy, respectively) and the rate of using HPV testing as a stand-alone screening method is relatively lower than in other countries (10% vs 22% vs 25%; Turkey, US and Italy, respectively). Nevertheless, the data from all three countries reveal that primary HPV testing is not yet widespread among the OBGYNs. One notable finding of the current study was that OBGYNs working at secondary-care public hospitals

Table 4. Practice behaviors of OBGYNs regarding the management of women with an HPV-positive test result

Variables	Frequency, n (%)
What is your recommendation for women who are HPV16/18-positive and cytology-negative (negative for intraepithelial lesion or malignancy - NILM)?	
Co-testing at 12 months	29 (8.5)
Referral directly to colposcopy	270 (78.7)
Others	
Referral to gynecologic oncologist	21 (6.1)
Cytology or co-testing within less than 12 months	19 (5.5)
Cytology or co-testing within more than 12 months	1 (0.3)
HPV vaccination	1 (0.3)
LEEP	2 (0.6)
What is your recommendation for women who are positive for HPV genotypes other than HPV16/18 and cytology-negative?	
Co-testing at 12 months	226 (65.9)
Referral directly to colposcopy	64 (18.7)
Others	
Referral to gynecologic oncologist	12 (3.5)
Cytology or co-testing within less than 12 months	28 (8.2)
Cytology or co-testing within more than 12 months	13 (3.8)
Adherence to joint ACS, ASCCP, ASCP guidelines (2012) and the Turkish Ministry of Health practice guidelines (2014)[1]	182 (53.1)

OBGYN: Obstetrician-gynecologist, HPV: Human papillomavirus, LEEP: Loop electro-excision procedure, ACS: American Cancer Society, ASCCP: American Society for Colposcopy and Cervical Pathology, ASCP: American Society for Clinical Pathology
[1]Adherence to joint ACS, ASCCP, ASCP guidelines (2012) and Turkish Ministry of Health practice guidelines (2014) denotes "referral directly to colposcopy" for women who are HPV16/18-positive and cytology-negative; and "co-testing at 12 months" for women who are positive for HPV genotypes other than HPV16/18 and cytology-negative

and those with >10 years of practice experience used HPV testing significantly less often as compared with those working at tertiary-care hospitals and private settings. In Turkey, the national cervical screening program is conducted by primary-level health staff trained by the Ministry of Health for sample collection and referral of women based on a national screening algorithm. According to the Turkish Ministry of Health Screening Algorithm, women with an HPV16/18-positive test result are referred to colposcopy centers, most of which are at tertiary-care public hospitals. Secondary-care public hospitals, therefore, have a relatively small role in this program. Besides, limitations in access to HPV tests in laboratories of secondary-care public hospitals can explain why physicians working at these hospitals use HPV testing less frequently. On the other hand, the decrease in the use of HPV testing as the years in specialty practice increased can be explained by the fact that HPV testing is a relatively new method, that it started to be included in the guidelines in 2012, and that it is difficult and requires time to replace settled practices with new ones.

Our findings also suggested that the "professional working setting" is the sole independent determinant of the adherence to guidelines in the management of women with HPV-positive test results. Although OBGYNs working at secondary-care public hospitals use HPV testing at lower rates, they reveal more compliance with the guidelines compared with those working at tertiary-care hospitals and private settings. The Cancer Control Department of Turkish Ministry of Health holds educational workshops periodically for physicians working in primary and secondary public healthcare regarding cervical screening and colposcopy. These workshops, however, do not include physicians working at private healthcare institutions. The lack of coverage of continuing medical education in physicians working in private settings is responsible for the lower rates of adherence.

Study Limitations

The main limitation of the study is that the survey was conducted in two of the most developed cities of Western Turkey, which limits the generalizability of findings. As with all survey studies, this study may also be a subject to non-response bias. Additionally, the inclusion of gynecologic oncologists might have affected the results. However, the number of gynecologic oncologists included in the study was limited (only 20 of 343

Table 5. Determinants of OBGYNs' adherence to practice guidelines[1] in the management of women with an HPV-positive test result.

Variables	Univariate			Multivariate		
	Adherence to guidelines, n (%)	Test value	p	OR	95% CI	p
Sex						
Female	93/183 (50.8)	1.011	0.315[a]	-	-	-
Male	90/160 (56.2)			-	-	-
Age, years						
≤41	105/174 (60.3)	6.937	**0.008**[a]	1.00		
>41	78/169 (46.2)			0.950	0.430-2.096	0.898
Years in specialty practice						
≤10	105/170 (61.8)	9.583	**0.002**[a]	1.00		
>10	78/173 (45.1)			0.619	0.277-1.382	0.242
Subspecialty						
General OBGYN	156/290 (53.8)	2.098	0.552[a]	-	-	-
Reproductive endocrinologist	12/21 (57.1)			-	-	-
Perinatologist	4/12 (33.3)			-	-	-
Gynecologic oncologist	11/20 (55.0)			-	-	-
Professional working setting						
Secondary-care public hospital	78/122 (63.9)	11.205	**<0.004**[a]	1.00		**0.034**
Secondary-care private hospital/outpatient clinic	50/118 (42.4)			0.490	0.285-0.842	**0.010**
Tertiary-care hospital	55/103 (53.4)			0.662	0.386-1.135	0.134
Type of practice						
Specialist	160/305 (52.5)	0.884	0.347[a]	-	-	-
Academic	23/38 (60.5)			-	-	-

OBGYN: Obstetrician-gynecologist, HPV: Human papillomavirus, OR: Odd's ratio, CI: Confidential interval
[1]Adherence to practice guidelines denotes "referral directly to colposcopy" for women who are HPV16/18-positive and cytology-negative; and " co-testing at 12 months" for women who are positive for HPV genotypes other than HPV16/18 and cytology-negative
[a]Pearson chi-square test
Variables with a p-value <0.20 were included in the multivariate logistic regression analysis
Bold values denote statistical significance at the p<0.05 level

participants), and being a gynecologic oncologist does not guarantee for adherence to guidelines, as is evident (55%) in our results. On the other hand, the strengths of the study include surveying nationally representative samples of OBGYNs and a high response rate of 68.6%. The study provided insights into the attitudes of Turkish OBGYNs towards HPV testing. The findings of the study are important for designing appropriate educational interventions to improve the knowledge of physicians about screening and the management of cervical premalignant and malignant lesions.

Conclusion

Primary HPV testing is not yet widespread among Turkish OBGYNs. Most OBGYNs continue to prefer using co-testing as the primary tool for cervical cancer screening. Moreover, adherence to the guidelines in the management of women with HPV-positive test results is relatively low, particularly in OBGYNs working in private settings. There is a clear need for continuing medical education in terms of cervical screening programs and the management of women with positive screening results.

Ethics

Ethics Committee Approval: The study was approved by the local ethics committee and it 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: Informed consent was obtained from all participants.

Authorship Contributions

Concept: T.T., A.U., Design: T.T., A.U., Data Collection or Processing: B.A.A., I.Ü., S.D., Analysis or Interpretation: T.T., Literature Search: B.A.A., Writing: B.A.A., T.T.

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Energy drinks may affect the ovarian reserve and serum anti-mullerian hormone levels in a rat model

Bir rat modelinde enerji içeceklerinin yumurtalık rezervi ve serum anti-müllerian hormon üzerine etkisi

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Abstract

Objective: Energy drinks have an impact on concentration levels, physical performance, speed of reaction, and focus, but these drinks cause many adverse effects and intoxication symptoms. The main goal of this study was to determine the effect of energy drink consumption on ovarian reserve and serum anti-mullerian hormone (AMH) levels.

Materials and Methods: Female Wistar albino rats (n=16) were included and randomized into two groups (n=8). Serum AMH levels were checked before and after energy drinks were given. Eight weeks later, the ovaries and uteruses of the rats were analyzed histopathologically. The number of follicles in the ovaries was counted.

Results: The total number of the preantral plus small antral follicles, which show the ovarian reserve, was decreased at the end of eight weeks in both the control group and the energy drink group. There was a statistical difference between them (p=0.021). Also, there was a statistically significant difference in the initial/final AMH (ng/mL) reduction levels between the control group and the energy drink group (p=0.002). AMH levels were decreased more in the energy drink group.

Conclusion: The consumption of energy drinks can lead to a decrease in ovarian reserve and AMH values and may cause weight gain.

Keywords: Energy drinks, anti-mullerian hormone, ovarian reserve, antral follicles

Öz

Amaç: Enerji içecekleri belli konsantrasyonlarda fiziksel performans, reaksiyon hızı ve odaklanma üzerine etkisi vardır. Ancak bu içecekler bir çok yan etkiye ve intoksikasyon semptomlarına neden olmaktadırlar. Enerji içeceği tüketiminin yumurtalık rezervi ve serum anti-müllerian hormon (AMH) düzeylerine etkisi bu çalışmanın temel amacıdır.

Gereç ve Yöntemler: Çalışmaya Wistar albino dişi sıçanlar (n=16) dahil edildi ve iki gruba (n=8) randomize edildi. Enerji içecekleri verilmeden önce ve verildikten sonra serum AMH seviyeleri kontrol edildi. Sekiz hafta sonra sıçanların overleri ve uterusları histopatolojik olarak incelendi. Overlerdeki folikül sayıları sayıldı.

Bulgular: Hem kontrol grubunda hem de enerji içeceği verilen grupta sekiz hafta sonunda yumurtalık rezervini gösteren preantral ve small antral foliküllerin toplam sayısı azaldı ve gruplar arasında istatistiksel olarak fark izlendi (p=0,021). Öte yandan, enerji içeceği verilen sıçanlar ile kontrol grubu arasında İlk/Son AMH (ng/mL) düzeylerindeki azalma istatistiksel olarak anlamlı fark vardı (p=0,002). Enerji içeceği verilen sıçanlarda AMH seviyeleri daha fazla düştüğü görüldü.

Sonuç: Enerji içeceklerinin tüketimi yumurtalık rezervinde ve AMH değerinde azalmaya yol açabilir ve kilo alımına sebep olabilir.

Anahtar Kelimeler: Enerji içecekleri, anti-müllerian hormon, yumurtalık rezervi, antral foliküller

PRECIS: Energy drinks became much more popular. These drinks are categorized as sugar sweetened beverages. People need to be careful about the energy drink consumption in terms of reproductive health.

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Introduction

Energy drinks (EDs) have become much more popular since the 1960s[1]. These drinks are categorized as sugar-sweetened beverages. They also contain caffeine, taurine, glucuronolactone, and other vitamins and mineral additives[2]. EDs have been the fastest growing area of the beverage industry to date. In advertisements, companies assert that these drinks have a good impact on concentration levels, physical performance, speed of reaction, focus, and wellness[3]. Despite the positive effects, these drinks cause many cardiovascular adverse effects and intoxication symptoms causing concerns about the health of the consumers[4]. From 2001 to 2008, the level of ED consumption in adolescents and adults was estimated to have increased from 24% to 56%, causing greater concern[5].

EDs have been completely prohibited or sold in low caffeine forms in some countries because of their adverse effects. Turkey is one of the countries that prohibit the high caffeine forms[6], but in most countries, EDs are qualified as nutrient support and there are no restrictions[6]. The United States of America Food and Drug Administration updated the classification of EDs as dietary supplements[6]. The percentages of each ingredient are different in every brand[6]. The most prevalent ingredients of EDs are caffeine, taurine, glucuronolactone, vitamin B complex, and other herbal stimulants, most of which have been studied little[6].

In terms of the reproductive system, studies have shown that caffeine probably decreases the estrogen and progesterone levels in the luteal phase and increases the risk of a shorter menstrual cycle (<25 days)[7]. However, studies have also shown that EDs have little effect on ovarian aging with their ovulation stimulant effect[7].

The second major component in EDs is taurine[8], which is the richest amino acid in mammalian cells and it plays a major role in many important biologic events. It acts as a neurotransmitter, and an osmoregulatory and antioxidant agent in many tissues[8]. In female rats, taurine exists in uterine tissue, uterine fluid, ovarian theca cells, and in cells that are responsible for androgen synthesis[8]. Taurine is a popular agent that is presented as a performance-enhancing agent and accepted to be safe, but many researchers think that the effects of high-dose taurine in EDs should be studied[9].

Other components are carbohydrates (glucuronolactone) and vitamin B complex. Although they show the effects of vitamins to caffeine and taurine, they act as coenzymes[10]. Carbohydrates, on the other hand, exist to provide energy to increase metabolism. However, consuming carbohydrate beverages may increase the risk of metabolic syndrome and weight gain may cause infertility[9]. It should be remembered that obese and overweight individuals have reduced fertility[11]. In this study, we aimed to analyze the effect of EDs on ovarian reserve in rats by examining AMH levels and ovarian histopathology.

Materials and Methods

The study was approved by the local ethics committee of the Yüzüncü Yıl University Faculty of Medicine Department in Van, Turkey, for the use of laboratory animals and was performed at the Experimental Surgery Training and Education Center at the same hospital (approval number: 2015-HIZ-TF290).

Animal Maintenance and Treatment

In this study, sixteen healthy adult female albino rats (8 to 10 weeks old) weighing 190±10 g were used. The animals were kept according to the institutional review board's guidelines for animal care, in a 14-hour light cycle at controlled temperatures (22-28 °C), and food and water were available ad libitum. The water consumption of the rats was not recorded. The weight of the rats was recorded daily and the food they consumed was recorded weekly. After the acclimation period, the stages of the estrus cycles of the rats were evaluated by performing daily vaginal smears.

The rats were randomly assigned to two study groups (8 rats each). In the control group (group I), the rats were kept on a normal diet and given water for 8 weeks. In the ED group (group II), the rats were kept on a normal diet and given water plus a daily single dose of ED. The dosing was calculated in comparison with the surface area of humans and rats (3.9 mL/kg b.w.)[12]. A 250-mL can of commercially available ED (A-5330 Fuschl am See, Austria) was opened daily between 09:00 and 10:00 and approximately 0.7 mL was given orally for each rat via flexible oral gavage tubes. A single dose of the ED is roughly equivalent to the minimal human dose [1 can (250 mL)/day], but of course it varies according to the animal's surface area. Each 100 mL of ED contains a mixture of water, taurine (0.4%) (400 mg), caffeine (0.032%) (320 mg/L), gluconolactone (0.24%), inositol, sucrose, glucose, sodium citrate, carbon dioxide niacin (8 mg), pantothenic acid (2 mg), vitamin B6 (2 mg), B12 (0.002 mg), caramel, riboflavin, and natural and artificial flavoring and coloring agents (these are listed ingredients on the label).

Blood Sampling, Tissue Collection, and Histopathologic Analysis

After the acclimation at the beginning of the study (initial) and following the 8-week period (final), blood samples (1 mL) were obtained from the right jugular vein of each rat to measure the serum AMH levels under general anesthesia. The animals were anesthetized by administering 50 mg/kg 10% ketamine hydrochloride (Ketasol; Richter Pharma) and 5 mg/kg 2% xylazine (Rompun; Bayer Healthcare) intramuscularly. All blood samples were immediately centrifuged at 4000 *g* for 10 minutes, and the collected sera were transferred to Eppendorf tubes. The samples were then transferred on ice and kept at -80 °C in a deep freeze until analysis using an automatic enzyme-linked immunosorbent assay (ELISA)[13] system with a commercially available kit (Cusabio Biotech Co, Wuhan, China). The AMH assay measured concentrations with an assay

range of 0.2-15 ng/mL; the manufacturer-specific mean inter and intra-assay coefficient of variation (CV) was less than 15% (CV<15%). All samples and standards were assayed in duplicate as recommended in the catalogue of AMH.

The rats were sacrificed in estrus cycles using cervical dislocation and bilateral oophorectomies and hysterectomies were performed in all rats. Histologic ovarian and uterus tissue samples were evaluated by a single histopathologist who was blinded to the origin of the samples. The volumes of the ovaries and uteruses were measured under a microscope. Tissues were fixed in 10% formaldehyde for 72 hours, underwent routine tissue processing, and then embedded in paraffin wax. Four-micron-thick sections were taken from the tissues and the tissues were completely consumed. All sections were stained with hematoxylin and eosin. All sections were investigated under a light microscope (Zeiss Axioskop 40 Carl Zeiss Göttingen, Germany) and the pieces were photographed (AxioVision 3.1 Zeiss Axioplan 2 imaging Germany, Göttingen). These sections were evaluated for follicle counting, with one in four sections in the order of sections. The histologic examination method was performed according to the model of Durlinger et al.[14,15].

Primordial follicles are nongrowing follicles and consist of an oocyte partially or completely encapsulated by flattened squamous pregranulosa cells. Early primary follicles have initiated development and contain at least one cuboidal (enlarged) granulosa cell[16]. In addition, primordial follicles and early primary follicles, which can be distinguished by their size: follicles with a mean diameter less than or equal to 20 µm are classified as primordial follicles, and follicles with a mean diameter greater than 20 µm are classified as growing follicles [preantral (PA), small antral, and large antral follicles][14,15]. The follicles were divided into four groups according to the average diameter determined by measuring two vertical diameters in the section where the oocyte nucleolus was[14,15]; primordial follicles (≤20 µm) (Figure 1a, PF (20-220 µm) (Figure 1b,c), SF (221-310 µm) (Figure 1d,e), and LF (311-370 µm) (Figure 1f). Atretic follicles were excluded from the count.

Stereologic methods were used as the assessment method of ovarian and uterine volume measurements. Stereology is a method by which random and quantitative information is obtained by using random systematic sampling. From the stereologic analysis, the modified method of the dissector Cavalieri principle was used[17]. The endometrium and ovarian volume ratio was measured using the dotted area ruler given in the Shetereom Ver. 1.5 package program[17,18].

Vaginal smears were taken at the same time daily in over 8 weeks. The length and layout of the estrous cycles were evaluated using vaginal smears. Dried smears were examined microscopically and the estrus cycle stage was determined according to the criteria of Allen[19]. No differences were found between the two groups of rats in either the length or the regularity of the estrous cycle; regular estrous cycles with a length of 4-5 days were found (results not shown).

The rats were weighed individually at the beginning and eight weeks later. The rats that drank the ED weighed more compared with those that drank water.

Statistical Analysis

Descriptive statistics for the studied variables (characteristics) are presented as median, mean, standard deviation, minimum, and maximum values. The Mann-Whitney U test was performed to compare the groups. A statistical significance level was considered as 5% and the Statistical Package for the Social Sciences (SPSS) (Ver. 22) statistical program was used for all statistical computations.

Result

There was statistical significance between the means of the weight changes of the two groups (p=0.002) (Table1). The weekly consumed pellets of the rats showed a statistical difference between group I and group II in regards to food consumption at the end of the 8 weeks (p=0.001) (Table1).

The uterus (n=16) and ovarian tissues (n=32) excised from the rats after fourteen cycles were evaluated morphologically. When the ovarian tissues of the rats that were given EDs were compared with the control group, their mean ovarian volume was smaller (10.78±2.9 mm^3) but there was no statistically significant difference (p=0.99). The endometrium, myometrium, and serosa layers of the uteruses of both groups were histologically normal. Endometrial volumes (mm^3) of both groups were evaluated in stereology in terms of endometrium thickness. The mean volume of endometrium in the ED group (38.83±21.2) was more than in the control group (29.28±14.48) (Table 2), but there was no statistically significant difference (p=0.565) (Table 2). According to these findings, the ED that was used in the experimental group did not affect the endometrium or other layers of the uterus.

The total follicles in the ovaries were evaluated. There was no statistically significant difference between the follicles of the two groups (p=0.283). In terms of ovarian reserve analysis, the number of PF, SF, and PA plus small antral follicles (PSF) were counted. Even though the means of PF and SF were decreased more in the subject group, there was no statistically significant difference between the two groups (p=0.026, p=0.057). Furthermore, the means of the total number of PSFs were decreased more in the subject group (160±30.9) than in the control group (133±28.6) and a statistical significance was shown between the two groups (p=0.021) (Table 3).

No statistically significant difference was seen between the mean levels of AMH taken from the jugular veins of the rats at the beginning and eight weeks later, but a statistically significant difference was shown between the means of decrease in the levels of AMH (p=0.002). The decrease was shown to be more in the subject group (Table 4).

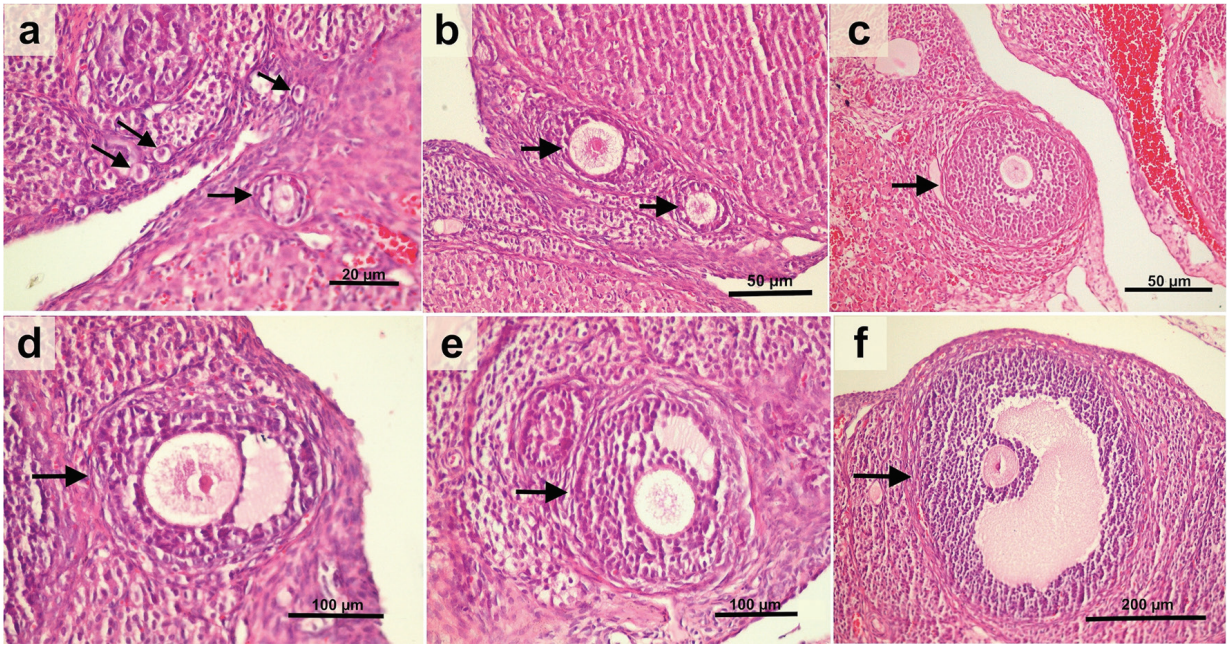

Figure 1(a). Light micrograph showing primordial follicles (≤20 µm) (Bar=20), (b) and (c) arrowheads indicate preantral follicle (20-220 µm) (Bars=50), (d) and (e) arrowheads indicate antral follicle (221-310 µm) (Bars=100 µm), (f) arrowhead indicate large antral follicle (311-370 µm) (Bar=200 µm). hematoxylin and eosin

Table 1. Means of weight changes between the groups

		Group-I (Control) (n=8)	Group-II (Energy drink) (n=8)	p-value
Initial (g)	Mean ± SD Med (min-max)	191±5.58 190 (184-200)	190.2±6.9 191 (179-201)	0.833
Final (g)	Mean ± SD Med (min-max)	212±9.4 210 (203-228)	230±10 227 (213-243)	**0.006***
Change (g) (Final-Initial)	Mean ± SD Med (min-max)	21±3.4 22 (18-28)	39.7±9.4 42 (24-54)	**0.002***
Total consumed feed (g)	Mean ± SD Med (min-max)	1127.7±37.42 1110 (1036-1151)	1283.6±54.99 1290 (1185-1353)	**0.001***

(*)Significantly different from group I and group II ($p<0.05$), SD: Standard deviation, min: Minimum, max: Maximum

Discussion

Ovarian function is very important for reproductive health. Follicles play a key role in the reproductive function of the ovaries[20]. In the development of follicles, along with several local factors, systemic (hypothalamus and/or pituitary) mediators affect their functions at a certain stage[20]. AMH is the most important mediator indicating the functions of follicles and providing information about their reserves. PF and AF counts are responsible for the synthesis of this mediator[20,21]. It was reported that AMH levels were a better indicator for ovarian reserve than age, follicle-stimulating hormone (FSH), luteinizing hormone (LH), inhibin-B, and, estradiol (E_2)[21,22]. AMH is a dimeric glycoprotein that belongs to the transforming growth factor family[23]. AMH protein expression starts immediately after the follicle recruitment and continues to the antral stage of the follicle[22,24]. PFs are the main source of folliculogenesis in the ovaries[20]. As soon as PF development begins, AMH plays a protective role by slowing down the rate of consumption of the local primordial follicle pool from the granulosa cells[14]. AMH also regulates the growth rate of follicles by inhibiting FSH-related follicle growth in the early antral period[14]. Three-quarters of all AMH is found in PFs and SA follicles[22]. In follicles without AMH in rats, preliminary estrus cycle loss was observed due to the rapid depletion of the primordial pool[15]. Loss of PFs causes irreversible infertility[10,15].

The common active ingredients used in EDs are caffeine, taurine, sugar, and a vitamin complex. The biggest difference between these drinks and sugary drinks is that they contain caffeine and taurine.

Table 2. Comparison of ovarian and uterine volumes of two groups

	Group-I (Control)	Group-II (Energy Drink)	p-value
Ovarian volume (mm^3) (n=16) Mean ± SD Med (min-max)	11.13±2.63 11.18 (8.27-15.97)	10.78±2.9 9.28 (8.71-15.96)	0.990
Endometrium volume (mm^3) (n=8) Mean ± SD Med (min-max)	29.28±14.48 22.3 (16.6-50.1)	38.83±21.2 40.5 (10.7-65.8)	0.565

(*)Significantly different from group I and group II (p<0.05), SD: Standard deviation, min: Minimum, max: Maximum

Table 3. Classification and comparison of ovarian follicles in terms of dimensions

		Group-I (Control) (n=16)	Group-II (Energy Drink) (n=16)	p-value
Primordial follicle	Mean ± SD Med (min-max)	761.3±310 708 (369-1505)	653.13±2.76 629.5 (59-1176)	0.439
Preantral follicle (PF)	Mean ± SD Med (min-max)	105±33.3 82 (82-164)	89.6±30.7 79 (41-164)	0.206
Small antral follicle (SF)	Mean ± SD Med (min-max)	55.8±17 61 (30-78)	43.9±12.79 44.5 (24-68)	0.057
Large antral follicle	Mean ± SD Med (min-max)	16±9.4 14 (3-31)	17.1±10.7 15 (4-35)	0.763
Preantral follicle plus small antral follicle (PSF)	Mean ± SD Med (min-max)	160±30.9 152 (112-230)	133±28.6 132 (69-199)	0.021*
Total Follicles	Mean ± SD Med (min-max)	939±325 855 (545-1352)	803.8±288.8 787 (399-1380)	0.283

(*)Significantly different from group I and group II (p<0.05), SD: Standard deviation, min: Minimum, max: Maximum

Table 4. Comparison of AMH levels between the groups

		Group I (Control) (n=8)	Group-II (Energy Drink) (n=8)	p-value
Initial AMH (ng/mL)	Mean ± SD Med (min-max)	6.19±0.92 6.39 (4.45-7.35)	5.97±1.31 6.1 (4-8)	0.713
Final AMH (ng/mL)	Mean ± SD Med (min-max)	5.93±0.86 6.2 (4.21-6.5)	5.32±1.33 5.28 (3.33-7.44)	0.294
Initial/Final AMH (ng/mL) reduction	Mean ± SD Med (min-max)	-0.26±0.13 -0.24 (-0.5/-0.05)	-0.65±0.22 -0.63 (-0.99/-0.36)	0.002*

(*)Significantly different from group I and group II (p<0.05), SD: Standard deviation, min: Minimum, max: Maximum, AMH: Anti-mullerian hormone

Caffeine acts as a phosphodiesterase (PDE) inhibitor[10]. PDE 2 is the enzyme that destroys the signal path stimulant cyclic adenosine monophosphate (cAMP). cAMP is a mediator in intracellular signaling pathways[10]. The most common stimulation effect of caffeine is causing the increase of cAMP values in neurons and the discharge of neurotransmitters[10]. In

some publications, it was suggested that caffeine might have a minimal effect on the menstrual cycle when taken as 300 mg/day or more, and could also stimulate ovulation[7]. A study performed on rats in puberty concerning the effects of caffeine on the ovary suggested that rather than indirectly, it increased estrogen release directly and inhibited the development of follicles[25]. It was stated that, with caffeine consumption, even though E_2 levels increased, the effectiveness of estrogen in the target tissue decreased due to the direct effect of caffeine[25]. In another study, it was stated that high cAMP levels prevented germinal vesicle destruction and further oocyte maturation including polar body release[10]. Most PFs wait, dormant in the follicle pool, some develop, and proceed to the primary follicle phase[20]. In a study performed on postnatal rats, the number of PFs decreased in the caffeine-treated group and the size and number of secondary and AFs did not differ compared with the control group. This was attributed to the atresia of PFs due to the toxic effect of caffeine and to the replacement of developing secondary follicles with PFs[26]. In a subsequent study, the authors proposed the hypothesis that caffeine induced this damage by causing DNA damage in cells during cell division[10]. Caffeine has been reported to inhibit DNA repair and suppress meiosis in female mice[23,27]. In our study, no significant decrease was observed in the PF pool (p=0.439). We think that caffeine slows the growth of PFs. Although there is a decrease in AMH levels, we think that the insufficient growth of PFs is due to caffeine. This increased inhibition may cause cell damage in PFs in the long term.

Taurine is an important amino acid that has many functions in the body and is found in many organs[8]. Taurine is found in oocytes, granulosa, and theca cells, especially in the epithelial cells of the ovary and uterus. It was reported that cells achieve this through cysteine sulfinic acid decarboxylase[28]. However, there is mRNA-carrying taurine in the ovaries[29]. It was reported that in rats, taurine stimulated follicular development indirectly by the release of FSH, LH, and E_2 through the hypothalamic-pituitary axis, or directly through E_2 produced in granulosa cells by increasing androgen synthesis in the osmoregulator or theca cells[30]. In an ***in vitro*** study, it was reported that taurine could directly stimulate follicular development, as well as work as an osmotic regulator in embryos, mouse and human oocytes, and maintain the development of follicles and embryos[31]. In another ***in vitro*** study on rats, it was suggested that taurine directly stimulated the development of follicles through several ambiguous ways[32]. In an ***in vitro*** study on cattle, it was concluded that taurine was not useful in the embryonal development of bovine oocytes as a direct effect. In this study, we propose that the hormonal effect and growth affect the PSF follicle pool, which is the highest, the number of follicles due to stimulation in the PSF pool decreases (p=0.021) and accelerates the preovulatory oocyte passage with a decrease in AMH. We suggest that PFs, whose functions are inhibited, do not provide a reduced PSF pool despite the decrease in AMH, and taurine does not affect PFs because it has no intracellular function. In our study, the number of large AFs increased in subjects given ED. However, we suggest that taurine accelerated the development of oocytes that entered the growth cycle and increased the number of preovulatory oocytes due to the increased LF count (group I; 16±9.4, group II; 17.1±10.7), even though there was no statistical difference (p=0.283).

Some authors suggest that lipids are stored in adipose tissue as a result of increased lipogenesis due to excessive stimulation of insulin due to sugar components of EDs and increased carbohydrate metabolism[33], and other authors suggested that the increased sugar level inhibited the satiety center and that there was weight gain due to the increase in energy intake[34,35]. However, no weight gain was observed in approximately 4-week studies on rats and rabbits[36,37]. In our study, significant weight gain was observed at the end of eight weeks in the ED group (p=0.002). However, a significant increase in food intake was also observed in the ED group (p=0.001). Therefore, like previous hypotheses[33], we think that the sugar in the ED increases the level of insulin, causing an increase in catabolism and high lipid storage rates in adipose tissues, and that the increase in insulin level increases food consumption by causing a feeling of hunger. However, further studies are needed to explain this situation clearly.

Study Limitations

This study is an animal experiment and there is no literature on the dose for ED use. Another difficulty of the study is that there are many components in EDs. The limitation of our study in the histologic examination is that immunohistochemical staining showing PF activity could not be performed. The effect of EDs on fertility could not be evaluated because the reproduction of the rats during the study period could not be controlled.

Conclusion

The substances in EDs have a dominant effect on ovarian function in certain periods, for example, caffeine for inhibition, taurine as a stimulant and increased catabolism, and sugar for weight gain. However, we think that further studies are needed on how and in which ways they act separately and in combination for each substance in EDs.

Ethics

Ethics Committee Approval: The study was approved by the local ethics committee of the Yüzüncü Yıl University Faculty of Medicine Department in Van, Turkey, for the use of laboratory animals and was performed at the Experimental Surgery Training and Education Center at the same hospital (approval number: 2015-HIZ-TF290).

Informed Consent: Stereology is a method by which random and quantitative information is obtained by using random systematic sampling.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: S.S., Concept: S.S., Design: R.Y., Data Collection or Processing: G.G.E., N.Ç., Analysis or Interpretation: İ.A., Literature Search: E.E., Writing: E.E.

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

Financial Disclosure: Authors have no financial interests about the research.

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The effect of embryo transfer technique on pregnancy rates in *in vitro* fertilization-intracytoplasmic sperm injection cycles: A prospective cohort study

İn vitro fertilizasyon intrasitoplazmik sperm enjeksiyon sikluslarında embriyo transfer tekniğinin gebelik sonuçlarına etkisi

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Abstract

Objective: To investigate whether embryo transfer affects pregnancy rates in *in vitro* fertilization-intracytoplasmic sperm injection (IVF-ICSI) treatment.
Materials and Methods: A total of 2,257 patients who underwent IVF-ICSI treatment between 2012 and 2017 were included in this study. Subjects were categorized according to the embryo transfer technique that was required: group 1 (n=1,657) underwent easy transfer with a soft catheter; group 2 (n=548) received external guidance transfers; and group 3 (n=52) experienced difficult transfers with a stylet. Basal parameters, clinical and laboratory IVF-ICSI outcomes, and clinical pregnancy rates (CPR) were compared between the groups.
Results: There were no differences between the groups in terms of age, body mass index, smoking status, duration and etiology of infertility, baseline follicle-stimulating hormone, luteinizing hormone, estradiol (E_2), thyroid-stimulating hormone, prolactin levels, antral follicle count, duration of stimulation, stimulation protocol, total gonadotropin dose required, peak E_2 levels, progesterone levels, and endometrial thickness on human chorionic gonadotropin administration and transfer days (p>0.05). The numbers of oocytes retrieved, MII and 2PN, fertilization rate, day of embryo transfer, and CPRs were also comparable between the groups (p>0.05).
Conclusion: Our data suggest that embryo transfer has no impact on pregnancy rates in patients who undergo IVF-ICSI treatment. Further studies with more participants are required to elucidate this situation.
Keywords: Embryo transfer, infertility, clinical pregnancy rate, assisted reproductive technology

Öz

Amaç: *İn vitro* fertilizasyon-intrasitoplazmik sperm enjeksiyonu (IVF-ICSI) tedavisinde embriyo transfer tekniğinin gebelik oranları üzerinde bir etkisi olup olmadığını araştırmak.
Gereç ve Yöntemler: 2012-2017 yılları arasında IVF-ICSI tedavisi gören toplam 2.257 hasta çalışmaya dahil edildi. Denekler embriyo transfer tekniğine göre üç gruba kategorize edildi: grup 1 (n=1657) yumuşak kateterle kolay transfer edilenler, grup 2 (n=548) dış harici kateterle transferleri edilenler, ve grup 3 (n=52) zor transfer yapılanlar. Gruplar arasında bazal parametreler, klinik ve laboratuvar IVF-ICSI sonuçları ve klinik gebelik oranları (KGO) karşılaştırıldı.
Bulgular: Gruplar arasında yaş, vücut kitle indeksi, sigara içme durumu, infertilite süresi ve etiyolojisi, folikül uyarıcı hormon, lüteinizan hormon, östradiol (E_2), tiroid uyarıcı hormon, prolaktin düzeyleri, antral folikül sayısı, infertilite süresi, stimülasyon günü, stimülasyon protokolü, gerekli toplam gonadotropin dozu, tepe E_2 seviyeleri, progesteron düzeyleri ve endometriyal kalınlık (human kayronik gonadotropini uygulanan gün) açısından fark yoktu (p>0,05). Alınan oosit sayısı, MII ve 2PN, döllenme oranı, embriyo transferi günü ve KGO'da gruplar arasında benzerdi (p>0,05).
Sonuç: Verilerimiz, embriyo transfer tekniğinin IVF-ICSI tedavisi gören hastalarda gebelik oranları üzerinde hiçbir etkisi olmadığını göstermektedir. Bu durumu açıklamak için daha fazla katılımcıyla daha fazla çalışma yapılması gerekmektedir.
Anahtar Kelimeler: Embriyo transfer tekniği, infertilite, klinik gebelik oranı, yardımcı üreme teknolojisi

PRECIS: Embryo transfer technique has no impact on pregnancy rates in patients who have undergone IVF-ICSI treatment.

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Introduction

Despite all the developments in assisted reproductive technology (ART) since the first live birth following *in vitro* fertilization (IVF) in 1978, pregnancy rates have remained at around 35-45%[1-4]. In ART cycles, the method of embryo transfer (ET) is important to clinical pregnancy success in addition to features such as age, the endometrial receptivity of the infertile woman, and embryo quality[5-8]. It has been claimed that faulty ET is responsible for 25-30% of failed implantations, relating either to the catheter application technique or the experience of the physician performing the ET procedure[9-12].

It has also been asserted that performing mock ET before IVF-intracytoplasmic sperm injection (ICSI) can improve the success of the real ET[9], but studies have also shown that a mock procedure does not reflect all problems that may occur during the actual ET[5,9]. Consequently, afterloading ET with external guidance has been suggested to avoid problems that may be encountered[5,11]. In the afterloading technique, a catheter with an inner sheath is inserted into the external cervical os, passed through the cervical canal to exit the internal os, and then advanced to 10 mm of the uterine fundus by gentle movement and guided by ultrasonography (USG). A second catheter with embryo-loaded inner sheath is inserted along the same pathway from the first retracted catheter and advanced to 10 mm of the uterine fundus, and the embryos are released into the endometrium[11]. The process is intended to minimize embryonic and endometrial trauma[5].

In this study, we aimed to investigate whether the ET technique used affects the pregnancy rates of patients who undergo IVF-ICSI treatment.

Materials and Methods

Study Participants and Data Collection

A prospective cohort study was conducted at Ali Kemal Belviranlı Women's Health and Children's Hospital, IVF Unit. Outcomes of 2257 fresh ICSI cycles were included consecutively between January 2012 and December 2017. Women were included in the study if they were aged 20-44 years. All of the patients had a body mass index (BMI) between 18 and 35 kg/m^2, regular menstrual cycles, no uterine abnormalities in an ultrasound examination, and normal baseline hormonal levels. Participants were excluded from the study if they were aged ≥45 years, BMI ≥35 kg/m^2, any significant illness or metabolic disorders. Ethics board approval was given from the institutional review board (2012/57). Written and oral informed aggrement was given from the participants.

Data were obtained for age, BMI (kg/m^2), smoking status, infertility period, cause of infertility, the baseline at day 3 for follicle-stimulating hormone (FSH), luteinizing hormone (LH), and estradiol (E_2) levels, thyroid-stimulating hormone (TSH), prolactin, antral follicle count, stimulation parameters, IVF-ICSI outcomes, and clinical pregnancy rates (CPR).

Ovarian Stimulation and Oocyte Retrieval

Controlled ovulation stimulation was negotiated using the gonadotropin-releasing hormone agonist (GnRHa) or the flexible gonadotropin-releasing hormone antagonist (GnRHant) protocol.

The GnRHa Protocol: First, pituitary down-regulation was achieved with a GnRH agonist. Then, the exogenous gonadotropins (Puregon; Organon, Oss, the Netherlands, or Gonal F; Serono, Istanbul, Turkey) were used for ovarian stimulation. The GnRH agonist leuprolide acetate (Lucrin; Abbott Cedex, Istanbul, Turkey) was preferred subcutaneously daily from day 21 of the preceding luteal phase (0.5 mg/day, sc) until menstruation, and then the dose was decreased to 0.25 mg/day until ovulation was triggered. The initial gonadotropin dose used for ovarian stimulation was started according to the patient's age, baseline serum FSH concentrations on day 3, BMI, and previous response to ovarian stimulation. The starting regimen was fixed for the first 3 days (100-225 IU recombinant FSH/day). Thereafter, the dose of gonadotropin was adjusted according to the individual ovarian responses, which were monitored by measuring serum E_2 levels and transvaginal USG (LOGIC 200 PRO, GENERAL ELECTRIC, Seoul, South Korea). The administration of 250 IU recombinant human chorionic gonadotropin (hCG) (Ovitrelle, Serono, Istanbul, Turkey) was preferred for the ovulation triggering when at least two follicles reached 18 mm in diameter. Oocytes were retrieved 36 h after the hCG injection, and ICSI was performed for all patients undergoing IVF-ET.

Microdose Flare-up Protocol: Recombinant FSH (Puregon; Organon, Oss, the Netherlands, or Gonal F; Serono, Istanbul, Turkey) and the GnRH agonist leuprolide acetate daily together (Lucrin; Abbott Cedex, Istanbul, Turkey) were administered subcutaneously (0.5 mg/day, subcutaneously for 5 days) on day 3 of a withdrawal bleed following at least 3 weeks of oral contraceptive use. The initial gonadotropin dose used was individualized according to the patient's age, baseline serum FSH concentration on day 3, BMI, and previous response to ovarian stimulation. The starting regimen was fixed for the first 3 days (100-225 IU recombinant FSH/day). Thereafter, the dose of gonadotropin was adjusted according to the individual ovarian responses, which were monitored by measuring serum E_2 levels and transvaginal USG (LOGIC 200 PRO, GENERAL ELECTRIC, Seoul, South Korea). Ovulation was triggered by the administration of 250 IU recombinant hCG (Ovitrelle, Serono, Istanbul, Turkey) when at least two follicles reached 18 mm in diameter. Oocytes were retrieved 36 h after the hCG injection, and ICSI was performed for all patients undergoing IVF-ET.

The GnRHant Protocol: The flexible GnRHant protocol was used for the pituitary down-regulation. Recombinant human FSH (r-FSH; Gonal-F, Merck-Serono, or Puregon, MSD) or human menopausal gonadotropin (hMG; Menogon or Menopur; Ferring) was preferred for COH. The initial gonadotropin

dose used for ovarian stimulation was started according to the patient's age, baseline serum FSH concentrations on day 3, BMI, and previous response to ovarian stimulation. The starting regimen was fixed for the first three days (150-225 IU rec FSH/day), and thereafter, the gonadotropin dose was adjusted according to the individual's ovarian response. Serial estrogen levels were measured and two-dimensional follicle measurements by transvaginal USG (LOGIC 200 PRO, GENERAL ELECTRIC, Seoul, South Korea) were assessed. A daily dose of 0.25 mg of GnRHant (Cetrotide, Merck-Serono, or Orgalutran, MSD) was started when the leading follicle diameter was ≥13 mm or the serum E_2 level reached ≥300 pg/mL. When at least two dominant follicles reached dimensions of 18 mm or greater in diameter, hCG (250 µg, Ovitrell, Merck-Serono) was administered, and oocytes were retrieved 36 hours after the hCG injection. ICSI was then applied following our clinical procedures.

ET Procedure

All ETs were performed with a full bladder under USG guidance (Logiq 200 Pro, General Electric, Seoul, South Korea) using an ET catheter system (Rocket Genesis R57630-00-23 and R57591-00-23). The degree of difficulty of each ET was determined according to the opinion of two physicians as either easy (with a soft catheter), moderate (with external guidance), or difficult (with stylet). With the patient in the lithotomy position, a sterile speculum was introduced to the vagina and the cervix visualized. If mucus or other debris were present, these were cleared using sterile cotton swabs.

An embryologist loaded the embryos into a soft transfer catheter, which was passed to the ET physician who deposited the embryos approximately 10 mm from the uterine fundus under USG guidance. The catheter was gently removed after 5 seconds. In cases of ET with external guidance, an initial catheter with an inner sheath was inserted into the external cervical os, and then advanced through the cervical canal and internal os to 10 mm of the uterine fundus using USG. The internal sheath was withdrawn, and a second catheter loaded with embryos was introduced in its place and advanced to approximately 10 mm from the uterine fundus where the embryos were deposited. "Difficult transfer" was defined if the use of a stylet was required in addition to this form of external guidance.

All catheters were immediately flushed with media to check for embryos, blood, or mucus, and the patient remained in the Trendelenburg position for about 10 minutes. Patients in whom tenaculum was used were excluded from the study. Progesterone in the form of Crinone 8% gel (Serono, Istanbul, Turkey) at a daily dosage of 90 mg for 14 days was given for luteal phase support. Subjects were categorized according to the ET technique required: group 1 (n=1657) experienced an easy transfer with the soft catheter; group 2 (n=548) required afterloading external guidance; and group 3 (n=52) underwent a difficult transfer using a stylet. Basal parameters, clinical and laboratory IVF-ICSI outcomes, and pregnancy rates were compared between the groups.

Statistical Analysis

Statistical analyses were performed using the SPSS 15.0 for Windows software package (SPSS, Chicago, IL, USA). The Shapiro-Wilk test was used for examining the continuous variables with normal and abnormal distributions, and One-Way analysis of variance (ANOVA) was used for normally distributed continuous variables. The Kruskal-Wallis test was used for abnormally distributed continuous variables. When the Kruskal-Wallis test indicated statistically significant differences, the causes of those differences were determined using a Bonferroni-adjusted Mann-Whitney U test. Categorical data were analyzed using Pearson's chi-square test, and Fisher's Exact test was used if the expected frequency was less than 5 in >20% of all cells. Continuous variables are presented as mean ± standard deviation and categorical variables are presented as the number of cases and percentages. For all possible multiple comparisons, Bonferroni-adjustment was performed to control for type I errors. Statistical significance was accepted at $p<0.05$. The sample size calculation was performed using the DSS statistical software package for research sample size calculations [13]. The primary aim of this study was to compare the differences in CPR between the groups. It was calculated that a minimum of 50 participants in each group would be required to demonstrate a difference of at least 10% between the groups, with a power of 80% at the 5% significance level. This difference of 10% was taken both from a pilot study and our clinical experiments.

Results

A total of 163 patients were excluded from the study, specifically those with aged ≥45 (n=51), BMI ≥35 kg/m^2 (n=37), systemic disease (n=31), endocrine or metabolic disorders (n=27), and concomitant medication (n=17). The remaining 2,257 patients were classified into the three ET groups and their outcomes analyzed (Figure 1).

A comparison of the sociodemographic and stimulation characteristics of the participants is provided in Table 1. There were no differences between the groups in terms of age, BMI, smoking status, duration and etiology of infertility, baseline FSH, LH, E_2, TSH, prolactin levels, antral follicle count, duration of stimulation, stimulation protocol, total gonadotropin dose required, peak E_2 levels, progesterone levels, and endometrial thickness on hCG administration and transfer days ($p>0.05$).

The laboratory and reproductive outcomes of the participants are summarized in Table 2. The numbers of oocytes retrieved, MII and 2PN, fertilization rate, the day of ET, and the CPR were comparable between the groups ($p>0.05$).

Discussion

The current study aimed to investigate whether the ET technique required during IVF-ICSI treatment affected pregnancy rates. We found no such impact in our study population.

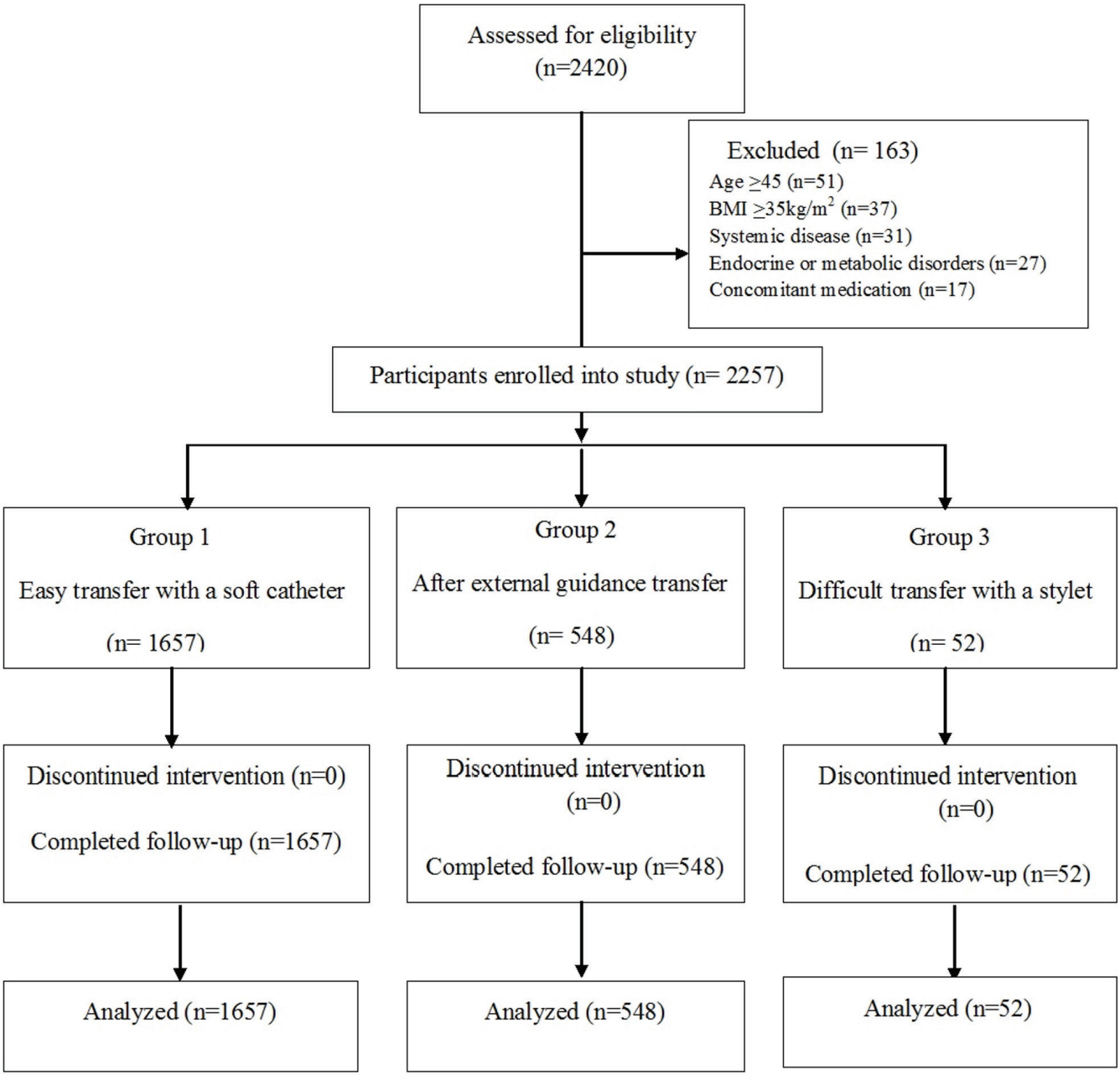

Figure 1. Enrollment and follow-up of the study subjects

Some existing studies have shown that the type of ET used can have as significant an effect on pregnancy rates in IVF-ICSI cycles as clinical and embryologic features, but different results have been reported[5-10]. In particular, Yılmaz et al.[5] retrospectively compared the effects of undergoing easy, moderate, or difficult ET on CPR in 313 IVF-ICSI cycles and found that difficult ET was associated with lower CPR but with no statistically significant difference. Elsewhere, Burke et al.[14] compared 159 cases of fresh ET with 46 frozen ETs and found that the technique and type of catheter used did not affect CPR. Cervical dilatation was performed under general anesthesia in IVF-ICSI cycles of 57 patients who had previously experienced difficult ET; easy ET was performed with a soft catheter in 70% of the participants with the remaining 30% experiencing difficult transfer, and an increased pregnancy rate was observed in those who underwent soft catheter ET[15]. Similarly, a meta-analysis of 23 randomized controlled trials reported that ET performed with soft catheters had an overall positive effect on CPR, with the authors suggesting this was due to minimizing uterine contractions[16].

In a retrospective analysis comparing easy and difficult ET with a total of 7,714 ETs included, CPR was found to be significantly

Table 1. Demographic and stimulation characteristics of the patients

		Group 1 (n=1657)	Group 2 (n=548)	Group 3 (n=52)	p
Age (years)		29.50±4.70	29.64±4.59	30.51±4.55	0.383
BMI (kg/m²)		25.63±4.29	25.99±4.74	26.24±4.37	0.616
Smoking rate (%)		8.5%	6.5%	11.8%	0.320
Duration of infertility (years)		6.05±3.48	6.01±3.46	6.17±3.72	0.945
Etiology of infertility (%)	**Male factor**	46.1%	34.6%	31.4%	0.424
	Tubal factor	1.9%	2.1%	2.0%	
	Unexplained	38.8%	41.5%	45.1%	
	Poor responder	18.2%	21.8%	21.5%	
Baseline-FSH (IU/mL)		6.72±1.99	7.13±2.38	7.22±2.21	0.136
Baseline-LH (IU/mL)		5.56±2.76	5.61±3.01	4.82±2.22	0.182
Baseline-Estradiol (pg/mL)		42.18±15.62	45.15±16.60	41.12±14.85	0.055
Antral follicle count		6.76±2.61	6.37±2.47	6.86±2.60	0.328
TSH (µIU/mL)		2.14±1.11	2.18±1.11	2.32±1.21	0.638
Prolactin (ng/mL)		16.15±7.35	16.24±8.88	18.35±13.18	0.289
Stimulation protocol (%)	**Long**	25.5%	22.6%	21.6%	0.223
	Antagonist	74.5%	77.4%	78.4%	
Duration of stimulation (days)		9.76±1.39	9.78±1.58	9.69±1.60	0.920
Gonadotropin dose (IU)		2000.22±848.46	1941.04±882.68	2019.12±855.00	0.667
Estradiol levels on day hCG (pg/mL)		1981.16±1009.09	1963.72±1156.54	2009.76±1473.55	0.956
Progesterone levels on day hCG (pg/mL)		0.76±0.34	0.84±0.40	0.81±0.34	0.130
Endometrial thickness on day hCG (mm)		10.30±1.80	10.23±1.63	9.87±1.85	0.287
Endometrial thickness on transfer day (mm)		10.66±1.76	10.73±1.95	10.07±1.99	0.127

BMI: Body mass index, FSH: Follicle stimulating hormone, LH: Luteinizing hormone, TSH: Thyroid stimulating hormone, hCG: Human chorionic gonadotropine

Table 2. Laboratory and reproductive outcome parameters of the patients

		Group 1 (n=1657)	Group 2 (n=548)	Group 3 (n=52)	p
Number of oocytes retrieved		10.43±5.28	9.28±5.70	8.90±4.46	0.058
Number of MII oocytes		8.16±4.32	7.30±4.44	7.55±3.92	0.154
2 Pronucleus		5.32±3.01	4.82±3.31	4.43±3.36	0.136
Fertilization rate (%)		68.06±24.09	69.09±23.49	68.29±27.32	0.885
Grade I embryo (%)		1075 (64.9)	374 (68.2)	32 (61.1)	0.386
The days of embryo transfer (%)	**2**	108 (6.5)	29 (5.3)	4 (5.9)	0.704
	3	1420 (85.7)	460 (83.9)	41 (80.4)	
	5	129 (7.8)	59 (10.8)	7 (13.7)	
Clinical pregnancy rate (%)		648 (39.1)	198 (36.1)	14 (27.5)	0.322

higher in the easy ET group[17]. In this study, the authors classified direct ET that did not require any intervention as easy ET, and ET that required afterloading external guidance as difficult. They claimed the possible reasons for low CPR in difficult ET as endometrial lesions and the induction of uterine contractions caused by the intervention. However, when

they performed subgroup analysis, no statistically significant difference was found in terms of CPR in the group requiring easy ET and afterloading external guidance, only ET duration was found longer in the intervention group.

In contrast, Sallam et al.[18] evaluated 784 IVF cycles, including 655 IVF-ICSI treatments, and found that negotiation of the cervix, the use of a vulsellum, and the presence of blood on the catheter wall or cervix did not affect CPR. Neithard et al.[11] reported that ET performed with afterloading external guidance increased pregnancy rates when compared with difficult ETs, but this increase did not reach a statistically significant level in their retrospective evaluation of 127 IVF-ICSI cycles. It was also observed that blood and mucus were present in the catheters of the difficult ET group, which the authors suggested could stimulate uterine contractions and negatively affect embryos. These negative conditions, the paper claimed, could have been prevented by using ET with external guidance.

Possible confounding factors on CPR, such as the presence of blood or mucus, were excluded in the current study. Nevertheless, there was no difference between the groups in terms of possible confounding factors that could have affected CPR, and we consequently explored ET type alone and found that it did not affect CPR.

Study Limitations

The strengths of the present study include its prospective design, the sufficient number of participants in each group, and the representative sample from central Turkey. The results of the study can be generalized to the majority of the country's population. Another strength is that the same two senior physicians performed all ETs, which helps minimize external human effects on CPR. However, the potential weaknesses of the study are that it was conducted in a tertiary care institution and that the cumulative CPR was not evaluated because no frozen ETs were included.

Conclusion

Our data showed that there was no impact of ET technique on the pregnancy rates of these patients who underwent IVF-ICSI treatment. Further studies with more participants are required to elucidate this situation.

Ethics

Ethics Committee Approval: Ethics board approval was given from the Necmettin Erbakan University Meram Faculty of Medicine institutional review board (2012/57).

Informed Consent: Written and oral informed aggrement was given from the participants.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

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Myometrial thickness overlying cesarean scar pregnancy is significantly associated with isthmocele formation in the third month of the postoperative period

Sezaryen skar gebeliği üzerindeki miyometriyal kalınlık, postoperatif dönemin üçüncü ayında istmosel oluşumu ile anlamlı ilişkilidir

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Abstract

Objective: To determine some associated factors for isthmocele formation 3 months after the treatment of cesarean scar pregnancy (CSP).

Materials and Methods: This is a prospective consecutive case series of CSP managed by fertility preservation modalities at a single tertiary care center from May 2016 to March 2019 (n=95). Patients with a diagnosis of CSP were identified and followed prospectively to collect data on the patients' demographics; detailed medical, surgical, and social history; symptoms; imaging and laboratory parameters at the time of CSP diagnosis and during treatment; treatment modalities, myometrial thickness; and outcomes in terms of isthmocele formation.

Results: Mean myometrial thickness overlying scar pregnancy was significantly lower in the group with isthmocele formation, and the mean gestational age of scar pregnancy was also significantly lower in the group with isthmocele formation following treatment of scar pregnancy ($p<0.05$). Multivariate regression analysis was conducted to determine associations between certain variables and isthmocele development, which revealed that the gestational age of scar pregnancy and myometrial thickness were significantly associated with isthmocele formation.

Conclusion: Myometrial thickness and gestational age of scar pregnancy were significantly associated with isthmocele formation 3 months after treatment.

Keywords: Isthmocele, scar pregnancy, cesarean, myometrial thickness

Öz

Amaç: Bu çalışmanın amacı, sezaryen skar gebeliğinin (SSG) tedavisinden 3 ay sonra istmosel oluşumuyla ilişkili bazı faktörleri belirlemekti.

Gereç ve Yöntemler: Bu, Mayıs 2016'dan Mart 2019'a kadar tek bir üçüncü basamak bakım merkezinde fertilite koruma modaliteleri ile yönetilen tüm SSG'lerin prospektif ardışık bir olgu serisidir (n=95). SSG teşhisi konan hastalar belirlendi ve hastaların demografik özelliklerine ilişkin verileri toplamak için ileriye dönük olarak izlendi; ayrıntılı tıbbi, cerrahi ve sosyal geçmiş; semptomlar; SSG teşhisi sırasında ve tedavi sırasında görüntüleme ve laboratuvar parametreleri; tedavi modaliteleri, miyometriyal kalınlık ve isthmosel oluşumu açısından sonuçları.

Bulgular: Skar gebeliğini örten ortalama miyometriyal kalınlık, istmosel oluşumu olan grupta anlamlı olarak daha düşüktü, yine skar gebelik tedavisi sonrası isthmosel oluşumu olan grupta da skar gebelik ortalama gestasyonel yaşı anlamlı olarak daha düşüktü ($p<0,05$). Bazı değişkenler ile istmosel gelişimi arasındaki ilişkileri bulmak için çok değişkenli regresyon analizi yapıldı, analiz, skar gebeliğinin gebelik yaşı ve miyometriyal kalınlığın istmosel oluşumu ile anlamlı şekilde ilişkili olduğunu ortaya koydu.

Sonuç: Miyometriyal kalınlık ve skar gebeliğinin gebelik yaşı, tedaviden 3 ay sonra istmosel oluşumu ile anlamlı olarak ilişkili bulunmuştur.

Anahtar Kelimeler: İstmosel, skar gebelik, sezaryen, miyometriyal kalınlık

PRECIS: Myometrial thickness and gestational age of scar pregnancy were significantly associated with isthmocele formation 3 months later after treatment.

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Introduction

Pregnancy in a previous cesarean scar occurs in approximately 1 in 2.000 pregnancies and constitutes 6% of ectopic pregnancies among women who had a previous cesarean delivery[1-3]. The incidence does not appear to be related to the number of cesarean births, similar pregnancies have been reported in the literature, including pregnancies implanted in previous myomectomy scars[4]. Pregnancy is located on the scar and is surrounded by myometrium and connective tissue. The mechanism of implantation to this site is believed to be the migration of the embryo through a wedge defect in the lower uterine segment or through a microscopic fistula in the scar[5,6]. In symptomatic patients, the clinical appearance ranges from painful or painless vaginal bleeding to uterine rupture and hypovolemic shock[7,8]. The diagnosis is made sonographically (transvaginal and transabdominal) by visualizing an enlarged hysterotomy scar with a buried mass that can extend beyond the anterior contour of the uterus[9,10]. The optimal treatment for a cesarean scar pregnancy (CSP) is unclear and therapy should be tailored to the patients' clinical presentation. Treatment options include wedge resection of the ectopic pregnancy via laparotomy or laparoscopy, or possible hysterectomy, dilatation, and curettage or methotrexate therapy. In subsequent pregnancies, recurrent scar implantation may occur[11]. There are reports of successful term pregnancy after a CSP[11]. Isthmocele is a myometrial defect that looks like a pouch on the anterior wall of the uterine isthmus above the previous cesarean scar[12]. Isthmocele formations were shown to be a risk factor for cesarean scar ectopic pregnancy[13].

This study aimed to determine some associated factors for isthmocele formation at the third month following treatment of scar pregnancy.

Materials and Methods

This is a prospective consecutive case series of CSP managed by fertility preservation modalities at a single tertiary care center (University of Health Sciences Turkey, Zeynep Kamil Women and Children's Health Training and Research Hospital) from May 2016 to March 2019 (n=95). The study protocol was approved by the institutional review board (University of Health Sciences Turkey, Zeynep Kamil Women and Children's Health Training and Research Hospital Ethics Committee -2017/05) and written informed consent was obtained from each participant. The patients with a diagnosis of CSP were identified and followed prospectively to collect data on patients' demographics: detailed medical, surgical, and social history; symptoms; imaging and laboratory parameters at the time of CSP diagnosis and during treatment; treatment modalities, myometrial thickness and outcomes in terms of the presence of isthmocele formation at third postoperative month and successful pregnancy following treatment. All diagnoses were made based on the patient's history of prior cesarean delivery, positive pregnancy test, presence of a gestational sac in the area of the scar, and otherwise empty uterine cavity on transvaginal ultrasonogram. Either a medical or surgical method was used as a treatment modality determined based on demographic and clinical characteristics (ultrasonography findings, beta-human chorionic gonadotropin (hCG) level, fetal cardiac activity ±) of each individual. Medical management consisted of a single dose methotrexate regimen (50 mg/m^2 body surface area); the second systemic methotrexate dose was given to the patients who declined surgical management when their first dose had failed. Dilatation and curettages were performed under general anesthesia with ultrasonography guidance, following ensuring removal of gestational material, a 20-F Foley catheter was placed in the uterine cavity to control heavy bleeding if it occurred. Postoperatively, the patient was monitored in the intensive care unit. As alternative management of CSP, hysteroscopy was performed after cervical dilatation, and hysteroscopic scar pregnancy removal was performed with bipolar energy. In patients who required an extracavitary approach, a laparoscopy was performed following a uterine manipulator insertion. The bladder and adhesions were dissected. CSP was removed using bipolar energy. The scar edges were expanded with scissors without any energy modalities. The incision was closed using continuous double-layer sutures.

All participants underwent myometrial thickness measurement of the scar area (Mindray DC-7) by the same sonographer. Myometrial thickness was defined as the minimum thickness overlying the amniotic cavity at the level of the uterine scar. In the postoperative third month, participants were reevaluated for isthmocele and risk of scar pregnancy recurrence.

The diagnosis of isthmocele formation at postoperative third month was established using transvaginal ultrasound as previously described[14], performed using a 5-MHz transvaginal transducer (Mindray DC-7) 3 to 6 days after the last menstruation by the same sonographer. An anechoic triangle defect in the myometrium with the base communicating to the uterine cavity, or a deformity on the anterior isthmus was considered to be isthmocele[15].

Statistical Analysis

Data analysis was performed using the SPSS version 15.0 package (Chicago, IL). Student's t-test and the Mann-Whitney U test were used to compare continuous variables. The chi-square and Fisher's exact tests were used for categorical variables. Receiver operating characteristics analysis was used to determine predictive values. Multivariate regression analysis was used to show adjusted associations. P-values <0.05 were accepted to be statistically significant.

Results

Comparison of Outcome Variables

There were 56 (58.9%) cases with isthmocele formation detected in the third postoperative month. There were 23 (24.2%) healthy pregnancies among the study population after

postoperative follow-up. The rate of isthmocele formation in the third postoperative months was significantly higher in the group without pregnancy (65% vs 39.1%, p=0.03).

Comparison of Variables with and Without Isthmocele

Groups with and without isthmocele formation following treatment of scar pregnancy were compared in terms of age, gravidity, parity, number of previous cesarean deliveries, and body mass index, and analysis of the data revealed no differences between the groups in terms of these variables (Table 1, p>0.05). A comparison of groups with and without isthmocele formation following treatment of scar pregnancy in terms of myometrial thickness and gestational sac diameter resulted in significant differences between the groups. The mean myometrial thickness was significantly lower in the group with isthmocele following treatment of scar pregnancy, and the mean gestational age of scar pregnancy was also significantly lower in the group with isthmocele formation following treatment of scar pregnancy (Table 2, p<0.05). Myometrial thickness was a significant predictor for isthmocele formation following treatment of scar pregnancy [area under the curve (AUC)=0.693, p=0.002]. The optimal cut-off value was 4.1 mm with 70% sensitivity and 70% specificity (Figure 1).

Table 1. Groups with and without isthmocele formation following treatment of scar pregnancy compared in terms of age, gravidity, parity, number of previous cesarean deliveries, and BMI

	Group with isthmocele (n=56)			Group with no isthmocele (n=39)			p (MWU)
	Median	SD	IQR	Median	SD	IQR	
Age (years)	33	4.6	4.8	34	4.4	5.5	0.8
Gravidity	3	1.2	1	4	1.4	2	0.6
Parity	2	0.5	0.8	1	0.9	1	0.1
Number of previous cesareans	2	0.6	1	1	0.9	1	0.2
BMI (kg/m^2)	25.8	7.8	7	27.8	7.5	9	0.7

BMI: Body mass index, SD: Standard deviation, IQR: Interquartile range

Table 2. Comparison of groups with and without isthmocele formation following treatment of scar pregnancy in terms of myometrial thickness and gestational sac diameter

	Group with isthmocele (n=56)			Group with no isthmocele (n=39)			p (MWU)
	Median	SD	IQR	Median	SD	IQR	
Gestational age (days)	42	4.6	3	42	12.1	7	0.002
Myometrial thicknress (mm)	3.8	0.8	1	5	2.4	2.8	0.002

SD: Standard deviation, IQR: Interquartile range

Treatment Modalities

A comparison of groups with and without further treatment following failed curettage revealed significant differences between the groups in terms of gravidity and age (Table 3, p<0.05). Among 26 patients who required further intervention secondary to failed curettage, the management modalities were as follows: laparoscopic scar pregnancy removal and scar closure (n=14), methotrexate (n=8), and hysteroscopic scar pregnancy removal and uterine cavity revision (n=4). Gravidity was a significant predictor for the failure of treatment with uterine curettage alone (AUC=0.660, p=0.02). The optimal cut-off value was 3.5 with 57% sensitivity and 73% specificity (Figure 2). The rate of isthmocele at the third postoperative month was 65.4% in the group that underwent further intervention following failed uterine curettage, whereas it was 56.5% in patients who were treated with uterine curettage (p=0.434).

Multivariate Regression Analysis to Show Adjusted Associations

Multivariate regression analysis was conducted to determine associations between certain variables and isthmocele formation, and the analysis revealed that the gestational age of scar pregnancy [odds ratio (OR): 0.4, 95% confidence interval (CI): (0.2-0.89), p=0.005] and myometrial thickness [OR: 0.6, 95% CI: (0.5-0.9), p=0.006] were significantly associated with isthmocele formation.

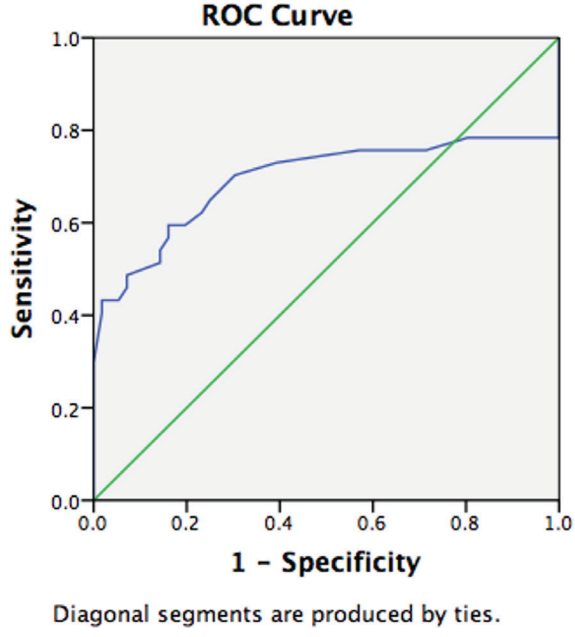

Figure 1. ROC analysis of myometrial thickness to predict postoperative isthmocele

ROC: Receiver operating characteristics

Associations Between Variables and Symptoms at Admission

A comparison of the groups with and without vaginal bleeding before intervention showed a significant difference between the two groups in terms of age, gravidity, parity, and preoperative beta-hCG levels ($p<0.05$). Parity (AUC=0.650, p=0.04, optimal cut-off value=1.5 with 73.4% sensitivity, 57% specificity) and preoperative beta-hCG levels (AUC=0.667, p=0.02, optimal cut-off value=10.700 with 69% sensitivity, 71% specificity) were significant predictors for vaginal bleeding before intervention (Figure 3).

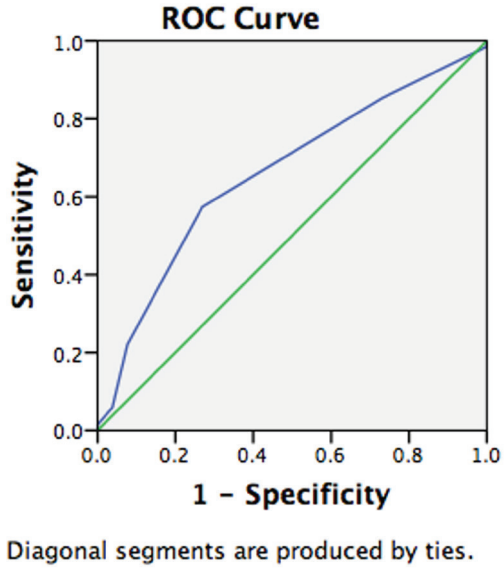

Figure 2. ROC analysis of gravidity to predict the need for further intervention following failed uterine curettage

ROC: Receiver operating characteristics

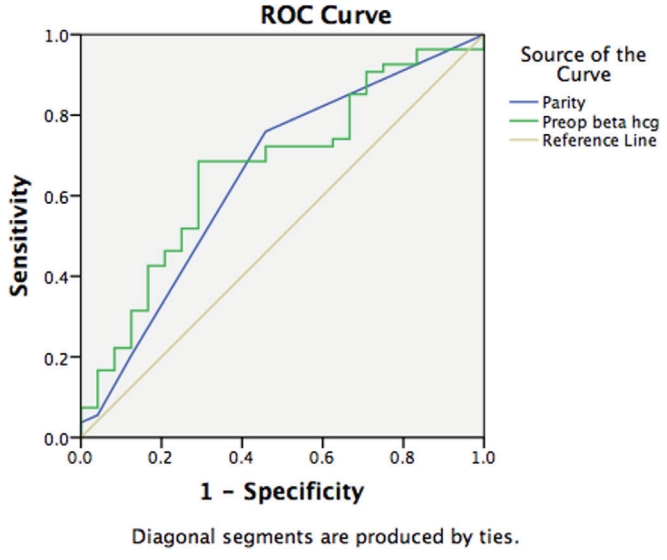

Figure 3. ROC analysis of parity and beta-hCG to predict vaginal bleeding as the symptom of the first admission

ROC: Receiver operating characteristics

Discussion

In this case series, we aimed to determine some associated factors for isthmocele formation in the third month following treatment of scar pregnancy. Our data analysis revealed that myometrial thickness overlying CSP and gestational age of scar pregnancy were significantly associated with isthmocele formation 3 months after treatment. The incidence and diagnosis of CSP are rapidly increasing, mainly due to higher cesarean rates and increased use of ultrasound in early pregnancy. Early diagnosis and treatment of CSP are important to increase the success rate of treatment and prevent complications[16]. Vaginal bleeding after amenorrhea was the most common but non-specific symptom. Some patients with CSP may experience low abdominal pain and vaginal bleeding simultaneously[17]. There were 28 (29.5%) cases of vaginal bleeding at first admission in our study population; a comparison of groups with and without vaginal bleeding before intervention showed a significant difference between the two groups in terms of age, gravidity, parity, and preoperative beta-hCG levels. Parity and preoperative beta-hCG levels were significant predictors for vaginal bleeding before the intervention.

Various methods have been proposed for the management of CSP without consensus on the optimal treatment method. Options include local and/or systemic medical therapy with methotrexate, uterine artery embolization, and surgical procedures such as D&C, laparoscopic or hysteroscopic gestational mass resection and hysterectomy[18,19]. Surgical treatment has been described with a success rate of 83%, but with a complication rate of 18% compared with 7% for medical treatment[18]. In a recent national cohort study in the United Kingdom, surgical treatment was identified with a 96% success rate but a 36% complication rate[20].

Given that there is no consensus on the optimal treatment modality of CSP, several treatment modalities have been compared in the literature in terms of success and complication rates. The most frequently assessed modalities were expectant management, D&C with the guidance of ultrasound, direct injection of potassium chloride into the embryonic sac with the guidance of ultrasound, local or systemic injection of methotrexate[21], uterine artery embolization, hysteroscopy, and laparotomy or laparoscopic excision[22,23]. However, none of these treatments was found to be entirely satisfactory. Success and complication rates of three different modalities including transvaginal clearance, endoscopic surgery, uterine artery embolism were compared in a study by Fei et al.[17], and the authors concluded that early detection of CSP and conservative treatment greatly improved the prognosis of patients and suggested transvaginal pregnancy tissue clearance may be the preferred option for a fertility protection approach. In their study, blood loss was the lowest with transvaginal pregnancy tissue clearance among the three groups. For this procedure, there is no need to enter the pelvic cavity so pelvic adhesions have no adverse effect during the surgical course. On the other

Table 3. Comparison of groups with and without further treatment following failed curettage

	Group of scar pregnancies needed further intervention following failed D&C (n=26)			Group of scar pregnancies manages by D&C alone (n=69)			p (MWU)
	Median	SD	IQR	Median	SD	IQR	
Age (years)	32	4.1	5.5	35	4.6	7	0.04
Gravidity	3	0.6	1	4	1.4	1	0.01
Parity	2	0.5	1	2	0.7	1	0.06
Number of previous cesareans	1	0.7	1	2	0.6	1	0.08
BMI (kg/m²)	27.7	5.9	9	25	7.9	7	0.4
MT (mm)	4.2	1.1	1.1	3.8	1.4	1.5	0.1
Preop b-hCG	12,309.5	17,294.6	28.595	16.545	56,973.6	24.629	0.5
GA (days)	42	4.7	3.5	42	14	6.3	0.2

D&C: Dilatation and curettage, BMI: Body mass index, MT: Myometrial thickness, GA: Gestatitional age, b-HCG: Beta-human chorionic gonadotropin, SD: Standard deviation, IQR: Interquartile range

hand, it was shown that resection of the old scar with a new uterine closure may reduce the recurrence of scar dehiscence[24]. Endoscopic surgery includes hysteroscopy or laparoscopy or a combination of these two modalities. In the majority of cases in our series, D&C was successful in the management of scar pregnancy cases. Surgical resection of the scar may be considered to be associated with a lower risk of scar pregnancy recurrence or isthmocele formation; however, our analysis failed to show any difference among different management modalities in terms of isthmocele formation.

Uterine artery embolization was also suggested as a treatment option for CSP, which could block the blood flow of uterine arteries, decrease vascularization, and induce trophoblastic degeneration. In previous studies, uterine artery embolization resulted in satisfactory results when combined with local methotrexate[25]. Because uterine artery embolization may interfere with the ovarian reserve, it cannot be a suitable choice for women who want to preserve fertility[26].

In a study published in 2016, the efficacy of ultrasound-guided suction curettage for the management of pregnancies implanted into the lower uterine segment cesarean section scar was assessed in 232 women with cesarean section scar pregnancy. The authors showed that ultrasound-guided transcervical surgical evacuation was an effective method for the treatment of first-trimester CSP. There were no cases of uterine perforation in their series, but the proportion of women diagnosed with retained products of conception on postprocedure ultrasound examination was higher when compared with women who underwent surgical evacuation of failed intrauterine pregnancies[27].

In another study, the risk of bleeding was shown to be increased with advancing pregnancies, but the authors stated that vascularity of the pregnancy on Doppler examination was the most significant predictor of excessive blood loss, obstruction of the cervical canal using a Foley catheter helps to control bleeding following the evacuation of CSPs[28].

In the majority of the cases in our series, D&C was successful in the management of scar pregnancy cases. Gravidity was a significant predictor for the failure of treatment, the success rate of D&C increased with higher gravidity. A Foley catheter was used in only a few cases to control bleeding.

It is now well known that one of the most common gynecologic sequelae of c-section is a uterine scar with deficient healing, known as an isthmocele or c-section defect[29]. The poor contractility of the myometrium around the isthmocele caused by the presence of fibrotic tissue can produce a blood drainage deficiency with the accumulation of blood during the menstrual cycle at the level of the scar and subsequent spotting[29], normally during the first week of the cycle.

Previous data showed that isthmocele contributes to the development of cesarean scar ectopic pregnancy[13] and isthmoplasty was suggested to be an option to prevent the occurrence of a scar ectopic pregnancy, thereby preventing massive blood loss and allowing the conservation of the uterus to maintain fertility, health, and quality of life[30]. The success of hysteroscopic surgery on isthmocele associated with CSP was reported by some authors. It was shown that hysteroscopic surgery was effective in increasing the residual myometrial thickness and reducing the size of isthmocele[31]. As the women in our series desired to preserve their fertility, isthmocele formation was critical for the possible future pregnancy. In our case series, myometrial thickness overlying scar pregnancy and gestational age of scar pregnancy were found to be significantly associated with isthmocele formation. On the other hand, the type of scar pregnancy management modality was not associated with the isthmocele formation at the postoperative third month.

This was a prospective cohort study of scar pregnancies, the relatively large sample size based on a single-center data and the evaluation of various outcomes of interest and independent variables were the major advantages of this study. The major disadvantage of this study was the lack of data regarding the rate of isthmocele before scar pregnancy.

Conclusion

Myometrial thickness and gestational age of scar pregnancy were significantly associated with isthmocele formation 3 months after treatment. Myometrial thickness measurement overlying scar pregnancy may be used to select candidates for further intervention following treatment of scar pregnancy.

Ethics

Ethics Committee Approval: The study protocol was approved by the institutional review board (University of Health Sciences Turkey, Zeynep Kamil Women and Children's Health Training and Research Hospital Ethics Committee -2017/05).
Informed Consent: Written informed consent was obtained from each participant.
Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: R.K., Concept: R.K., Design: R.K., Data Collection or Processing: R.K., S.S.K., B.G., G.Ü., Analysis or Interpretation: E.Ö., Literature Search: E.Ö., Writing: E.Ö.
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Blake's pouch cyst: Prenatal diagnosis and management

Blake's poş kisti: Prenatal tanı ve yönetim

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Abstract

Objective: This study aimed to present the characteristic features of 19 patients who were diagnosed as having Blake's pouch cyst (BPC) at our center.

Materials and Methods: Nineteen patients diagnosed as BPC between 2015 and 2019 were included in this retrospective study. Follow-up examinations were performed using ultrasonography (US) every three weeks up to 35 weeks of gestation. Prenatal magnetic resonance imaging (MRI) was performed at the time of diagnosis or during follow-up in 13 patients. MRI or transfontanellar US was performed to confirm the diagnosis of BPC after delivery. Karyotype results of eight patients were recorded.

Results: Isolated BPC was observed in 9 (47%) patients, and associated anomalies were detected in 10 (53%) patients, including seven (36%) with the central nervous system and four (21%) with cardiac anomalies. Two fetuses had abnormal karyotype analysis as trisomy 21 and 13. The MRI report of eight patients was "differential diagnosis required for Dandy-Walker complex" and only in five (26%) patients, it was reported to be compatible with BPC. Spontaneous resolution was seen in four patients. Postnatal MRI was performed in five patients, and transfontanellar US in two patients, and all MRI and US results were consistent with BPC. During the neonatal period, abnormal neurologic development was observed in four (21%) patients, and one (5%) died.

Conclusion: Although the prognosis of isolated BPC is very good with healthy neurologic development until advanced ages, death in the early neonatal period and abnormal neurologic development may be observed depending on the condition of the associated anomalies.

Keywords: Blake's pouch cyst, posterior fossa, cerebellar vermis, prenatal diagnosis

Öz

Amaç: Bu çalışmada merkezimizde Blake's poch kisti (BPK) tanısı alan 19 hastanın karakteristik özellikleri sunuldu.

Gereç ve Yöntemler: Bu retrospektif çalışmaya 2015-2019 yılları arasında BPK tanısı alan 19 olgu dahil edildi. Takip muayeneleri 35. gebelik haftasına kadar her üç haftada bir ultrasonografi (US) kullanılarak yapıldı. On üç olguya prenatal manyetik rezonans görüntüleme (MRG) tanı anında veya takip sırasında yapıldı. Doğumdan sonra BPK tanısını doğrulamak için MRG veya transfontanellar US yapıldı. Sekiz hastanın karyotip sonuçları kaydedildi.

Bulgular: Dokuz olguda (%47) BPK izole olarak gözlenirken merkezi sinir sistemi anomalisi olan 7 olgu (%36) ve kalp anomalisi olan 4 olgu (%21) dahil olmak üzere 10 olguda (%53) ilişkili anomaliler saptandı. İki fetüste trizomi 21 ve 13 olmak üzere anormal karyotip bulgusu saptandı. Sekiz olgunun MRG raporuna göre Dandy-walker kompleksi için ayırıcı gerekli oldu ve sadece 5 olguda (%26) BPK ile uyumlu olduğu bildirildi. Dört olguda spontan rezolüsyon izlendi. Postnatal dönemde 5 olguya MRG, 2 olguya transfontanellar US yapıldı ve bunların sonuçları BPK ile uyumlu idi. Yenidoğan döneminde 4 olguda (%21) anormal nörolojik gelişim gözlendi ve bunlardan biri (%5) öldü.

Sonuç: İzole BPK'nin prognozu ileri yaşlara kadar sağlıklı nörolojik gelişim ile çok iyi olmakla birlikte, ilişkili anomalilerin durumuna bağlı olarak erken neonatal dönemde ölüm ve anormal nörolojik gelişim gözlenebilir.

Anahtar Kelimeler: Blake's Poch kisti, arka fossa, serebellar vermis, prenatal tanı

PRECIS: Isolated Blake's Pouch cyst has an excellent prognosis, with a high possibility of intrauterine resolution and healthy intellectual development.

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Introduction

Blake's pouch is a rudimental embryologic structure of the fourth ventricular tela choroidea and it perforates in the 9th or 10th weeks of embryogenesis. Perforation ordinarily occurs in the foramen of Magendie. If perforation of Blake's pouch does not occur in the foramina during embryogenesis, it leads to a ballooning of the superior medullary velum into the cisterna magna resulting in Blake's pouch cyst (BPC) formation. During embryologic development, foramina of Luschka, having a smaller diameter than the foramen of Magendie, open later than this foramen[1,2]. As the perforation in the foramina of Luschka does not occur during the formation process of BPC, the fourth ventricle continues to expand with supratentorial structures until the foramina of Luschka opens and provides cerebrospinal fluid (CSF) flow from the ventricles to the cisterna magna. BPC may sometimes disappear in the third trimester due to late fenestration at the 24th to 26th weeks of gestation[3].

Cystic malformations of the posterior fossa are frequently revealed with neuroimaging studies. An abnormal amount of CSF in the posterior fossa is classified within the spectrum called Dandy-Walker complex (DWC) or as arachnoid cysts[1,4]. Tortori-Donati et al.[5] claimed that BPC was a different entity from cysts in DWC or arachnoid cysts. mega cisterna magna and arachnoid cysts are usually incidental findings, whereas cysts in DWC are associated with cerebellar hemisphere and other developmental anomalies related to vermis, and most commonly found together with hydrocephalus[1,6]. BPC has been less recognized in the radiologic spectrum among the posterior fossa's cystic malformations because it was considered as a separate entity. However, BPC interestingly presents a broad spectrum of symptoms between showing all signs of hydrocephalus and being asymptomatic[7].

This study aimed to present the associated anomalies, karyotype analysis, ultrasonographic (US) and magnetic resonance imaging (MRI) findings of 19 patients who are diagnosed as having BPC at our center over the last five years and to review the literature about BPC.

Materials and Methods

This study was conducted retrospectively on patients admitted to the Kanuni Sultan Suleyman Research and Training Hospital Perinatology Clinic between 2015 and 2019. Nineteen patients who were diagnosed as having BPC after suspicion of posterior fossa anomalies were included in the study. The US criteria used to diagnose BPC were used as recommended by Paladini et al.[3]: (1) normal anatomy and normal size of vermis, (2) slight to medium rotation counterclockwise of vermis, (3) normal size of cisterna magna, (4) evidence of the wall of the BPC in the cisterna magna; the first three criteria were considered necessary for the diagnosis, and the 4th criterion is supportive for diagnosis. Multiplanar 3-dimensional US (GE Voluson E6 Wide Band Convex Transducer) was used to examine the BPC and its neighboring vermis and posterior fossa. The vermian size was measured using the nomograms recommended by Viñals et al.[8]. Fetuses suspected of BPC before 20 weeks of gestation were re-evaluated after 20 weeks of gestation. Follow-up examinations were performed every three weeks up to 35 weeks. Prenatal MRI exams were performed at the time of diagnosis or during the follow-up period in 13 patients. All MRI examinations were conducted at a single center. In some patients where no termination was performed, MRI or transfontanel US was performed to confirm the diagnosis of BPC after delivery. US results obtained at diagnosis and associated anomalies encountered during follow-up were recorded. Karyotype results of eight patients were obtained and recorded. Delivery mode and week, postnatal neurologic development results of the fetuses were recorded. The neurologic examination included the head shape assessment, the head circumference measurement, and the cranial nerve evaluation. The upper and lower limbs (deep tendon reflexes, pathologic reflexes, movement, strength, muscular tension), abdominal reflexes, meningeal signs, superficial and deep feeling, and involuntary movements were also evaluated[9].

This study was conducted after the Kanuni Sultan Suleyman Research and Training Hospital Clinical Research Ethics Committee's gave approval and written informed consent was obtained from all participants.

Statistical Analysis

We used the IBM SPSS 21.0 for Windows (SPSS Inc., Chicago, IL, USA) statistical package for statistical evaluation of our research data. A descriptive analysis of the records was performed following completion of the audit. Continuous variables are presented as median. Categorical variables are presented as frequencies and percentage.

Results

This study consisted of 19 patients who were diagnosed as having BPC through prenatal US examinations after referral to our clinic due to suspicion of posterior fossa anomaly. The median gestational age at diagnosis was 23 weeks, with only two patients referred in the third trimester. Isolated BPC was observed in nine of 19 patients (47%), and associated anomalies were detected in 10 (53%) patients. Seven (36%) of the patients with multiple anomalies had central nervous system (CNS) anomalies, and 4 (21%) had cardiac anomalies. The detailed associated anomalies and other follow-up results are summarized in Table 1.

MRI was performed in 13 (68%) of 19 patients diagnosed as having BPC using US. The MRI report of eight (42%) patients was "differential diagnosis required for DWC," and only in five (26%) patients, it was reported to be compatible with BPC.

When the spontaneous resolution of BPC was examined between the 24th and 26th weeks in the US follow-up, a spontaneous resolution was seen in four of 16 patients (21%). However, it could not be evaluated in three (15%) patients due

Table 1. USG, MRI and follow-up results and associated anomalies of 19 patients diagnosed with BPC

Case	Associated anomaly	GA	Prenatal MRI diagnosis	Spontaneous resolution between 24 and 26 weeks	Karyotype	Outcome	Compliance with BPC on postnatal MRI	Postnatal transfontanel USG
1	None (Isolated BPC)	24	DWC suspicion	None	None	NSVD (39^{th} w) NND	None	None
2	None (Isolated BPC)	22	DWC suspicion	Spontaneous resolution	None	NSVD (40^{th} w) NND	None	None
3	None (Isolated BPC)	24	DWC suspicion	None	None	C/S (39^{th} w) NND	None	Compatible with BPC
4	None (Isolated BPC)	21	DWC suspicion	Spontaneous resolution	None	C/S (39^{th} w) NND	None	None
5	None (Isolated BPC)	22	BPC	None	None	C/S (39^{th} w) NND	Compatible	None
6	Dysmorphic face and polydactyly	20	None	None	Normal	C/S (39^{th} w) AND	Compatible	None
7	Hydrocephalus, polydactyly and interhemispheric cyst	25	None	None	Normal	C/S (39^{th} w) Abnormal development, neonatal ex	Compatible	None
8	Hydrocephalus and vermian hypoplasia	23	None	None	Normal	C/S (39^{th} w) AND	Compatible	None
9	Aortic coarctation and hypospadias	24	DWC suspicion	None	None	C/S (39^{th} w) Not known neurological development	None	Compatible with BPC
10	Skeletal dysplasia, hypertelorism and oligodactilia	23	None	Not known	None	TOP	TOP	TOP
11	CC dysgenesis, subaortic VSD, muscular VSD and nasal bone hypoplasia	25	BPC	None	Trisomy 21	TOP	TOP	TOP
12	None (Isolated BPC)	20	DWC suspicion	Spontaneous resolution	Normal	C/S (39^{th} w) NND	None	None
13	Vermian agenesis and premature ventricular contractions	26	BPC	Not known	Normal	TOP	TOP	TOP
14	None (Isolated BPC)	21	DWC suspicion	None	Normal	NSVD (40^{th} w) NND	None	None
15	Tricuspid valve failure, pericardial effusion, increased nuchal fold	23	None	Not known	Trisomy 13	TOP	TOP	TOP
16	None (Isolated BPC)	22	None	Spontaneous resolution	None	NSVD (40^{th} w) NND	None	None
17	Dysgenesis of corpus callosum	23	BPC	None	None	NSVD (40^{th} w) AND	Compatible	None
18	None (Isolated BPC)	23	DWC suspicion	None	None	C/S (39^{th} w) NND	None	None
19	Dysgenesis of corpus callosum	26	BPC	Not known	None	TOP	TOP	TOP

NSVD: Normal spontaneous vaginal delivery, C/S: Caesarean section, DWC: Dandy-Walker complex, BPC: Blake's pouch cyst, VSD: Ventricular septal defect, TOP: Termination of pregnancy, w: Week, GA: Gestational age, CC: Corpus callosum, NND: Normal neurological development, AND: Abnormal neurological development, MRI: Magnetic resonance imaging, USG: Ultrasonography

to the termination of pregnancy was performed before these weeks. It was recorded that six (75%) of eight patients had a normal karyotype, and one was trisomy 13, and another patient was trisomy 21 (Down syndrome). To confirm the prenatal diagnosis of BPC in 14 patients, MRI was performed in five patients, and transfontanel US in two patients and all MRI and US results were consistent with BPC. However, the MRI or US results of other patients could not be obtained.

Termination of pregnancy was performed in five (26%) patients with multiple anomalies, 14 (74%) patients reached term. The delivery of five (35%) patients was normal spontaneous vaginal delivery at 40 weeks, and the delivery of nine (65%) patients was cesarean section (C/S) at 39 weeks. In the postnatal follow-up of the patients, no information was obtained about the neurologic development of one patient, healthy neurologic development was observed in nine (47%) patients, and abnormal neurologic development was observed in four (21%) patients. One (5%) patient died during the neonatal period due to the multiple anomalies. If the patients with healthy neurologic development after birth were examined, all patients had isolated BPC.

Discussion

Studies on BPC have focused on the non-perforation of BPC in the foramen of Magendie. According to this theory, when the perforation of BPC in the foramen of Magendie does not occur, the cerebellar hemisphere and vermis are compressed due to increased CSF. Still, this increased pressure does not occur in the development of BPC. Therefore, most authors agree that DWC originates from a defect in the anterior membranous region, and BPC and mega cisterna magna originate from a defect in the posterior membranous region[1,10].

Modern MRI methods provide essential information in identifying concomitant malformations in the differential diagnosis of BPC[11]. Typical radiologic findings of BPC are infra or retrocerebellar localization of the cyst, a well-developed and non-rotated cerebellar vermis, cystic dilatation of the fourth ventricle, compression of the cerebellar hemispheres to some extent, and continuity of the choroid plexus on the cyst wall[1,11,12]. In our study, only 5 (39%) of the 19 patients who underwent MRI in their follow-up were reported to be compatible with BPC. The reason it was reported in this way may be because radiologists have not received adequate training in the differential diagnosis of BPC. It is still challenging to discriminate mild hypoplasia from slight deformation of the cerebellar vermis in fetal and postnatal MRI[13]. We re-examined the MRI images and found that the images were compatible with BPC. Postnatal MRI or transfontanellar US confirmed the diagnosis of BPC in all seven patients who underwent MRI and USG after delivery.

It is seen that most articles written on BPC in the literature investigated other developmental anomalies, and most focused on embryogenesis rather than clinical results. However, the clinical presentation of BPC is extensive; it can be detected incidentally in adulthood and fatal complications can occur in the neonatal period. Cornips et al.[1] presented a case series of six patients with BPC. In the case series, both a case of BPC detected incidentally in MRI screening in adulthood in a 51-year-old and a patient who died of high-pressure hydrocephalus and cholestatic anomalies at the age of one month were present. It was detected that hydrocephalus slowly developed in two patients. However, neurologic development was normal and treated with endoscopic third ventriculostomy, and there was a patient who had listeria meningitis due to compensated hydrocephalus and had healthy neurologic development. In a case report of Calabrò et al.[12], two patients with BPC showed healthy neurologic development until the age of 61 and 62. They were diagnosed as having BPC when syncope attacks developed in the first patient, and headache and vertigo were seen in the second. Bontognali et al.[14] presented a patient with BPC who started to show signs of cerebellar dysfunction in the 18th month despite having healthy neurologic development. In the case report of Iuculano et al.[15], it was reported that a patient diagnosed with prenatal BPC had no associated anomaly and showed healthy neurologic development after delivery. In our results, normal neurologic development was observed in nine (47%) of the 13 patients that reached term, which was isolated BPC; four (21%) had abnormal neurologic development and one (5%) died in the neonatal period. One of four fetuses with abnormal neurologic development had polydactyly and a dysmorphic face. One had hydrocephalus, polydactyly, and interhemispheric cyst. One had hydrocephalus and vermian hypoplasia, and the last had dysgenesis of the corpus callosum. It can be seen that fetuses with abnormal postnatal neurologic development in the postnatal period are more likely to have CNS anomalies.

In a case series of 19 patients diagnosed as having BPC using prenatal USG, Paladini et al.[3] found major anomalies in eight of 19 patients (42%), and five (26%) were associated with congenital heart disease. In 12 of 19 patients, karyotype analysis results were normal, but only two were abnormal (trisomy 21). A termination was performed in eight patients (42%) and neonatal death was seen in two (10%). Eight (48%) patients reached term, and all had healthy neurologic development. The results of Paladini et al.[3] were in parallel with our results. Among our fetuses with associated anomalies, seven (36%) had CNS anomalies four (21%) had cardiac anomalies, and termination of pregnancy was performed in five (26%) due to multiple anomalies. According to a case report and a meta-analysis of case series about posterior fossa anomalies, D'antonio et al.[16] detected BPC in 86 fetuses from nine studies. Among these patients, the rates of associated anomalies of CNS and other than the CNS were found as 11.5% and 23.5%. Trisomy 21 was detected in only one of the 45 patients who underwent karyotype analysis. In our results, trisomy 21 and 13 were detected in two patients with BPC, and associated anomalies were observed in 10 (53%) patients. Seven (36%) of associated

anomalies were CNS anomalies, and four (21%) were cardiac anomalies. In the second part of the same meta-analysis on 46 patients with BPC regarding neurologic development outcomes, no significant relationship was found between BPC and the abnormal neurologic development results[17]. In a study examined 105 fetuses with posterior fossa anomalies by Gandolfi-Colleoni et al.[18], 32 fetuses were diagnosed as having BPC using prenatal USG, associated anomalies were detected in eight of these 32 patients, fluid accumulation in the posterior fossa with neurologic development disorder in one of 20 patients who reached term, and in one patient, abnormal neurologic development related to other anomalies were observed. Healthy neurologic development was observed in 90% of patients in 1 to 5 years of follow-up. In our results, similar to these studies, patients with BPC were most frequently associated with CNS and cardiovascular system anomalies, and all patients with isolated BPC had healthy neurologic development in post-natal life.

In the study conducted by Gandolfi-Colleoni et al.[18], spontaneous resolution of BPC was observed in about one-third of 32 fetuses. In the study of Paladini et al.[3], BPC regressed in 6 (55%) of the 11 patients that reached term and the vermis returned to the normal position between 24 and 26 weeks. In a case report reported by Ramaswamy et al.[2], it was seen that the patients diagnosed as BPC in prenatal USG at 25 weeks had completely regressed on MRI after delivery. In our study, as in these studies, three (15%) patients could not be evaluated because termination of pregnancy was performed before 24th and 26th weeks, but it was observed that spontaneous resolution of BPC occurred in four (21%) of the remaining 16 patients and it continued in 12 (63%) patients.

Conclusion

BPC, whose embryology, clinical findings, imaging characteristics, and outcomes are not known sufficiently by medical professionals, is one of the posterior fossa cystic lesions. Therefore, it may be misdiagnosed by radiologists and physicians in patients with BPC. Prognosis is excellent in patients with isolated PBC, and healthy neurologic development may be observed without any evidence until advanced adult ages. However, depending on the condition of the associated anomalies, complications such as termination, death in the early neonatal period, and abnormal neurologic development may be observed.

Ethics

Ethics Committee Approval: This study was conducted after the Kanuni Sultan Suleyman Research and Training Hospital Clinical Research Ethics Committee's gave approval.

Informed Consent: Written informed consent was obtained from all participants.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: M.B., S.C.O., F.Ö., Z.G.Ö., S.S.Ç., Y.B., S.S., K.E., M.A.Y., İ.Ö., Design: M.B., S.C.O., F.Ö., Z.G.Ö., S.S.Ç., S.S., K.E., M.A.Y., İ.Ö., Data Collection or Processing: M.B., Z.G.Ö., S.S.Ç., Y.B., Analysis or Interpretation: M.B., S.C.O., F.Ö., Z.G.Ö., S.S.Ç., Y.B., K.E., İ.Ö., Literature Search: M.B., S.C.O., Writing: M.B., S.C.O., Critical Review: S.C.O.

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Repeated dose of prostaglandin E2 vaginal insert when the first dose fails

İlk doz başarısız olduğunda prostaglandin E2 vajinal insertin tekrar kullanımı

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Abstract

Objective: To compare the obstetric and neonatal outcomes of patients treated with repeated-dose prostaglandin E2 (dinoprostone) vaginal insert when the first dose fails.

Materials and Methods: This retrospective study included 1.043 pregnant women who received dinoprostone for labor induction between November 2012 and August 2015. Pregnant women were divided into two groups according to the number of dinoprostone administrations: group 1, single-dose dinoprostone (n=1.000), and group 2, repeated-dose dinoprostone (n=43). Intrapartum, postpartum, and neonatal outcomes of the pregnant women were compared.

Results: Vaginal delivery rate was 65% in group 1 and 30.2% in group 2 (p=0.001). The need for the neonatal intensive care unit was found in 44 pregnant women (4.4%) in group 1 and 6 pregnant women (13.6%) in group 2 (p=0.006).

Conclusion: When obstetric and neonatal data were evaluated in our study, we observed that dinoprostone administration was associated with increased cesarean rates and adverse neonatal outcomes with repeated-dose dinoprostone when the first dose failed.

Keywords: Dinoprostone, labor induction, second-dose dinoprostone

Öz

Amaç: İlk doz başarısız olduğunda tekrarlayan dozda prostaglandin E2 (dinoproston) vajinal ovül ile tedavi edilen gebelerin obstetrik ve neonatal sonuçlarını karşılaştırmayı amaçladık.

Gereç ve Yöntemler: Bu retrospektif çalışmaya, Kasım 2012 ile Ağustos 2015 arasında doğum indüksiyonu için dinoproston alan 1,043 gebe dahil edildi. Gebeler dinoproston uygulama sayısına göre; grup 1: tek doz dinoproston, grup 2: tekrarlanan doz dinoproston olmak üzere 2 gruba ayrıldı. Tek doz dinoproston (n=1,000) ve iki dinoproston (n=43) alan gebelerin intrapartum, postpartum ve neonatal sonuçları karşılaştırıldı.

Bulgular: Grup 1'de vajinal doğum oranı %65 iken, grup %30,2 idi (p=0,001). Yenidoğan yoğun bakım ihtiyacı grup 1'de 44 gebede (%4,4), grup 2'de 6 gebede (%13,6) görüldü (p=0,006).

Sonuç: Çalışmamızda obstetrik ve neonatal veriler değerlendirildiğinde, ilk doz başarısız olduğunda tekrarlayan dinoproston dozu uygulanmasının artmış sezaryen oranları ve olumsuz neonatal sonuçlar ile ilişkili olduğunu gözlemledik.

Anahtar Kelimeler: Dinoproston, doğum indüksiyonu, ikinci doz dinoproston

PRECIS: Dinoprostone administration was associated with increased cesarean rates and adverse neonatal outcomes in repeated dose of dinoprostone when first dose fails.

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Introduction

Labor induction is widely used for various maternal and fetal indications and is likely to be effective when the cervix is favorable. However, when the cervix is unfavorable (e.g. low Bishop score), iatrogenic cervical ripening is usually employed to increase the probability of vaginal delivery. For this purpose, there are two major options, which include the application of cervical ripening agents such as prostaglandins (PG), and the insertion of mechanical dilators such as cervical ripening balloons[1,2]. PGs efface the cervix by increasing the water content and dissolving collagen bundles[3,4]. In addition to these processes, myometrial contraction occurs, an advantage of PG over the use of mechanical dilatators, and collectively, these lead to cervical ripening. The efficacy of PGs for cervical ripening and labor induction has been established by randomized trials and recently by a Cochrane review[5].

Dinoprostone is a prostaglandin E2 (PGE2) analogue and is approved for cervical ripening by the United States Food and Drug Administration. Endocervical gel and vaginal insert forms of dinoprostone are available[6]. The vaginal insert form is approved for use up to 12 and 24 hours in the United States and Europe, respectively. If labor does not ensue, or the expected Bishop change does not occur after administering PG (i.e. the cervix is still unfavorable), there is no consensus as to the preferred methods of labor induction. As a result, there are options such as repeating the PG dose, switching to mechanical dilators, oxytocin induction, or cesarean delivery[7].

There are insufficient data in the literature regarding the repeated dose of dinoprostone and its safety. Our hypothesis was that repeated PGE2 administration would be associated with poor maternal and fetal outcomes. We aimed to compare the obstetric and neonatal outcomes of pregnant women treated with repeated doses of dinoprostone when the first dose fails.

Materials and Methods

This retrospective study included 1.043 pregnant women treated with dinoprostone for labor induction at Etlik Zübeyde Hanım Women's Health Training and Research Hospital between November 2012 and August 2015. The protocol used in this study was approved by the Institutional Review Board of Etlik Zübeyde Hanım Women's Health Training and Research Hospital and performed in accordance with the ethical standards established by the 1964 Declaration of Helsinki.

Pregnant women with singleton pregnancies, vertex presentation, a Bishop score ≤6, and normal fetal heart rate tracing were retrospectively reviewed. A previous history of uterine surgery (e.g. cesarean section, myomectomy, septum resection), known fetal anomalies, fetal malpresentation, diagnosis with placenta previa/vasa previa, PG allergy, asthma, abnormal fetal monitorization finding, vaginal delivery contraindication and estimated fetal weight of 4.000 g or more in the ultrasonography were excluded from the study. A vaginal insert (Propess®, Ferring, Saint-Prex, Switzerland) with 10 mg slow-release dinoprostone was inserted high in the posterior vaginal fornix for cervical ripening. We recorded the insertion and retrieval times of the dinoprostone vaginal insert. After 24 hours, pregnant women with a Bishop score less than 6 were considered as non-responsive to dinoprostone. Dinoprostone was administered to pregnant women with non-responsive to dinoprostone after 24 hours. Continuous fetal monitoring was performed from the onset of the vaginal insert. Fetal heart rate classification and management were defined according to the American College of Obstetricians and Gynecologists guidelines[8]. Cervical opening ≥3 cm and active uterine contractions were considered active labor. Dinoprostone was retrieved in cases of active labor, uterine tetanus/tachysystole (more than five contractions in a 10-minute interval), or the presence of abnormal fetal heart monitorization. Pregnant women were divided into two groups according to the number of dinoprostone administrations: group 1 had a single dose of dinoprostone, and group 2 had a repeated dose. Intrapartum, postpartum, and neonatal outcomes of the groups were compared by reviewing patient files.

Outcome Measures

The primary outcome was vaginal and cesarean section rates. Other outcomes considered were the interval from the start of induction to active labor and delivery, the length of the first stage of labor and the total length of labor, the need for oxytocin augmentation, the occurrence of hyperstimulation, postpartum hemorrhage, and neonatal outcomes.

Statistical Analysis

Patient information was recorded in the Statistical Package for the Social Sciences (SPSS) Version 17.0 program. The distribution of the variables was analyzed using the Kolmogorov-Smirnov test. Continuous variables are expressed as mean ± standard deviation median (range), and categorical variables are expressed as percentages and frequencies. The independent sample t-test was used for comparisons of parametric variables, the Mann-Whitney U for non-parametric variables, and the chi-square test and Fisher's exact tests for intermittent variables. A value of $p<0.05$ was considered statistically significant.

Results

Our study included 1.064 pregnant women who underwent dinoprostone induction for labor in the perinatology unit of our hospital between November 2012 and August 2015. Twenty-one pregnant women did not meet the criteria and were excluded from the study. The remaining 1.043 pregnant women were divided into two groups as group 1 (n=1.000), which received only one dose of dinoprostone, and group 2 (n=43), which received a repeated dose. The characteristics of the groups are summarized in Table 1. Age, body mass index (BMI), mean gestational week, number of pregnancies, and amniotic fluid measurements were similar between the two

Table 1. Characteristics and pre and post-Bishop scores of groups and reasons for retrieval of dinoprostone

		Group 1 (single dose) (n=1.000)	Group 2 (repeated dose) (n=43)	p
***Age (years)**		24.4±4.2	24.7±4.8	0.435
****Parity**		0 (0-4)	0 (0-1)	0.789
Nulliparous % (n)		84.3% (843)	93% (40)	0.240
***Body mass index (kg/m²)**		29.1±4.5	29.4±5	0.666
***Gestational age (weeks)**		39.6±1.7	38.7±2.9	0.053
Amniotic fluid index % (n)	Oligohydramnios	42.6% (426)	41.9% (18)	0.617
	Normal	55.5% (555)	58.1% (25)	
	Polyhydramnios	1.9% (19)	-	
Dinoproston Indication % (n)	Postmaturity (>41 weeks' gestation)	34.6% (346)	27.9% (12)	0.001
	FGR	6% (60)	16.3% (7)	
	Oligohydramnios	25.2% (252)	20.9% (9)	
	PROM	2.5% (25)	14% (6)	
	Hypertension	6.7% (66)	9.3% (4)	
	Postmaturity + oligohydramnios	10.9% (109)	7% (3)	
	FGR + oligohydramnios	5.2% (52)	2.3% (1)	
	Other	8.9% (89)	2.3% (1)	
****Bishop score**	At 1st application	0 (0-4)	0 (0-1)	0.878
	at 2nd application	-	0 (0-4)	-
****Bishop score**	at 1st removal	6 (0-10)	1 (0-4)	0.001
	at 2nd removal		2 (0-9)	-
Reason for 1st retrieval of dinoporostone % (n)	Completed 24 hours	10.5% (105)	76.7% (33)	0.001
	Active labor	64.1% (641)	-	
	Uterine tachysystole	5.1% (51)	18.6% (8)	
	Non-reassuring fetal status	7.4% (74)	-	
	Spontaneous dislocation	5.8% (58)	2.3% (1)	
	Other	71 7.1%	2.3% (1)	
Reason for 2nd retrieval of dinoporostone % (n)	Completed 24 hours	-	39.5% (17)	-
	Active labor	-	34.9% (15)	
	Uterine tachysystole	-	4.7% (2)	
	Non-reassuring fetal status	-	14% (6)	
	Spontaneous dislocation	-	4.7% (2)	
Oxytocin augmentation % (n)		13.6% (136)	11.6% (5)	0.069

*Results are expressed as mean ± standard deviation, **Results are expressed as median (minimum-maximum), PROM: Preterm rupture of membrane, FGR: Fetal growth restriction

groups (p>0.05). The most frequent indication for dinoprostone in both groups was post-term pregnancy. The Bishop scores during the first dinoprostone application were similar between the groups (p=0.878), whereas the Bishop score after the first application of dinoprostone was 6 (0-10) in group 1, and 1 (0-4) in group 2 (p=0.001). There was no difference in the need for oxytocin augmentation after dinoprostone retrieval in both groups (p=0.669) (Table 1). Also, 650 (65%) pregnant women in group 1 and 13 (30.2%) in group 2 had vaginal deliveries (p=0.001).

The most frequent cesarean indication was non-reassuring fetal status in group 1, and failed induction in group 2. The second stage of delivery time was similar between the two groups (p>0.05). However, latent phase and active phase durations were longer in group 2 (p=0.001, p=0.033, respectively). Ten (1%) women in group 1 and two (4.5%) in group 2 needed postpartum blood transfusions (p=0.031) (Table 2). When neonatal outcomes were evaluated, the mean APGAR score at 1 minute was 8.8±0.5 in group 1 and 8.6±0.7 (p=0.023) in group 2. Furthermore, need for neonatal intensive care unit (NICU) treatment was found in 44 (4.4%) women in group 1 and six (13.6%) women in group 2 (Table 2).

In our study, only one patient in group 1 developed a severe complication (uterine rupture). The cause of dinoprostone retrieval in this patient was active delivery and her uterus ruptured at the second stage of delivery.

Discussion

In this study, we compared the obstetric and neonatal outcomes of pregnant women who underwent labor induction once or twice with dinoprostone. To the best of our knowledge, there is no much information in the literature about the repeated administration of dinoprostone and there are scant data on reliability and efficacy[7,9,10]. Our center is a tertiary teaching hospital and a reference center that has more than 16.000 deliveries annually. Additionally, our hospital is one of the largest centers of the induction of labor in Turkey. The most important finding of this study is the high cesarean rate in repeated dinoprostone administrations for women non-responsive to dinoprostone. Therefore, pregnant women should be informed that the process may result in a cesarean delivery before the second administration and other labor induction methods such as mechanical dilators and oxytocin could be offered.

Table 2. Obstetric and neonatal outcomes of groups

		Group 1 (single-dose) (n=1.000)	Group 2 (repeated-dose) (n=43)	p
Vaginal delivery % (n)		65% (650)	30.2% (13)	0.001
Cesarean indication % (n)	Failed induction	11.4% (40)	63.3% (19)	0.001
	Fetal distress	47.4% (166)	23.3% (7)	
	CPD	34.3% (120)	13.3% (4)	
	Cord prolapse	1.1% (4)	-	
	Chorioamnionitis	0.6% (2)	-	
	Other	5.2% (18)	-	
***Time to delivery (hours)**		16.91±9.12	58.46±16.50	0.001
***Latent phase duration of labor (hours)**		12.67±7.40	50.38 ±12.97	0.001
***Active phase duration of labor (hours)**		4.01±3.74	6.26±4.47	0.033
***Second stage duration of labor (min)**		44.99±21.56	48.46±15.19	0.564
***Estimated blood loss (mL)**		179.7±113.2	182±109.2	0.890
Transfusion requirement (+) % (n)		1% (10)	4.6% (2)	0.031
Number of transfusions (RBC concentrate) % (n)	2 units	80% (8)	100% (2)	0.787
	4 units	10% (1)	-	
	>4 units	10% (1)	-	
***Birth weight (g)**		3161.1±484.4	3030±604.2	0.186
***APGAR 1**		8.8±0.6	8.6±0.7	0.023
***APGAR 5**		9.3±0.5	9.2±0.5	0.081
Need for neonatal intensive care unit % (n)		4.5% (45)	14% (6)	0.006
Reason for neonatal intensive care unit % (n)	Respiratory distress	54.5% (24)	100% (6)	0.208
	Hyperbilirubinemia	15.9% (7)	-	
	Prematurity	11.4% (5)	-	
	Other	18.2% (8)	-	

*Results are expressed as mean ± standard deviation. CPD: Cephalo-pelvic disproportion, RBC: Red blood cell

In our study, most of the pregnant women were nulliparous and the most frequent indication for labor induction was post-maturity. This can be explained by the fact that post-maturity is higher in nulliparous women. On the other hand, the gestational week of women who received dinoprostone for the second time was lower than those given it once, although it was not statistically significant. Probably, the effectiveness of the dinoprostone increases as the gestational week progresses, but more patients are needed to confirm this.

The use of labor induction especially in elective delivery has increased significantly in recent years[11]. Dinoprostone is one of the most commonly used pharmacologic methods for the induction of labor[12]. Cesarean delivery, mechanical dilatation, oxytocin induction, and repeated administration of dinoprostone are alternative methods for non-responsive patients to dinoprostone during labor induction. We use repeated dinoprostone for pregnant women who are non-responsive to single-dose dinoprostone in our center.

The success of labor induction depends on many factors such as the general characteristics of the population, age, gestational week, BMI, parity, estimated fetal weight, Bishop score, and labor induction method used[13-15]. In our study, the age, gestational week, BMI, parities of pregnant women were similar between the two groups. Antonazzo et al.[7] compared the results of patients who received repeated dinoprostone administrations and oxytocin induction in pregnant women who did not respond to dinoprostone. They reported that the cesarean section rate was 44.7% in 47 pregnant women who received repeated dinoprostone administration and 66% in 47 pregnant women who had oxytocin induction. In the literature, the average cesarean delivery rate for labor induction with dinoprostone is 25% (range, 10.5-38.6%), and the vaginal delivery rate within 24 hours is 59.4% (range, 38.2-81.1%) [16-19]. In our study, we observed that the cesarean section rate was 70.5% in 43 pregnant women who received repeated dinoprostone administrations. We speculated that this rate was extremely high, therefore patient selection for repeating dinoprostone should be performed more carefully before the second application.

Additionally, Antonazzo et al.[7] found no differences in terms of neonatal outcomes (5-minute APGAR score, umbilical artery pH) between the repeated dinoprostone group and the oxytocin group. In our study, the need for NICU treatment and the need for blood transfusion were higher in group 2. Despite of literature[7], our data showed that repeated administration of dinoprostone is not a safe therapeutic option when first dose fails.

Study Limitations

The present study has some limitations such as the relatively small population for repeated dinoprostone group, the absence of other labor induction method groups for patients non-responsive to dinoprostone in labor induction, and its retrospective design. Additional well-designed randomized controlled studies are required to improve our understanding of the efficacy and outcomes (neonatal and maternal) of repeated-dose dinoprostone for women non-responsive to dinoprostone in labor induction.

Conclusion

When we evaluated all these obstetric and neonatal data in our study, we observed that dinoprostone administration was associated with an increased rate of caesarean and adverse neonatal outcomes with the second dose compared with single-dose dinoprostone in unresponsive women. As an alternative to applying dinoprostone for the second time, mechanical or other pharmacologic methods should be tried.

Ethics

Ethics Committee Approval: The protocol used in this study was approved by the Institutional Review Board of Etlik Zübeyde Hanım Women's Health Training and Research Hospital and performed in accordance with the ethical standards established by the 1964 Declaration of Helsinki.

Informed Consent: Retrospective study.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: C.K., S.E., Design: S.E., E.S.Y., Data Collection or Processing: C.K., T.B., Analysis or Interpretation: Y.A.T., B.K., Literature Search: C.K., Y.A.T., Writing: C.K., B.K.

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

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Review / Derleme

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Systematic review and meta-analysis of ropivacaine use in laparoscopic hysterectomy

Laparoskopik histerektomide ropivakain kullanımının sistematik derlemesi ve meta analizi

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Abstract

To assess the efficacy of all forms of ropivacaine administration for the management of pain and opioid use, specifically in patients undergoing laparoscopic hysterectomy. We searched PubMed, Cochrane CENTRAL, Web of Science, and SCOPUS for relevant clinical trials matching our eligibility criteria. Outcomes of interest included: Pain intensity (measured either by visual analog scale score or by numerical rating scale score), QoR-40 score (Overall quality of recovery tool, designed to measure physical comfort, physical independence, pain, emotional status, and need for support), and the need for opioid rescue. We performed the analysis under the fixed-effects model for homogeneous data and random-effects model for heterogeneous data. Most heterogeneous data were solved by the leave-one-out method, in cases where this was not successful, we then proceeded to conduct at least one subgroup meta-analysis in an attempt to solve heterogeneity. We assessed the risk of bias using Cochrane's risk of bias tool. A total of five clinical trials were included. Regarding the pain score, there was no significant difference between either group [standardized mean difference=-0.17, 95% confidence interval (CI): (-0.56, 0.23); p=0.41]. The analysis of the overall RoQ40 scores favored the ropivacaine group over the control group significantly [mean difference (MD)=17.68, 95% CI: (1.48, 33.87); p<0.001]. Regarding the use of opioids, the analysis revealed no significant difference between either group [MD=-2.57, 95% CI: (-6.62, 1.49); p=0.21].

Ropivacaine administration by any method does not seem to be effective in reducing pain or reducing the need for opioid use after laparoscopic hysterectomy procedures; however, the administration did show a significant improvement in the patient's "overall quality of recovery," as measured using the QoR-40 tool.

Keywords: Laparoscopy, ropivacaine, hysterectomy, ERAS, MIGS

Öz

Özellikle laparoskopik histerektomi geçiren hastalarda ağrı ve opioid kullanımının yönetimi için tüm ropivakain formlarının etkinliğini değerlendirmek. Uygunluk kriterlerimizle eşleşen ilgili klinik araştırmalar için PubMed, Cochrane CENTRAL, Web of Science ve SCOPUS'u taradık. Aradığımız sonlanımlar şunlardı: Ağrı yoğunluğu (vizuel analog skala skoru veya sayısal değerlendirme ölçeği skoru ile ölçülen), QoR-40 skoru (fiziksel rahatlık, fiziksel bağımsızlık, ağrı, duygusal durum ve destek ihtiyacını ölçmek için tasarlanmış genel iyileşme kalitesi aracı) ve opioid ihtiyacı. Analizi homojen veriler için sabit etkiler modeli ve heterojen veriler için rastgele etkiler modeli altında gerçekleştirdik. Cochrane'nin bias riski aracını kullanarak bias riskini değerlendirdik. Toplam beş klinik çalışma dahil edildi. Ağrı skoru açısından her iki grup arasında anlamlı bir fark yoktu [standartlaştırılmış ortalama fark=-0,17 (%95 güven aralığı (GA)=-0,56, 0,23); p=0,41]. Genel RoQ40 skorunun analizi, kontrol grubuna kıyasla ropivakain grubu lehine sonuçlandı [ortalama fark (MD)=17,68 (%95 GA=1,48, 33,87); p=0,001]. Opioid kullanımı açısından her iki grup arasında anlamlı bir fark yoktu [MD=-2,57 (%95 GA=-6,62, 1,49); p=0,21]. Herhangi bir yöntemle ropivakain uygulaması ağrıyı azaltmada veya laparoskopik histerektomi prosedürlerinden sonra opioid kullanımına olan ihtiyacı azaltmada etkili görünmemektedir; bununla birlikte ropivakain uygulaması, QoR-40 aracı ile ölçülen hastanın "genel iyileşme kalitesinde" önemli bir düzelme göstermiştir.

Anahtar Kelimeler: Laparoskopi, ropivakain, histerektomi, ERAS, MIGS

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Introduction

Hysterectomy is the most common gynecologic surgical procedure undergone by women in the United States, with over 600,000 performed annually[1]. In gynecologic surgery, we have seen a persistent increase in the rate of hysterectomies performed via laparoscopic techniques over time[2,3]. About 30% of hysterectomies are performed using minimally invasive laparoscopic techniques[4]. Laparoscopic surgeries have many benefits over abdominal approaches: they ensure faster recovery, fewer complications, less pain, and shorter hospital stay[5]. Many trials have been conducted to develop strategies to facilitate laparoscopic hysterectomy as an outpatient procedure when feasible[6]. However, pain control is still a major problem in postoperative care. Obtaining adequate postoperative analgesia can increase the advantages of the laparoscopic approach over abdominal surgery, and at least in theory, a painless recovery from laparoscopic surgery is possible. For this reason, many trials have been performed to find the best strategies to relieve pain after laparoscopic surgery[7].

Patients undergoing laparoscopic hysterectomy have substantial pain and may require a large dose of opioids in the first 24 hours after the procedure[8]. Administration of opioids has many adverse effects such as nausea, vomiting, constipation, and respiratory depression[9]. The opioid-related adverse effects may impair the postoperative quality of recovery of patients undergoing this procedure. Administration of local analgesics either intraperitoneally or through the transversus abdominis plane (TAP) may reduce the total need for opioids in the first 24 h postoperatively[10].

Ropivacaine is a long-acting amino amide local anesthetic agent with a duration of action that may extend to 8 hours[11]. Ropivacaine is commonly used for nerve block and intraperitoneal use. It produces its analgesic effect via reversible inhibition of sodium ion influx in nerve fibers[12]. Ropivacaine is less lipophilic than other local analgesic agents and less likely to penetrate large myelinated motor fibers, resulting in a relatively reduced motor blockade. The reduced lipophilicity is associated with less undesirable central nervous system toxicity and cardiotoxicity. For this reason, it is suitable for immediate pain control after uterine surgeries[13,14].

TAP block is widely used as a pain management approach after various abdominal surgical procedures[15,16]. The TAP block consists of an injection of a local anesthetic agent between the internal oblique abdominal muscle and the transverse abdominal muscle[17]. This procedure interrupts the sensory innervation to the anterior abdominal wall and peritoneum[18]. Some trials have shown that TAP blocks lead to a significant reduction in narcotic consumption and recovery times in both open and laparoscopic surgery[19].

In our meta-analysis, we aimed to estimate the effect of ropivacaine infiltration in the reduction of postoperative pain and the total need for opioids in the first 24 h postoperatively.

This systematic review and meta-analysis were performed with strict adherence to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) Statement[20]. In addition, we followed the guidelines reported in the Cochrane Handbook for Systematic Reviews of Interventions[21].

Literature Search

We searched for published studies in four online databases: PubMed, Web of Science, Scopus, and Cochrane Central Register of Controlled Trials (CENTRAL) in August 2020. Our search was performed using the following keywords: ropivacaine, naropin, laparoscop*, and hysterectom* and combining these words with "AND" or "OR" as was necessary according to the search engine being used.

Eligibility Criteria

For eligibility, we included all studies that met all of the following criteria: (1) Patients: women undergoing laparoscopic hysterectomy, (2) Intervention: ropivacaine, (3) Comparator: placebo, (4) Outcomes: pain intensity [measured either using visual analog scale (VAS) scores or numerical rating scale (NRS) scores], overall quality of recovery (QoR-40 score), which is measured by physical comfort, physical independence, pain, emotional status, and support, and the need for opioid rescue. Type of Study: we only included randomized clinical trials (RCTs). Studies with other criteria were excluded, including (1) non-RCTs, (2) single-armed trials or with different comparators, (3) trials involving animals, and (4) studies for which there was no availability of a full-text copy of the paper.

Screening and Studies Selection

Our next step was to export the search results from our databases into Endnote X8.0.1 (Build 1044) and perform automatic removal of any duplicates. Following this, we screened the search results manually in two steps: first, we performed title and abstract screening, then we went on to perform full-text screening for the preliminary studies included in the first step. We included articles based on our criteria for eligibility and removed studies that did not fulfill these criteria.

Data Extraction and Analysis

After the screening step, we extracted data from the eligible studies. Data extracted were categorized into two main groups: (1) Demographic and baseline data of patients in each study including age, body mass index (BMI), sample size, dose of intervention, surgery time, blood lose, number of patients diagnosed by fibroid, number of patients diagnosed with endometriosis, number of patients diagnosed with prolapse, number of patients diagnosed with chronic pelvic pain. (2) Data for analysis include pain intensity (by VAS or NRS score), quality of recovery (QoR-40) score that includes physical comfort, physical independence, emotional status, pain RoQ40, and support. Additional outcomes included anti-emetic use and need for opioids. Data for continuous outcomes were extracted as a mean and standard deviation, and data for dichotomous outcomes were extracted as events and total.

Data Analysis

We performed this analysis using the Review Manager software (RevMan 5.3). Data for continuous outcomes are expressed using mean difference (MD) and standard deviations, and dichotomous outcomes are expressed using percentage and total relative to a fixed 95% confidence interval (CI). We used standardized mean difference (SMD) whenever outcomes were measured using different scores. Heterogeneity was assessed using a statistical I^2 test and p-value of the chi-square test, where outcomes with I^2 >50%, p<0.1 were considered heterogeneous, and outcomes with I^2 <50%, p>0.1 were considered homogeneous. Next, homogenous data were analyzed using a fixed-effects model, and the heterogeneous outcomes were analyzed using the random-effects model. In heterogeneous data not solved using the leave-one-out method, we then conducted a subgroup meta-analysis as the next step in attempting to solving heterogeneity.

Quality Assessment

We performed a quality assessment by an evaluation which used the GRADE Guidelines (Grading of Recommendations Assessment, Development and Evaluation). For our risk of bias (ROB) assessment, we used the Cochrane ROB tool for use in clinical trials[22]. The Cochrane ROB assessment tool includes the following domains: random sequence generation (selection bias), allocation sequence concealment (selection bias), blinding of participants and personnel (performance bias), blinding of outcome assessment (detection bias), incomplete outcome data (attrition bias), selective outcome reporting (reporting bias) and other potential sources of bias. The authors' judgment is categorized as "Low risk", "High risk" or "Unclear risk" of bias.

Results of Literature Search

Out of 144 studies included through our literature search and references, only nine studies were eligible and included in the full-text literature. Five studies fulfilled our eligibility criteria after full-text screening and were included in our meta-analysis. Figure 1 illustrates the PRISMA statement of our literature search.

Three hundred eight patients were included (166 in the ropivacaine group, and 142 in the control group). The mean age of patients in the study group was 50.2±10.9 years, and the mean age of the control group was 51.2±12.8 years. The mean BMIs in the intervention group and the control group were 26.7±5.8 and 27±5.3 kg/m^2, respectively. Detailed baseline characteristics for the included studies are shown in Table 1. The mean duration of surgery in the ropivacaine group and control group were 147.3 and 138.8 minutes respectively, and the mean blood loss was 92.5 and 72.5 mL, respectively. Table 2 summarizes the surgical duration and blood loss in each study.

Results of Quality Assessment

The overall ROB of included studies was of low risk according to the Cochrane ROB assessment tool. All studies were at low risk regarding random sequence generation (selection bias) and allocation concealment. Three studies[23-25] performed proper blinding of personnel and participants and therefore were considered as low risk, whereas the other two studies[26,27] were at high risk of performance bias. Outcome assessors were blinded in De Oliveira et al., 2011[23] and Torup et al.[25] 2015 and considered at low risk of detection bias. Three studies did not report whether outcome assessors were blinded and therefore were considered to have unclear risk of detection bias[24,26,27]. All studies were of low ROB regarding attrition bias and reporting bias. No other ROB was detected in any study. Figure 2 illustrates the ROB of included studies.

Results of Outcomes

Pain Score

All included studies reported pain score outcomes. Three studies reported pain score outcomes using VAS scores[25-27], whereas the others used NRS scores[23,24]. Therefore, we used the SMD. The analysis showed no significant difference between the ropivacaine and placebo groups [SMD=-0.17, 95% CI: (-0.56, 0.23); p=0.41] (Figure 3A). Data were heterogeneous (p=0.007, I^2=69%). In an attempt to solve the heterogeneity, we excluded one study[23] (0.50% mg) from the analysis. Pooled analysis did not favor any one group over any other [SMD=-0.00, 95% CI: (-0.30, 0.30); p=0.99]. Data were homogeneous (p=.17, I^2=38%). Figure 3B shows the analysis of pain score outcomes after the leave-one-out method.

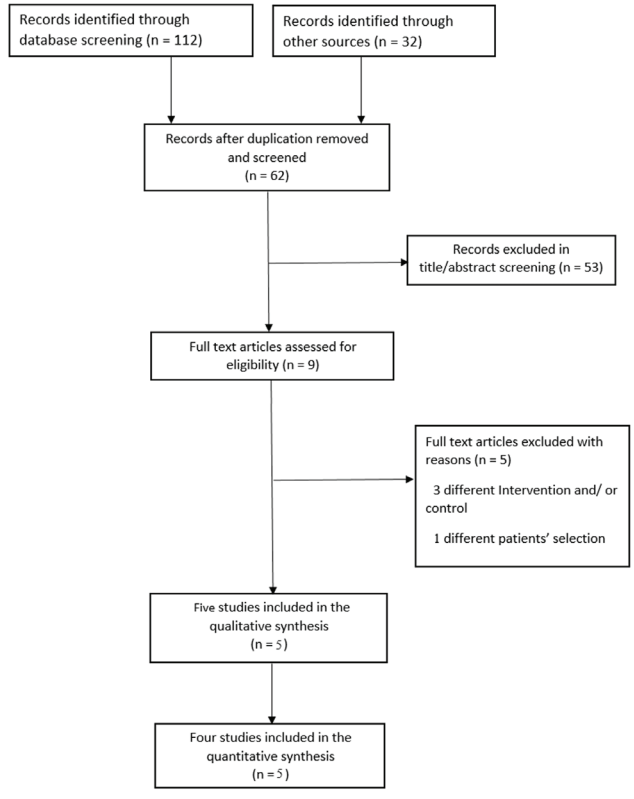

Figure 1. PRISMA flowchart diagram

QoR-40 Score

De Oliveria et al.[23] 2011 and Kane et al.[26] 2012 reported QoR-40 score outcomes. The analysis of overall RoQ40 score favored the ropivacaine group over the control group significantly [MD=17.68, 95% CI: (1.48, 33.87); p<0.001]. Data were heterogeneous (p=0.001, I^2=85%) (Figure 4A). Heterogeneity was best solved by employing the leave-one-out method to exclude De Oliveira et al.[23] 2011 (0.50%) (p=0.56, I^2=0%), and there was significant favoring of the ropivacaine group over the control group [MD=25.99, 95% CI: (18.20, 33.77); p<0.001] (Figure 4B).

Detailed analysis for each item of the QoR-40 score (physical comfort, physical independence, emotional status, pain, and support) is shown in Figure 5; there was no significant difference between the two groups regarding each item of QoR-40.

Opioid Rescue

All studies reported opioid rescue outcomes. The analysis showed no significant difference between the groups [MD=-2.57, 95% CI: (-6.62, 1.49); p=0.21]. Data were heterogeneous (p<0.001, I^2=79%) (Figure 6A). Heterogeneity was best solved using the leave-one-out method excluding De Oliveira et al.[23] 2011 (0.50% mg) (p=0.18, I^2=36%). The net result of the analysis showed no significant difference between the groups [MD=-0.31, 95% CI: (-3.00, -2.38); p=0.82]. Figure 6B shows the analysis of opioid rescue outcomes after the leave-one-out method.

Discussion

Our analysis found that the ropivacaine neither significantly reduce pain following laparoscopic hysterectomy nor opioid consumption in the first 24 h. It significantly controlled overall RoQ40, but there was no difference between ropivacaine and

Table 1. Baseline characteristics of the included studies

Study ID	Number		Age (M ± SD)		BMI (M ± SD)		Fibroids total (%)		Endometriosis total (%)	
	RPV	C	RPV	C	RPV	C	RPV	C	RPV	C
De Oliveira et al.[23] 2011 (0.50% mg)	22	23	45±4	47±9	25.3±4.5	27±4.4	18 (81.8)	17 (73.9)	1 (4.5)	2 (8.7)
De Oliveira et al.[23] 2011 (0.25% mg)	21	23	46±6	47±9	27±5.2	27±4.4	16 (76.2)	17 (73.9)	4 (19)	2 (8.7)
Kane et al.[26] 2012	28	28	46.2±5.1	43.5±7.9	31.1±7.5	29.9±7.3	17 (60.7)	14 (50)	5 (17.9)	4 (14.3)
Kwack et al.[24] 2018	20	20	50.75±6.7	49±5.5	24.89±2.61	25.2±2.8				
Torup et al.[25] 2015	34	31	51.3±14.1	54.7±9.6	24.7±3	27.3±5.2	3 (8.8)	8 (25.8)		
Kilpio et al.[40] 2019	41	40	56.7±13.3	57.3±17.8	26.7±6.7	25.7±4.4				

RPV: Ropivacaine, C: Control (placebo), M: Mean, SD: Standard deviation

Table 2. Surgery time and blood loss estimation in each of the included studies

Study ID	Blood loss (M ± SD)		Surgical time (M ± SD)	
	RPV	C	RPV	C
De Oliveira et al.[23] 2011 (0.5% mg)			225±85	198±68
De Oliveira et al.[23] 2011 (0.25% mg)			223±72	198±68
Kane et al.[26] 2012			138.05±43.5	155±40.5
Kwack et al.[24] 2018	155±120	52.5±22.5		
Torup et al.[25] 2015	66.7±51.9	50±74.1	113.7±51.9	121.7±65.2
Kilpio et al.[40] 2019	83.3±148.1	100±148.1	101±46.7	106.7±33.3

RPV: Ropivacaine, C: Control (placebo), M: mean, SD: Standard deviation

placebo in control items of the QoR-40 score (physical comfort, physical independence, emotional status, pain, and support). Of the five included studies, three administered ropivacaine through (TAP)[23,25,26], one through vaginal cuff infiltration[27], and one simply administered it vaginally[24]. Kwack et al.[24] assessed pain score at different hours during the entire 24 hours and found that ropivacaine was superior to placebo in reducing postoperative pain intensity only at 2 h, but there was no significant difference in pain reduction at 6, 12, and 24 h. De Oliveira et al.[23] compared two different concentrations of ropivacaine (0.5% and 0.25%) with saline, concluding that there was no difference between the 0.25% ropivacaine group or the 0.5% ropivacaine group and the saline group in the reduction of postoperative opioid consumption[23].

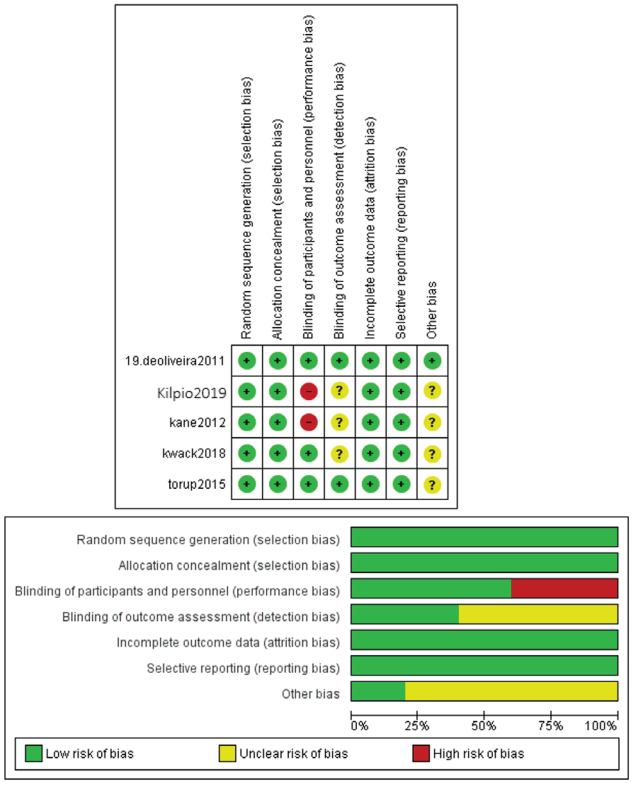

Figure 2. Risk of bias chart

The use of the TAP block in most of our studies could explain why ropivacaine did not significantly control post-laparoscopic hysterectomy pain, which usually arises from the perineum, shoulder, and abdomen. Abdominal pain originates from somatic and visceral components with the visceral pain being stronger[28]. A TAP block potentially covers somatic pain only because it blocks sensory nerves in the thoracolumbar region that supply the anterolateral abdominal wall[29-31]. Some authors theorized that for the reduction of postoperative pain, ropivacaine should be administered in such a way as to be absorbed systemically[32]. A review by Shin et al.[33] demonstrated that TAP block was not significant in pain reduction and morphine consumption in the first 24 h following laparoscopic hysterectomy. Kwack et al.[24] focused on control visceral pain through the injection of ropivacaine into the uterosacral area to block pelvic visceral plexus (uterine nerve plexus). They found that there was a reduction in early postoperative pain and the need for analgesics[33].

Acharya et al.[34] and Chiruvella et al.[35] concluded that adding dexmedetomidine to ropivacaine was effective in the management of postoperative pain and reduced analgesic consumption following laparoscopic hysterectomy. They also found that this combination was superior to using ropivacaine alone[34,35]. In comparison with lidocaine, Ghisi et al.[36] found

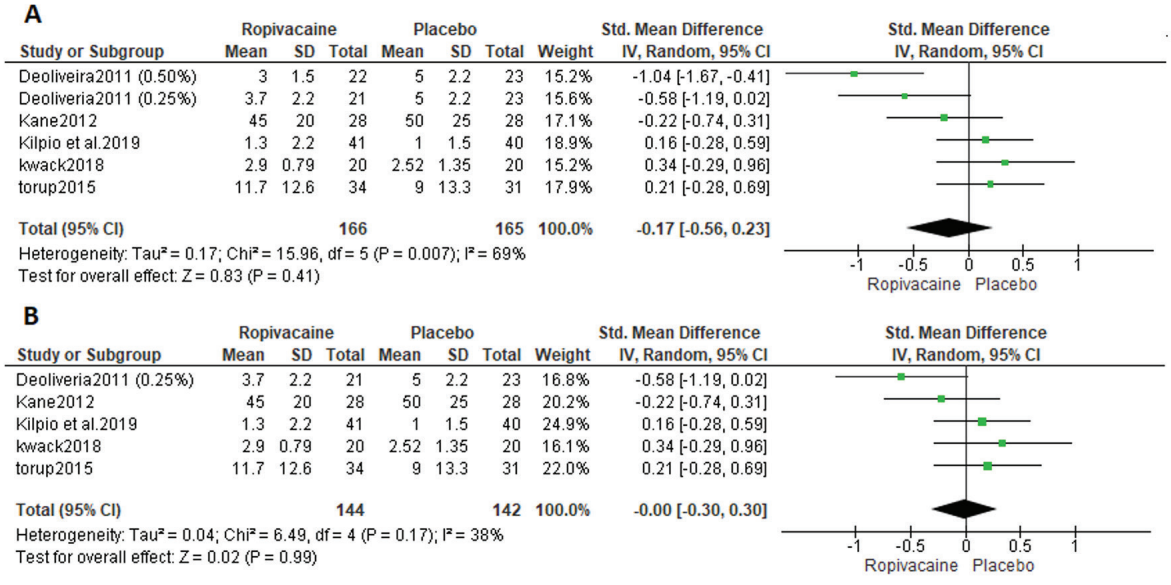

Figure 3. Postoperative pain in included studies

SD: Standard deviation, CI: Confidence interval

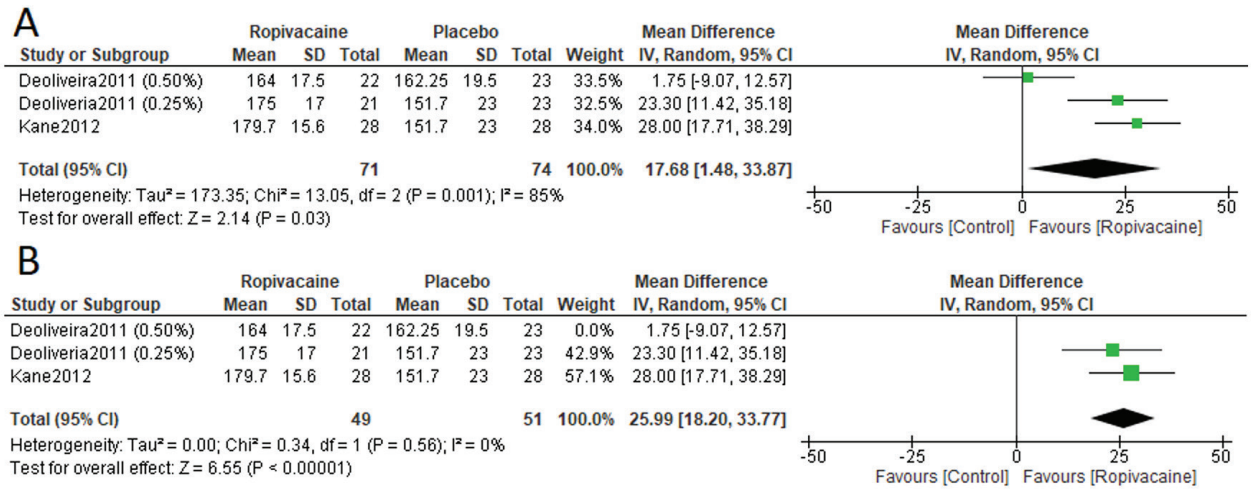

Figure 4. RoQ scores in included studies

SD: Standard deviation, CI: Confidence interval

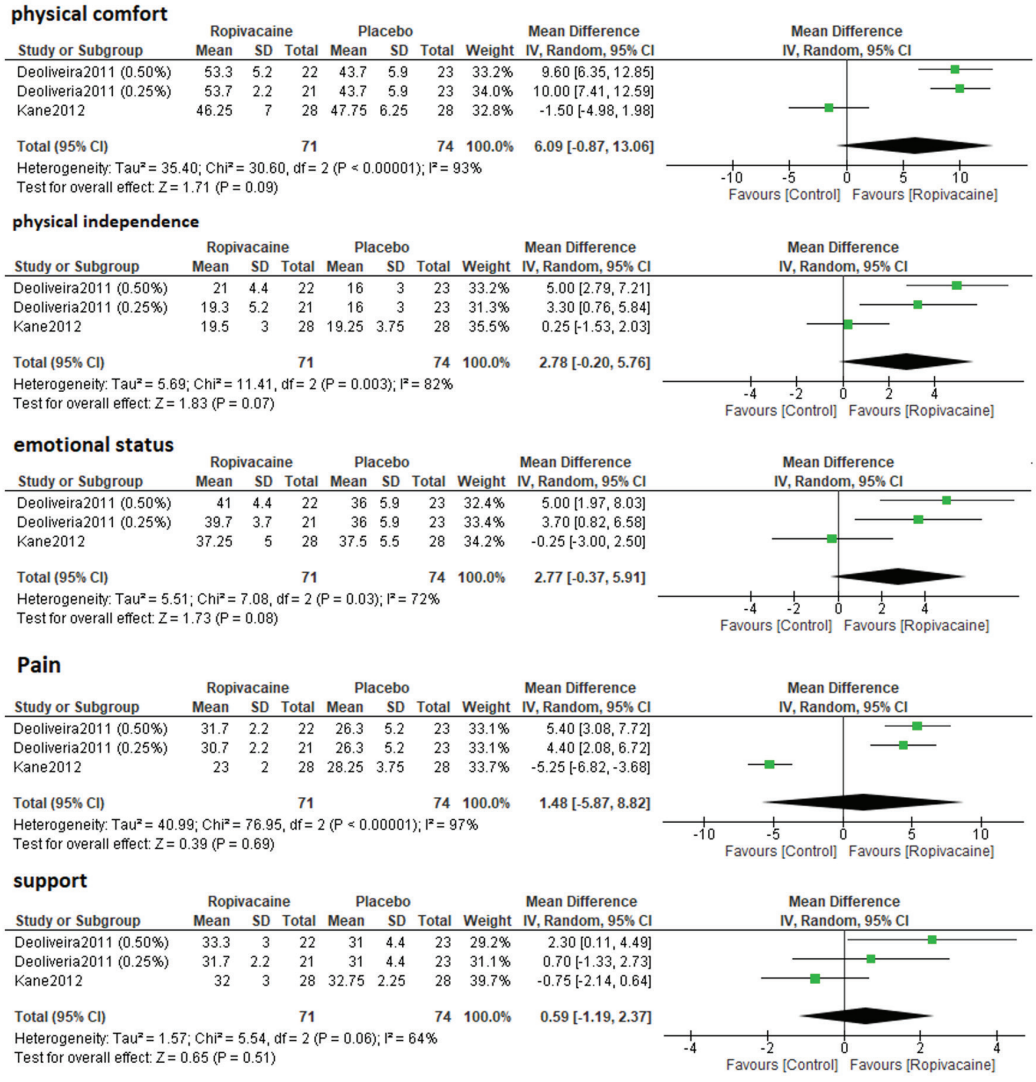

Figure 5. Components of RoQ scores

SD: Standard deviation, CI: Confidence interval

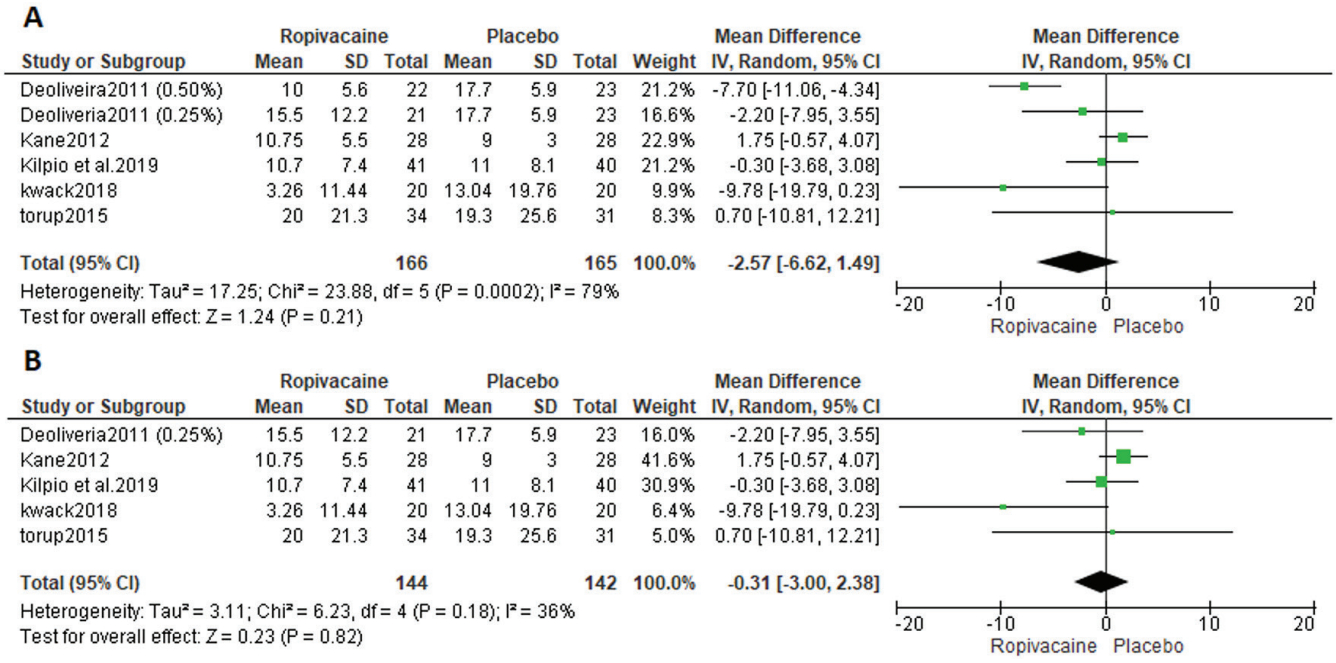

Figure 6. Incidence of use of opioid rescue

SD: Standard deviation, CI: Confidence interval

no difference in the analgesic effect of ropivacaine in pain control after laparoscopic abdominal surgeries, but the cost of lidocaine was lower than that of ropivacaine.

Chou et al.[28] showed that the use of ropivacaine via the intraperitoneal route was effective in pain control and reduced analgesic consumption after laparoscopic appendectomy. Thakur et al.[37] found that the ropivacaine significantly reduced postoperative pain and analgesic consumption following laparoscopic cholecystectomy through combined wound and intraperitoneal instillation. Likewise, Yong and Guang[38] found that ropivacaine could reduce pain following laparoscopic cholecystectomy, but only when administered through an intraperitoneal installation[39].

A RCT by Korkmaz et al.[39] showed that bupivacaine could significantly reduce VAS scores and tramadol consumption after laparoscopic hysterectomy[40]. However, Chatrath et al.[41] found that ropivacaine was better than bupivacaine in its analgesic effect with fewer adverse effects.

The main strength of our analysis was that it included only RCTs with low ROB. The main limitation of our study was the very low number of studies, the fact that we were forced to pool studies in which ropivacaine was injected as part of a TAP block along with those that administered or injected vaginal ropivacaine. More clinical trials are needed to investigate the efficacy of ropivacaine in pain relief after laparoscopic hysterectomy, and ultimately studies need to be performed to differentiate the efficacy and advantages of the different routes of administration. In conclusion, our analysis found that ropivacaine did not significantly reduce pain intensity and analgesic consumption after laparoscopic hysterectomy.

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Ethics

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: G.J.M., A.A., K.S., Concept: G.J.M., S.A., S.R., K.W., Design: A.K., K.C., A.S., J.L., Data Collection or Processing: S.H., Analysis or Interpretation: S.R., K.W., Literature Search: K.C., A.S., J.L., Writing: G.J.M., A.A., K.S., S.A., S.R., K.W., S.H., G.B., A.K., K.C., A.S., J.L.

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Gynecologic manifestations in Emberger syndrome

Emberger sendromunda jinekolojik belirtiler

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Abstract

Immune system vulnerability seems to play a significant role in the development and malignant transformation of pre-malignant squamous cell lesions. Emberger syndrome is a condition that affects the immune system, which is caused by *GATA2* gene mutations. Our objective was to present the gynecologic expressions of this rare syndrome in our case. Here, we discussed a relatively young patient with findings related to Emberger syndrome such as recurrent infections, myelodysplastic syndrome, lower extremity edema, and multifocal, multicentric premalignant/malignant genital lesions. Sequencing of the *GATA2* gene was accomplished for suspected Emberger syndrome and a point mutation in intron5, c1143+8C >T was detected. Gynecologists may play an important role in the early detection of Emberger syndrome and guiding multidisciplinary treatment options as the initial signs related to this rare entity can appear on the genitalia.
Keywords: Emberger syndrome, GATA2, HPV, immunodeficiency, vulvar cancer

Öz

İmmün yetmezlik, pre-malign ve malign skuamöz hücre lezyonlarının gelişiminde ve premalign lezyonların malignitelere dönüşümde önemli bir rol oynamaktadır. Emberger sendromu, *GATA2* gen mutasyonlarının neden olduğu, immün sistemi etkileyen böyle bir durumdur. Bu nadir sendromun jinekolojik belirtileri olan bir olgumuzu sunduk. Burada, yıllar içinde gelişen tekrarlayan enfeksiyonlar, miyelodisplastik sendrom, alt ekstremite ödemi ve multifokal, multisentrik premalign/malign genital lezyonlar gibi Emberger sendromu ile uyumlu bulguları olan, nispeten genç bir hastayı tartışmayı amaçladık. Olası Emberger sendromu için *GATA2* geninin sekanslaması gerçekleştirildi ve intron5, c1143+8C >T'de nokta mutasyonu tespit edildi. Jinekologlar, bu sendromun erken teşhisinde ve multidisipliner tedavi seçeneklerinin yönlendirilmesinde önemli bir rol oynayabilir, çünkü ilk belirtiler genital organlarda ortaya çıkabilir.
Anahtar Kelimeler: Emberger sendromu, GATA2, HPV, immün yetmezlik, vulva kanseri

Introduction

Emberger syndrome is an autosomal dominant condition with incomplete penetrance and is caused by mutations in GATA-binding protein 2 (*GATA2*) gene[1-3]. GATA2 is located on the long arm of chromosome 3 (3q21). Its protein product belongs to a family of transcription factors known for having a role in the differentiation and proliferation of hematopoietic cell progenitors.

Familial aggregation and sporadic occurrences have both been reported[1,4]. GATA2 protein deficiency can lead to lymphopenia, and monocytopenia resulting in a tendency to viral, mycobacterial, and fungal infections[5]. Myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) are among the common presentations of Emberger syndrome. Along with immunodeficiency, hematologic abnormalities and lower extremity lymphedema, some other physical features such as sensorineural deafness, hypotelorism, webbed neck, and condylomas may co-occur[1,3,4,6].

The predisposition to certain infections due to immune and hematologic system abnormalities observed in this syndrome can be difficult to manage. Human papillomavirus (HPV)-related lesions may cause serious problems, especially in young patients with prominent immune deficiencies[4]. Clinically challenging disseminated genital condylomas have been reported. Although squamous cancer of the vulva is rarely encountered under the age of 40 years, patients with Emberger syndrome seem to tend to develop such neoplasms caused by HPV[7,8].

However, HPV-related premalignant or malignant diseases in patients with GATA2 mutations have been rarely discussed in gynecologic oncology literature. A few available reports have focused mostly on its hematologic representation. In this text, we presented a case of Emberger syndrome with multifocal/multicentric HPV-related premalignant and malignant genital

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lesions. Early diagnosis was emphasized to slow down disease progression.

Case Reports

A 41-year-old G1P1 woman was referred to our outpatient clinic with a history of an "abnormal smear result," which was dated more than a decade ago. She also had a history of occasional blood transfusions for chronic and severe anemia in the internal medicine department. Cervical biopsies were performed, and results were consistent with cervical carcinoma ***in situ***. The patient had opted for a hysterectomy due to her social and health insurance problems. A total abdominal hysterectomy was performed in 2008 and the final pathology was reported as carcinoma ***in situ*** of the cervix. She continued to need blood transfusions twice a year for anemia during her follow-up. Also, she was followed up for chronic hepatitis B.

After hysterectomy, the patient had vaginal smears at irregular intervals, which were reported as benign/low-grade results. Over the years, she had treatment for various infections, mostly of upper respiratory origin. Once, she had been hospitalized for pericardial tamponade of undetermined etiology in 2014. Subsequently, the patient was diagnosed as having vulvar squamous cell cancer and a radical vulvectomy was performed. The final pathology was vulvar squamous cancer (pT1b), positive surgical margins with vulvar intra-epithelial neoplasia (VIN) grade 3. The specimen was positive for HPV immunohistochemistry. Three months after the surgery, one skinning re-excision was performed for positive margins, which were later confirmed as VIN2+ lesions.

An investigation for the cause of anemia and thrombocytopenia revealed splenomegaly, a year after her last surgery. Subsequently, the patient had intractable hematuria, which led to a bone marrow biopsy resulting in a diagnosis of AML. The patient underwent chemotherapy without any complications and MDS developed in the following years.

Recently, she developed new VIN and anal intraepithelial neoplasia of various grades on perineal biopsies and a wide local excision was performed. The final pathology report revealed focal invasive squamous cell cancers with clear margins. Her last vaginal smear was low-grade squamous intraepithelial lesion, and was positive for HPV type 89. Meanwhile, the patient was treated with antiviral treatment for hepatitis B.

On physical examination, there was left lower extremity lymphedema, which she recalled having since her childhood (Figure 1). There were also brown macules, mainly on the edematous leg.

With many matching clinical findings, Emberger syndrome was suspected. Sequencing was accomplished for the *GATA2* gene and a point mutation in intron5, c1143+8C >T was detected.

Later, the patient was hospitalized at another hospital for pneumonia and she died of myocardial infarction. Her family history was negative among her five siblings and parents for similar conditions.

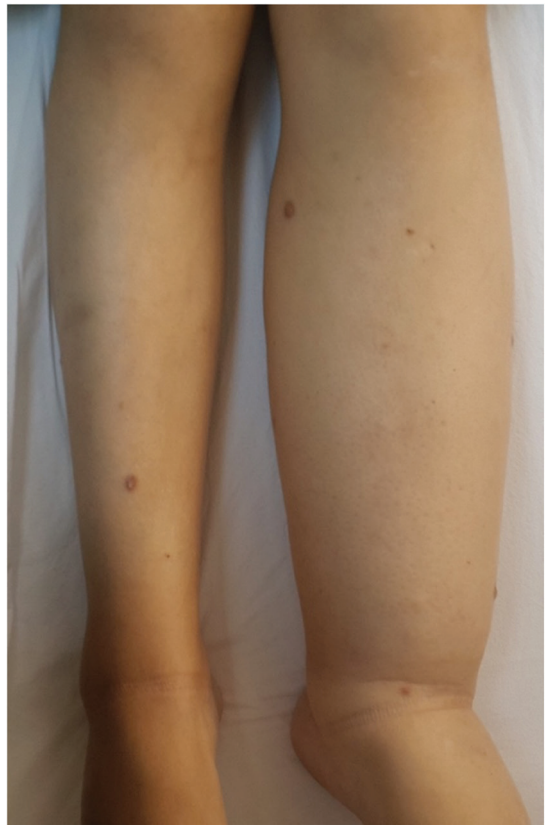

Figure 1. Lymphedema of the left lower extremity

Discussion

When the etiology of genital premalignant and malignant squamous lesions is questioned, we usually refer to the term of "immune deficiency" among many factors. However, this etiologic subtitle is not scrutinized except in few situations such as in immune suppressant use and HIV infection. Current knowledge is expanding with ever-advancing research and technology. Both ***in vitro*** and clinical genetic studies contribute to the recognition, classification, and treatment of diseases as in immune deficiencies. For instance, *CXCR4*, *DOCK8, EVER1, EVER2* and *GATA2* gene mutations are found to be linked to HPV infection susceptibility[9]. Therefore, in patients with such disseminated and severe findings, or with persistent, and recurrent lesions, early recognition of a possible immune deficiency and/or genetic syndrome can be important. Indeed, there is a need for clear algorithms to guide gynecologists as to when and where to consider such diagnoses and run these tests. Several mutations affecting the gene function have been reported in Emberger syndrome. Also, several different phenotypes of Emberger syndrome have been described among both familial and sporadic cases[1,10].

Susceptibility to HPV-related lesions and nontuberculous mycobacterial infections is common in Emberger syndrome. Ebstein-Barr virus-related cancers may also be encountered[4,8]. MDS is a clonal stem cell disease, which is a common finding in patients with Emberger syndrome. It may present with chronic

mild cytopenia or it can cause symptomatic anemia, infection, and bleeding. Progression to AML can be a serious problem. In addition to the predisposition to MDS/AML, some other features such as pulmonary alveolar proteinosis, congenital lymphedema, and sensorineural hearing loss can also be observed.

According to a study of a small group of 57 (31 women, 26 men) patients with GATA2 mutations, 82% of patients had infection-related presenting symptoms[4]. HPV was first among these infections: genital warts in 55%, genital dysplasias in 48% (vulvar, cervical, vaginal and anal), and cervical squamous cell cancers in 6% was reported in female patients. HPV-related head and neck cancers have also been reported.

It is difficult to give the exact prevalence of Emberger syndrome. To our knowledge, this is the first case to be reported from Turkey. The patient's slides revealed typical koilocytic changes and positive HPV staining and HPV type 89 was demonstrated via polymerase chain reaction in the last vaginal smear.

The patient had no family history of a similar phenotype; therefore, the case was accepted as sporadic. Sequencing and analysis of the *GATA2* gene identified an alteration in intron5, c1143+8C >T. A wide range of mutations and even whole-gene deletion can lead to the wide spectrum of clinical findings described in this syndrome. For instance, viral infections and lymphedema were reported to be more common in individuals with null mutations[4]. In a genome-wide study on endometriosis, GATA2 was found to be suppressed by hypermethylation in endometriotic cells but was normal in stromal cells[11]. This is an interesting point to keep in mind that, even though a certain gene's sequence looks intact and not mutated, that gene can still be "silenced" by certain mechanisms, mostly by hypermethylation.

Disease-causing alterations, namely mutations and pathogenic variants, are usually present in exons, the coding regions. However, there have been reports on mutations in introns, in enhancer elements, affecting the transcription process and causing Emberger syndrome[12]. In our patient, there were no coding region mutations. The only sequence change was found in the intron region.

Genotype-phenotype relations in rare syndromes are established better as more cases are reported, and as more variants/mutations are characterized. Even though this variant has not been reported before, the patient's clinical presentation strongly suggests the presence of such a genotype-phenotype correlation.

Conclusion

A predisposition to HPV-related infections and cancers is common in conditions with immunodeficiency. There seems to be a need for clear guidelines for early diagnosis and management of these immunocompromised conditions of unknown etiology. Gynecologists can encounter these conditions such as in this case presented here. Therefore, persistent, recurrent HPV lesions or their rapid malignant transformations may demand further investigation, especially for conditions or syndromes that affect the immune system.

Ethics

Informed Consent: Informed consent was obtained from the patient when she was alive.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

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

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

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Case Report / Olgu Sunumu

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Prenatal diagnosis and postnatal course in four fetuses with very rare pulmonary artery anomalies

Çok nadir görülen pulmoner arter anomalilerinin prenatal tanısı ve postnatal seyri

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Abstract

Pulmonary artery (PA) anomalies are very rare congenital cardiac malformations, a significant number of which remain unrecognized or misdiagnosed during the prenatal period. We report the prenatal diagnosis and outcome of pregnancy with fetal PA anomalies and discuss the related management issues. We identified four cases of prenatally diagnosed rare PA anomalies that were seen and confirmed in the newborn period by echocardiography and computed tomographic angiography at our center from 2018 to 2020. The course of the pregnancy, perinatal outcome, and the postnatal course in each case were analyzed. Three fetuses were born by repeat cesarean section approximately at 39 weeks of gestation and the other woman delivered vaginally. Of the abnormal origin of the left PA (LPA) in two patients, the first had right PA abnormalities derivating from the ascending aorta, and in the second, the LPA originated from the right PA. Two patients had agenesis of ductus arteriosus (DA), the first was accompanied with tetralogy of Fallot (TOF) and right aortic arch with a normal pulmonary valve, the second patient presented with an Absent Pulmonary Valve syndrome with TOF. Prenatal ultrasonography can be used to correctly diagnose the abnormal origin of the PA branches. Branching of the PA, presence of DA, location of the aortic, and ductal arch by the trachea should be routinely screened in the prenatal anatomic examination and the three-vessel and trachea view can determine the primary clues of PA malformations.

Keywords: Agenesis of ductus arteriosus, LPA originating from the ascending aorta, Absent Pulmonary Valve syndrome, LPA sling, right aortic arch, tetralogy of Fallot

Öz

Pulmoner arter anomalileri çok nadir görülen konjenital kalp hastalıklarından olup birçoğu prenatal dönemde farkedilmeyebilir veya yanlış tanımlanabilmektedir. Burada fetal pulmoner arter anomalilerinin prenatal tanısı ve sonuçlarını ve yönetimini tartışmayı amaçladık. 2018 ile 2020 arasında kliniğimize başvuran prenatal dönemde tanı konulan dört olgu tanımladık ve postnatal yenidoğan döneminde ekokardiyografi ve bilgisayarlı tomografik anjiyografi ile tanılarımızı doğruladık. Her olgunun gebelik seyrini, perinatal sonuçlarını ve postnatal takiplerini analize ettik. Sezaryan öyküsü olan 3 hasta 39. gebelik haftasında sezaryen ile, 1 hasta normal vajinal yolla doğurtuldu. Olguların ikisinde sol pulmoner arter anormal orjinli olup bu olguların ilkinde sol pulmoner arter asendan aortadan çıkarken, ikinci olguda sol pulmoner arter sağ pulmoner arterden kaynaklanmakta idi. İki olguda duktus arteriosus yokluğu olup ilk olguda Fallot tetraolojisi ve sağ aortik ark, ikinci olguda ise pulmoner kapak yokluğu ile birlikte Fallot tetrolojisi saptandı. Prenatal ultrasonografi ile anormal pulmoner arter dallanması doğru bir şekilde tanımlanabilir. Pulmoner arter dallanması, aort ve duktal arkın trakeaya göre yerleşimi prenatal ultrasonografik incelemede değerlendirilmeli ve 3 damar trakea kesiti pulmoner arter malformasyonlarında prenatal dönemde tanı konması için primer ipucu sağlamaktadır.

Anahtar Kelimeler: Duktus arteriosus yokluğu, sol pulmoner arterin asendan aortadan orjini, Pulmoner Kapak Yokluğu sendromu, sol pulmoner arter askısı, sağ aortik ark, Fallot tetralojisi

Introduction

Congenital pulmonary artery (PA) anomalies are extremely rare among cardiovascular anomalies[1]. These pathologies usually present with other complex cardiac diseases, when these anomalies are isolated, they are likely to go unrecognized during fetal life. Accurate prenatal diagnosis of congenital

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heart disease prevents neonatal morbidity and mortality and provides appropriate preoperative conditions, and improves surgical outcomes[2]. We describe four cases including left PA (LPA) sling, LPA originating from the ascending aorta (AOLPA/hemitruncus), and two cases of agenesis of ductus arteriosus (DA). Patients with DA agenesis had accompanying tetralogy of Fallot (TOF) - absent pulmonary valve (APVS) and TOF - right aortic arch (RAA), respectively. LPA sling, also known as aberrant LPA, derives from the superior and posterior aspect of the right PA instead of the main PA and then turns sharply leftward posterior to the trachea to enter the hilum of the left lung[3]. An LPA originating from the ascending aorta is characterized by the anomalous origin of left PAs from the posterolateral wall of the ascending aorta in the presence of two semilunar valves[4]. The DA is a unique vessel of fetal circulation that connects the pulmonary trunk and descending aorta. The underlying cause of DA agenesis is not well known and it is associated with other cardiac anomalies such as TOF, absent pulmonary valve, truncus arteriosus, and ventricular septal defect[5]. The aim of this study was to review our experience of prenatal ultrasonographic features of PA abnormalities. We emphasize that the three-vessel and trachea (3VT) view, which is the most important view in the diagnosis of PA anomalies, and in addition, LPA originating from the ascending aorta should be taken into consideration in the differential diagnosis when the fourth vessel is detected in the 3VT.

Materials and Methods

A total of 6752 women with singleton pregnancies from 18 to 30 weeks gestation were examined from September 2018 to February 2020 at the Department of Perinatology and Pediatric Cardiology, Istanbul Medeniyet University, Istanbul, Turkey. Fetal anatomic and echocardiographic examinations were performed using a Voluson E6 Expert ultrasound device, Samsung Ultrasound H60, and the Esaote MyLab™9 Platform. All patients underwent a complete fetal anatomic screening according to the International Society of Ultrasound in Obstetrics & Gynecology practice guideline. The standard four chambers and ventricular outflow tracts of the fetal heart were obtained routinely during the fetal anatomic screening for all patients in detailed two-dimensional, color Doppler echocardiography. After finding a cardiac anomaly, detailed echocardiographic examinations including 3VT, aortic and ductal arch, PA branches, aortic long axis, superior vena cava, inferior vena cava inflow, pulmonary venous return were assessed. We used color Doppler blood flow to track the PA branches and DA. Chromosome analysis and fluorescent *in situ* hybridization (FISH) analysis for microdeletion 22q11.2 were recommended to all patients. In addition to ultrasonography, demographic features and medical histories of all the mothers were reviewed and the perinatal outcomes were recorded. In the postnatal period, to verify the prenatal diagnosis of all PA anomalies different imaging modalities, such as echocardiography, cardiac computed tomography (CT) angiography, and angiography were used. The study was approved by the hospital ethics committee and all of the pregnant women with PA anomalies provided written informed consent.

Results

All four patients with a prenatal diagnosis of PA anomalies with or without cardiac malformations were reviewed for intrauterine course and outcome between 2018 and 2020. The mean gestational age at the time of admission was 24.8 (range, 22-28) weeks and the mean maternal age was 32.5 (range, 30-35) years. Three of four patients were referred to our center due to suspicion of congenital heart disease, and the remaining patient was detected during a routine ultrasonographic examination. All cases were singleton pregnancies and no extracardiac anomalies were identified. The four cases of the PA anomalies were LPA sling, LPA originating from the ascending aorta, DA agenesis with right aortic arch - TOF, and DA agenesis with absent pulmonary valve - TOF, respectively. Only one of the four patients accepted an invasive procedure and the results of chromosome analysis and FISH analyses for microdeletion 22q11.2 were normal. Three of four fetuses were delivered by cesarean section (CS) due to previous CS at term. A physical examination of the other three infants was performed and no dysmorphic findings were found in the postnatal period. Three infants except for one with DA agenesis and accompanying absent pulmonary valve - TOF, were clinically stable and they did not require surgery at the time of writing. A summary of the data from all four patients is shown in the Table and each clinical case report is described below and accompanied by supporting figures (Figures 1-9).

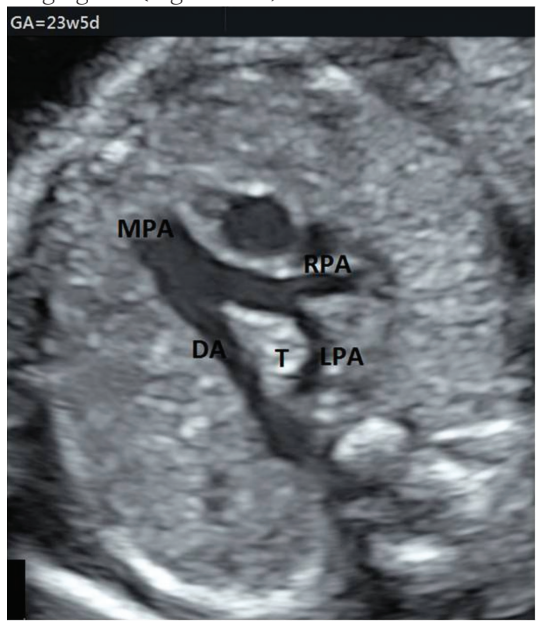

Figure 1. The LPA originates from the right pulmonary artery
LPA: Left pulmonary artery

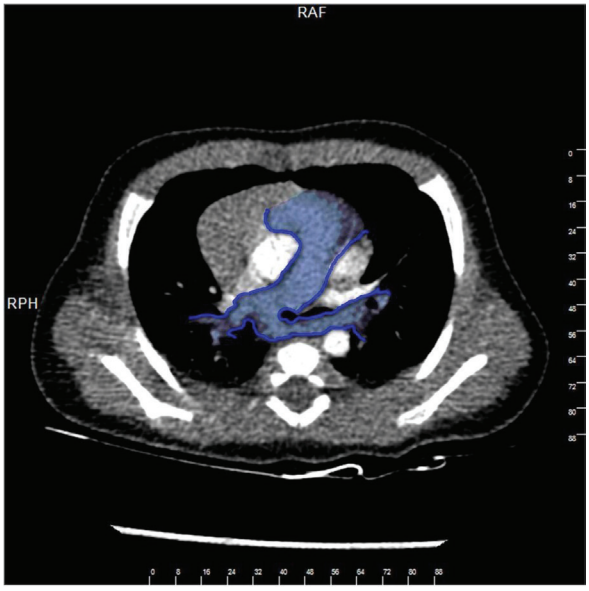

Figure 2. Axial contrast-enhanced computed tomography confirmed the aberrant LPA

LPA: Left pulmonary artery

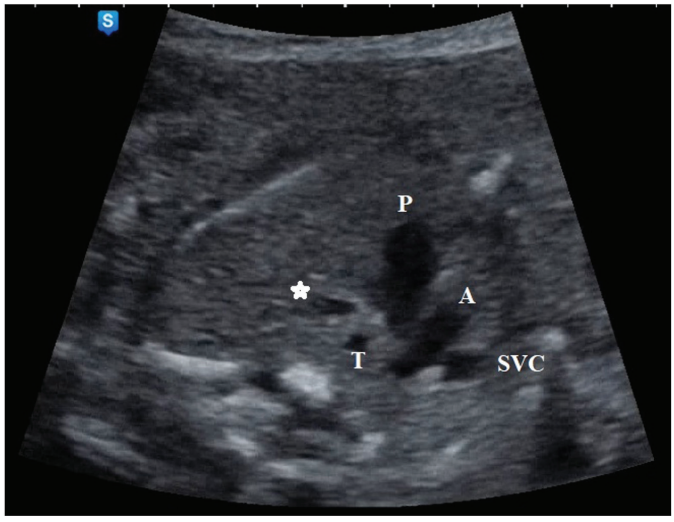

Figure 3. The aortic arch and ductal arch were located on the right side of the trachea, and four vessels were seen on the 3VT view

*P: Main pulmonary artery, A: Aorta, SVC: Superior vena cava, T: Trachea, *: LPA, LPA: Left pulmonary artery*

Case 1

A gravida (G) 5, parity (P) 1, abortus (Ab) 3, 33-year-old woman with a non-consanguineous marriage, underwent routine ultrasound examination at 22 weeks gestation and fetal echocardiography (Samsung Ultrasound H60) was performed for suspected cardiovascular malformation. The family history was not significant regarding congenital diseases or malformations. Prenatal echocardiography demonstrated that the LPA originated from the right PA and then, turning left, crossed midline behind the trachea, anterior esophagus, and descending aorta towards the hilum of the left lung (Figure 1). In the 3VT view, the trachea was encircled by the aberrant LPA and left-sided DA. The diameter of right and left PAs was equal and the thymus size was in the normal range for 22 weeks gestation. Neither congenital anomalies and polyhydramnios nor genetic abnormalities including abnormal karyotype and Di George syndrome were detected in amniocentesis. Due to placenta previa totalis, a 3480 g female baby was delivered by CS in a tertiary care center at 38 weeks of gestation. The APGAR scores

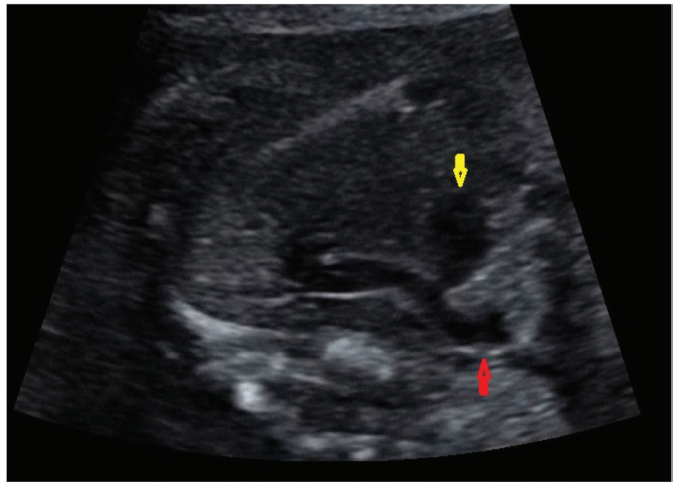

Figure 4. The LPA originated from the posterior aspect of ascending aorta and then turning left, crossed midline anterior the trachea towards the hilum of the left lung

Yellow arrow: Pulmonary artery, Red arrow: Aolpa, LPA: Left pulmonary artery

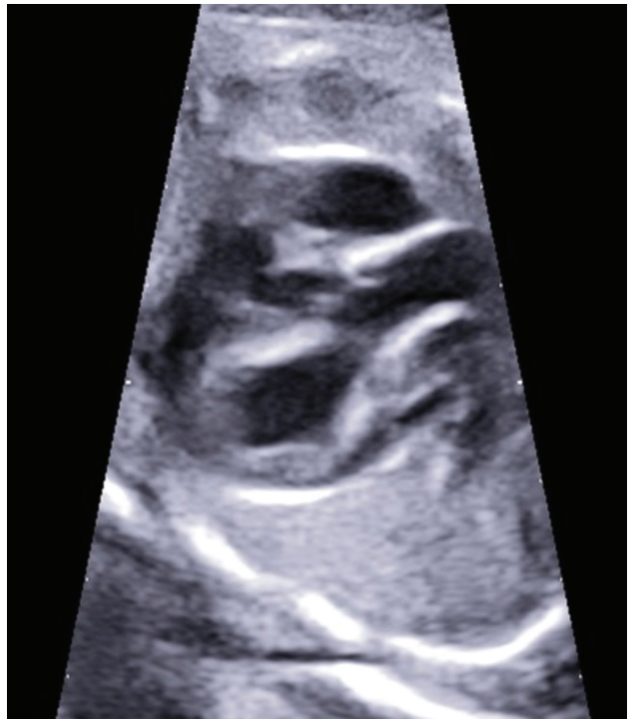

Figure 5. The right aortic arch, malalignment ventricular septal defect, overriding aorta in the five-chamber view were shown

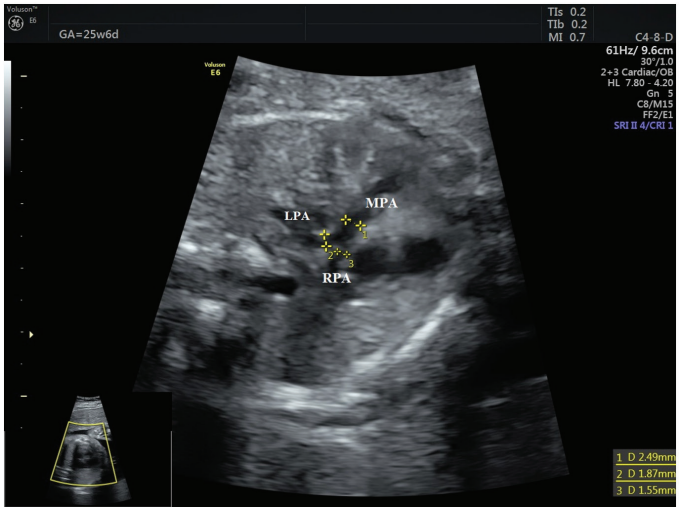

Figure 6. The diameters of the pulmonary annulus, right and LPA were seen on the 3VT view

LPA: Left pulmonary artery

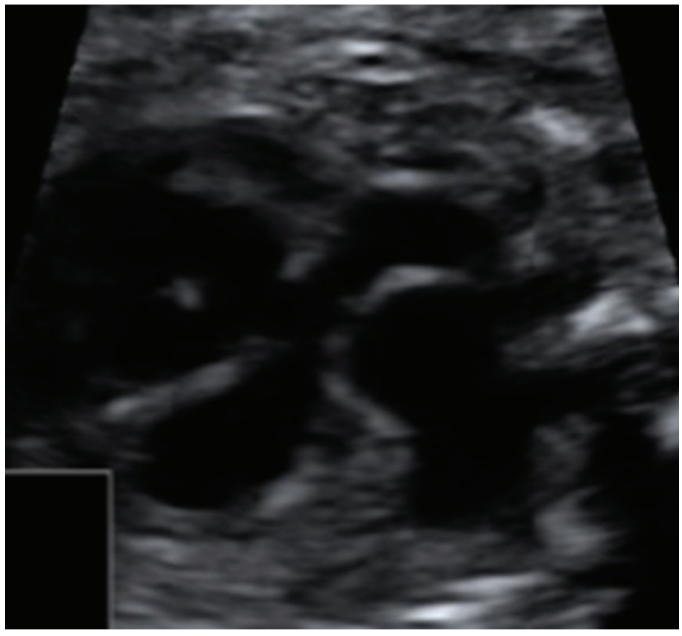

Figure 7. Hypoplasia of the pulmonary annulus, subaortic ventricular septal defect with the overriding aorta, severe dilatation of the pulmonary trunk were seen on the outflow tract of the right ventricular view

were 9 and 10 at 1 and 5 minutes, respectively. The diagnosis of LPA sling was confirmed using postnatal echocardiography on the first day after birth, and the peak systolic velocity of the left pulmonary was measured in the normal range, excluding the stenosis or hypoplasia of LPA. Postnatal axial contrast-enhanced CT confirmed the aberrant LPA and normal-appearing lungs and tracheobronchial anatomy (Figure 2). The asymptomatic baby was discharged from hospital on the third day after birth and she has been still symptom-free for eight months at the time of the writing.

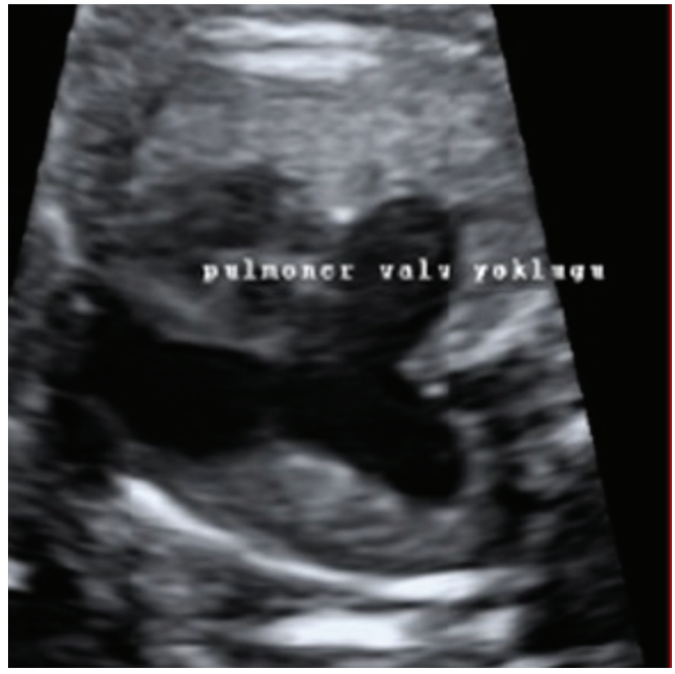

Figure 8. Absent pulmonary valve cusps, absent ductus arteriosus, and right aortic arch were determined on the 3VT view

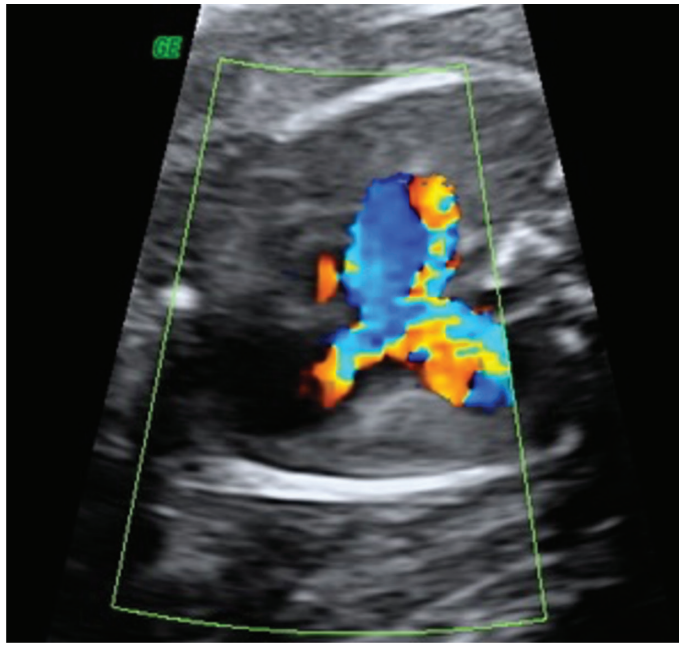

Figure 9. Increased peak flow velocity through the pulmonary valve annulus and a retrograde diastolic flow originating from the pulmonary valve annulus was revealed by color Doppler ultrasound

Case 2

A G2P1 32-year-old woman with a history of hyperthyroidism was referred to our perinatology clinic for second trimester ultrasonographic and fetal echocardiographic screening at 26-weeks gestation. The family history was negative for congenital heart defects, chromosomal abnormalities, or unexpected newborn death shortly after birth. There were no

other malformations outside the heart of the fetus (Samsung Ultrasound H60). Fetal echocardiography showed that visceroatrial situs solitus with normal systemic and pulmonary venous connections and normal four-chamber view. Fetal cardiac findings were as follows: the aortic arch and ductal arch were located on the right side of the trachea and four vessels on the 3VT view (Figure 3). Although the right PA arose from the pulmonary trunk, the origin of the LPA could not be seen clearly. To evaluate the anatomy in the transverse plane, Doppler ultrasonography was used, sweeping from inferior to superior and tracking the LPA from the hilum of the left lung, and anterior the trachea revealed its origin from around the vicinity of the ascending aorta closed to the brachiocephalic artery (Figure 4). The thymus was seen normally behind sternum at the level of the 3 vessels trachea view. The parents were consulted for genetic diseases but cordocentesis for karyotyping and analysis of 22q11 deletion was refused by the patient. At 40 weeks of gestation, a female infant of 3,565 g was born after an uncomplicated vaginal delivery with APGAR scores of 9 and 10 at 1 and 5 minutes, respectively. The physical examination of the baby was normal without any dysmorphic features and arterial oxygen saturation was normal. Neonatal echocardiography revealed LPA origin in the posterior aspect of ascending aorta, midsegment stenosis in the LPA with a peak pressure gradient of 30 mm Hg. Right-sided aortic arch, right-sided patent ductal arteriosus, and mild tricuspid regurgitation were seen in the postnatal echocardiography. Two days after birth, we performed CT angiography and confirmed the fetal diagnosis showing the described left pulmonary hemitruncus. The newborn was discharged at 3 days after birth without cardiac or respiratory symptoms. The baby was doing clinically well in her last clinic visit at 4 months of age.

Case 3

A 35-year-old G4P1Ab2 refugee woman was referred to our perinatology clinic due to a suspected a fetal heart defect. There was no family history of congenital heart disease nor did she have any risk factors leading to congenital heart disease. The results of a fetal echocardiogram at 23 weeks of gestation showed the right aortic arch, malalignment ventricular septal defect, and overriding aorta in five-chamber view (Figure 5). Further, DA could not be seen arising from the main PA and connecting to the descending aorta in the 3VT view and sagittal view, respectively (Samsung Ultrasound H60). Pulmonary valve, main PA, and the branches of the pulmonary valve were in the normal range. The diameters of the pulmonary annulus, right and LPA were 2.49 mm, 1.55 mm, and 1.87 mm, respectively (Figure 6). Color Doppler confirmed the presence of an overriding aorta with blood draining from both ventricles into the aorta. The antegrade flow was seen in the main and branch of the PA but DA could not be demonstrated using color Doppler. After a prenatal genetic consultation, amniocentesis for karyotyping and analysis of 22q11 deletion was declined by the parents. The female fetus was delivered by CS due to previous CS at 38 weeks gestation. The neonate did not need any cardiac or respiratory support and weighed 3175 g. There were no findings suggesting any genetic anomaly in the phenotype of the newborn baby. A postnatal transthoracic echocardiogram revealed a normal four-chamber view; however, the aortic root was slightly shifted in the right ventricle and located to the right of the trachea, DA was absent as in the fetal findings. The diameters of the pulmonary annulus, right and LPA were 5.8 mm, 4.5 mm, and 4.8 mm, respectively. The peak pressure gradient at the level of the pulmonary valve was approximately 23 mm Hg at discharge from the hospital. She has remained uneventful for two months at the time of the writing. Surgical correction is delayed until she reaches the appropriate age.

Case 4

A G2P1 30-year-old woman was sent to our clinic with a suspicion of congenital diaphragmatic hernia on obstetrical ultrasound at 28 weeks of gestation. The healthy pregnant women had no genetic disorders or fetal heart anomaly in her family history. Anatomic screening was normal except for the fetal heart (Samsung Ultrasound H60). The fetus presented with situs solitus, levocardia, and the atrioventricular connection were normal. A slightly larger right heart was shown on a four-chamber view; the pulmonary valve annulus was hypoplastic (4.5 mm); a subaortic ventricular septal defect with the overriding aorta, severe dilatation of the pulmonary trunk and its branches (right and LPA were 1.2 mm and 13.8 mm, respectively) were seen on the outflow tract of the right ventricular view (Figure 7); absent pulmonary valve cusps, absent DA, and right aortic arch were determined on the 3VT view (Figure 8). Increased peak flow velocity through the pulmonary valve annulus and a retrograde diastolic flow originating from the pulmonary valve annulus was revealed using color Doppler ultrasound (Figure 9). Absent pulmonary valve with TOF was diagnosed based on the following findings: absent pulmonary valve, absent DA, right aortic arch, presence of malalignment ventricular septal defect, and an overriding aorta. The main PA diameter was 5.63 mm, and the left and right artery diameters were 3.37 mm and 3.33 mm, respectively. The mother refused to undergo karyotype analysis and FISH analysis to detect the 22q11 microdeletion. Fetal hydrops did not develop with compensatory extension of the left and right PA and with the shunt through a patent foramen ovale during the follow-up from the 28 weeks of gestation to the delivery. A male fetus was born by repeat CS at 39 weeks of gestation. Postnatally, transthoracic echocardiographic findings were consistent with the prenatal diagnosis. Cardiac results included TOF, aneurysmal dilatation of PAs branches, and the right ventricle was slightly enlarged. When the baby presented early

with airway compression from aneurysmal PAs, early correction surgery was performed successfully at age 2 months.

Discussion

Reports of prenatal diagnosis of PA malformations, including agenesis of the DA, absent pulmonary valve, abnormal branching of pulmonary vessels, and origin of the PA, are extremely rare anomalies[1,6]. PA anomalies may be isolated or accompanied by other complex cardiac and extracardiac anomalies. Surgical treatment and prognosis are also variables because the pathologic anatomy is very different[2]. We described four cases of the PA anomalies that were identified in fetal echocardiography and confirmed in the newborn period using echocardiography and CT angiography (Table 1).

The PA derives from the right ventricle, courses towards the left of the more posterior ascending aorta, and branches after a short course in the normal manner. The first branch is the right PA, left branch subsequently. The PA continues distally towards the left side and into the DA, which connects to the descending aorta in anatomically normal fetuses. In healthy fetuses, ductal and transverse aortic arches merge with a course to the left of the trachea and no vascular structures exist posterior or surround the trachea at the level of three-vessel tracheal view, which is the most important view in the diagnosis of conotruncal abnormalities[7]. During the normal development of the cardiovascular system, the aortic arches develop from the aortic sac, with a pair of branches (right and left), and initially, six pairs of aortic arches are present and symmetrical. They develop and regress at different stages of development. In the process of normal embryogenesis, the sixth aortic arches separate into ventral and dorsal segments and the ventral part of the arches forms the proximal branch of the PAs bilaterally. The left ventral arch is responsible for the formation of the pulmonary trunk, the left dorsal sixth arc continues as the DA and connects the main pulmonary trunk with the left dorsal aorta[8]. The LPA originates as a branch of the left ventral sixth aortic arch and capillaries that arise from the pulmonary postbranchial plexus surrounding the lung bud by the 8th week of embryonic development[8].

The first prenatal diagnosis of LPA sling was demonstrated in 2011 during a fetal ultrasound examination at 32 weeks gestation by Yorioka[9]. To date, a few cases of prenatally diagnosed LPA sling have been reported[10]. For the development of aberrant LPA, it was proposed that if the connection between the left lung bud ventrally and left six arches fails, the connection occurs between right six aortic arch and the developing left lung bud dorsally, resulting in a LPA forming from the right PA, passing posterior to the trachea[11]. We described one prenatal case with LPA sling with follow-up from 22 weeks gestation to a postnatal age of 2 years. After birth, axial contrast CT and postnatal echocardiography findings confirmed the prenatal diagnosis of LPA sling. According to the Wells classification of LPA sling, CT demonstrated that the bronchial anatomy was type 1A, the LPA arose from the right PA, and coursed posteriorly between the trachea and esophagus[12]. The carina was typically located at the T4-T5 level and the aberrant LPA did not cause compression of the trachea, bronchus, and esophagus. Symptoms of this condition usually manifest clinically in the neonatal period in 50% of the patients, and in 65% they occur before 1 month of age[13]. The most common symptoms are related to the respiratory system, causing dyspnea wheezing and stridor. The symptoms are produced by compression of the tracheobronchial tree and esophagus, causing tracheobronchial stenosis, tracheomalacia, and airway compression in the postpartum period. About half of all cases are associated with other cardiac and non-cardiac defects such as atrial septal defect, persistence left superior vena cava, ventricular septal defect, abnormal pulmonary lobulations, and tracheal bronchus[13]. Morbidity and mortality often depend primarily on the presence of tracheobronchial tree anomaly and especially tracheal or bronchial stenosis other than LPA sling, with mortality rates as high as 50% without surgical

Table 1. Summary of cases with pulmonary artery anomalies

Case	Type of PA anomaly	Maternal Age (years)	G-P-Ab	Gestational week (GW) at diagnosis	Other cardiac anomalies	Time and mode of delivery	Postnatal follow-up
1	LPAS	33	5-1-3	22	Retrotracheal LPA	38GW-CS	Perform MR Angiography. Asymptomatic
2	AOLPA	32	2-1	26	Right AA Right DA	40GW-VB	Perform Angiography Asymptomatic
3	Agenesis of DA	35	4-1-2	23	ToF Right AA	38GW-CS	Perform echocardiography Asymptomatic
4	Agenesis of DA	30	2-1	28	APVS ToF	39GW-CS	Perform echocardiography Correction surgery at 2 months old

PA: Pulmonary artery, G: Gravida, P: Parite, Ab: Abortus, LPAS: LPA sling, CS: Caesarean section, AOLPA: Abnormal origin of LPA, AA: Aortic arch, DA: Ductus arteriosus, VB: Vaginal birth, TOF: Tetralogy of Fallot, APVS: Absent pulmonary valve syndrome

correction[11]. Although half of all patients have symptoms, in our case, LPA sling was an isolated anomaly without symptoms at the time of writing.

Anomalous origin of unilateral PA is a rare congenital pulmonary vascular malformation, with one branch of the PA originating from the ascending aorta and the other arising from the main PA. The embryogenesis and development of DA and the aortic arch is still uncertain. DA originates from the left sixth arch, presumably, and DA is connected with the first part of the LPA. As the 6th arches do not develop partially or completely on the left side, the LPA cannot attach to the main PA and the aortic sac persists from which the PA originates[14]. Right hemitruncus is more frequent than left hemitruncus[14]: however, left hemitruncus is more associated with other cardiovascular anomalies with either TOF or right aortic arch[15]. After birth, pulmonary vascular resistance decreases progressively in healthy babies; however, excessive blood flow into the anomalous origin of unilateral PA from the aortic arch leads to pulmonary over circulation and the affected lung is exposed to systemic pressure, whereas the other lung received the entire blood supply volume from the right ventricle. As a result of pulmonary over circulation, pulmonary hypertension, subsequently, and heart failure develops. To avoid irreversible pulmonary vascular damage and the development of pulmonary hypertension, early diagnosis and surgical correction are crucial in hemitruncus[16]. It has been reported that mortality rates are very high in patients who are not surgically treated[14]. Prenatal detection of hemitruncus is crucial in fetal echocardiography because early surgical correction improves survival[17]. In this case report, we documented the prenatal diagnosis of a patient with left hemitruncus, right-sided aortic arch, and right-sided ductal arch in the 3VT view. Postnatal echocardiography and axial contrast-enhanced CT confirmed the fetal diagnosis showing the described left pulmonary hemitruncus, and midsegment stenosis in the LPA with a peak pressure gradient of 30 mm Hg was determined, which was confirmed by cardiac catheterization. Detection of the fourth vessel in the 3VT should lead to a search for the differential diagnosis including abnormal pulmonary venous connections, persistent left superior vena cava, esophageal or bronchial tree anomalies, or cystic thoracic mass. We emphasized that left hemitruncus should be taken into consideration in differential diagnosis and the importance of demonstrating branching of both PAs.

In the normal fetal circulation, DA carries 78% of the right ventricular blood away from the lungs and joins the descending aorta to supply the lower part of the fetus. The underlying mechanism of DA agenesis is not yet clear. One of the mechanisms is agenesis of DA occurring unless the left dorsal sixth arc continues as the DA. The secondary mechanism is where blood in the right ventricle, which has high oxygen saturation, cannot be transported to the lungs due to the severe obstruction of the PA and the higher oxygen saturated aortic blood diverts from aorta to the DA, thereby inducing constriction[18]. Embryologically, the right aortic arch occurs due to the persistence of the right dorsal aorta instead of the left dorsal aorta. The embryologic process causing the development of TOF is not known; it is assumed that an anterior and cephalad deviation of the infundibular septum results in a malalignment ventricular septal defect, and the aortic root overrides the ventricular septal defect, causing a subsequent right ventricular outflow obstruction. Absent Pulmonary Valve syndrome may be accompanied by a ventricular septal defect called TOF with absent pulmonary valve, and this is generally associated with the absence of the DA. However, if the ventricular septal defect does not occur, DA is usually present. In the absence of DA, the main pulmonary trunk cannot connect the left dorsal aorta, PA blood pressure increases, severe pulmonary regurgitation develops, resulting in an enlarged PA, and this pathologic process interferes with the normal development of the pulmonary valve. Isolated agenesis or premature closure of the DA is an uncommon congenital cardiac malformation; they are usually associated with TOF, absent pulmonary valve, truncus arteriosus, or maternal use of prostaglandin synthetase inhibitors.

We demonstrated two cases of agenesis of DA and the first was accompanied by TOF and right aortic arch with a normal pulmonary valve at 23 weeks of gestation, the second presented with absent pulmonary valve with TOF. Although DA did not develop in the first case, pulmonary valve and branches of the PA were seen in the normal range because blood from the right ventricle to the aorta exited via a malalignment ventricular septal defect and pulmonary valve, and branches of the PA were not exposed to the high pressure of right ventricle. In TOF, agenesis of a DA would probably occur at the beginning of the pregnancy; patent foramen ovale and large ventricular septal defect would be able to carry the blood from the right ventricle into the descending aorta, thus, the pulmonary valve, pulmonary vascular bed, and PA branches could develop normally[19]. The component of absent Pulmonary Valve syndrome includes the absence or rudimentary pulmonary valve, stenosis of the pulmonary valve annulus, dilatation of the pulmonary trunk, and left and right branches of PAs. This condition is rarely isolated, it is most often associated with TOF and agenesis of DA. The fetal echocardiographic diagnosis was confirmed for both cases. The first patient is 4 months old at the time of writing and no therapeutic intervention has been performed yet. In the second case, early successful correction was performed due to significant airway compression when the baby was aged 2 months.

Fetal echocardiography is considered to be an important, non-invasive, and safe diagnostic modality for assessing congenital heart anomalies. Our cases highlight the variability of abnormalities of the PA. Although outflow tract anomalies have a characteristic echocardiographic appearance in the 3VT view, prenatal diagnosis of these anomalies may be missed or anomalies may be misdiagnosed, especially abnormal branching

of the PA[20]. Therefore, the detection of pulmonary anomalies prenatally may be challenging but is very important because postnatal careful follow-up of the neonate improves short and long-term outcomes. When the pulmonary branch cannot be displayed as a Y shape, it is vital to trace the course of left and right PA branch to diagnose the anomaly.

Conclusion

Branching of the PA, presence of DA, location of the aortic, and ductal arch should be routinely screened in the prenatal anatomic examination and the 3VT view determines primary clues of PA malformations. Physicians should increase their awareness in the prenatal diagnosis of PA anomalies to avoid unfavorable outcomes and the 3VT view needs to be performed excellently during routine fetal anatomic examinations.

Ethics

Informed Consent: All of the pregnant women with PA anomalies provided written informed consent.
Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: R.A., Concept: O.D., Ö.T., Design: O.D., Data Collection or Processing: Ö.A.T., Analysis or Interpretation: Ö.A.T., Literature Search: Ö.A.T., Ö.T., Writing: R.A.
Conflict of Interest: The authors declare no conflict of interest.
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Case Report / Olgu Sunumu

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Prenatal diagnosis of persistent cloaca accompanied by uterus didelphys: A case report

Uterus didelphys'ye eşlik eden persistan kloakanın prenatal tanısı: Bir olgu sunumu

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Abstract

Persistent cloaca is a rare abnormality that occurs usually in females and is characterized by direct communication between the gastrointestinal, urinary, and genital structures resulting in a single perineal opening. We report a case of persistent cloaca accompanying uterus didelphys that was diagnosed antenatally with fetal ultrasonography. A gravida 3, para 2, 35-year-old women at 22 weeks of gestation was referred to our hospital with a diagnosis of moderate pyelectasis of the fetal kidneys and fetal diffuse intestinal dilation. Detailed ultrasound scan findings were reported as a small thick-walled septated cystic pelvic mass of 5.2×5.5 cm size seen at the level of the fetal pelvic region. The target sign could not be visualized, it was considered as anal atresia. In the following weeks, the patient, who was evaluated together with meconium on the uterine septum, and monitoring of the neighboring bladder and anal atresia, was diagnosed as having persistent cloaca. Ultrasound findings showed that it could be persistent cloaca accompanying uterus didelphys. The fetus postnatally manifested persistent cloaca. On the first day after vaginal delivery, pelvic ultrasound in the neonatal intensive care unit showed bilateral 2nd-degree hydronephrosis, presacral enlarged bowel loops, uterus didelphis, vaginal septum, direct contact between urethra and vagina, proximal end in the rectum compatible with atresia. On the second day, colostomy was performed. Her renal condition continued to be stable. She is now waiting for definitive surgery for cloaca. Persistent cloaca should be considered in any female fetus presenting with hydronephrosis and a cystic pelvic mass lesion as diagnosed by ultrasound. Prenatal diagnosis allows time for parental counseling and delivery planning at a tertiary hospital for neonatal intensive care and pediatric surgery.

Keywords: Persistent cloaca, uterus didelphys, prenatal diagnosis, fetal pelvic mass

Öz

Persistan kloaka, genellikle kadınlarda görülen; gastrointestinal, üriner ve genital yapılar arasında doğrudan tek bir perineal açıklıkla karakterize nadir görülen bir anomalidir. Fetal ultrasonografi ile antenatal olarak tanı konulan uterin didelfisin eşlik ettiği bir persistan kloaka olgusunu sunuyoruz. Otuz beş yaşında kadın, gravida 3, para 2, fetal böbreklerde orta derecede piyelektazi ve fetal diffüz intestinal dilatasyonu olan 22 hafta gebelik tanısıyla hastanemize sevk edildi. Detaylı ultrason taraması bulguları, fetal pelvik bölge seviyesinde 5,2×5,5 cm boyutlarında küçük kalın duvarlı septasyonlu kistik pelvik kitle olarak rapor edildi. "Target sign" görüntülenemedi, anal atrezi olarak değerlendirildi. İlerleyen haftalarda uterin septumda mekonyum görülmesi, komşuluğunda mesanenin izlenmesi ve anal atrezisi olan hastaya persistan kloaka tanısı konuldu. Ultrason bulguları, uterin didelfisin de eşlik ettiğini gösterdi. Vajinal doğumdan sonrası persistan kloaka gösterildi, yenidoğan yoğun bakım ünitesinde yapılan pelvik ultrasonda bilateral 2. derece hidronefroz, presakral diffüz intestinal segmenti, uterus didelfis, vajinal septum, üretra ve vajina arasında direkt temas, rektumda proksimal uç atrezisi raporlandı. İkinci gün kolostomi açıldı. Renal durumu stabil devam eden hasta şimdi kloaka için cerrahisi bekliyor. Ultrason ile tanı konulmuş kistik pelvik kitle ve hidronefroz ile gelen her kız fetüste persistan kloaka düşünülmelidir. Prenatal tanı, yenidoğan yoğun bakım ve çocuk cerrahi olan üçüncü basamak bir hastanede ebeveyn danışmanlığı ve doğum planlaması için zaman sağlar.

Anahtar Kelimeler: Persistan kloaka, uterus didelfis, prenatal tanı, fetal pelvik kitle

Introduction

Cloacal anomalies represent the persistence of early embryonic development in which the urinary, genital, and gastrointestinal tracts remain confluent and communicate with the exterior through a single perineal opening. It is an extremely rare disorder, the incidence being 1 in 50,000 births[1]. It generally affects females. There may be co-existent anomalies of the gastrointestinal tract, cardiac, renal, uterine, skeletal and limbs. The presence of a cloaca is a normal phase of early human embryologic development. Between the 4th and 7th weeks

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of gestation, the cloaca undergoes subdivision to form the hindgut and urogenital sinus. Failure of this process results in the congenital anomaly termed persistent cloaca[2]. Herein, we report a case of persistent cloaca accompanying uterus didelphys that was diagnosed antenatally in fetal ultrasonography.

Case Report

A gravida 3, para 2, 35-year-old woman at 22 weeks of gestation was referred to our hospital with a diagnosis of moderate pyelectasis of the fetal kidneys and fetal diffuse intestinal dilation. The amniotic fluid level was normal. Detailed ultrasound scan findings were reported as a small thick-walled septated cystic pelvic mass of 5.2×5.5 cm size seen at the level of the fetal pelvic region. Except for two previous normal vaginal deliveries, her obstetric history was unremarkable. The glucose tolerance test performed at about 24 weeks during pregnancy led to the diagnosis of gestational diabetes. The target sign could not be visualized, and anal atresia was considered. In the following weeks, the patient, who was evaluated together with meconium on the uterine septum, and monitoring of the neighboring bladder and anal atresia, was diagnosed as having persistent cloaca (Figure 1). She was diagnosed as having intrahepatic cholestasis of pregnancy at 37 weeks of gestation. We decided to induce labor as determined by the Bishop score. After the initiation of misoprostol induction, in another 8 h, the patient gave birth to a live-born female infant weighing 2,520 g, a height of 47 cm, and an Apgar score of 9-10 points. The female neonate presented with an absent anal opening; she was passing stool from the vestibule. The newborn had abdominal distension, simulating a distended bladder, and rectal atresia with a single perineal opening between the labia majora. The fetus postnatally manifested as persistent cloaca. On the first day after vaginal delivery, pelvic ultrasound in the neonatal intensive care unit showed bilateral 2nd-degree hydronephrosis, presacral enlarged bowel loops, uterus didelphis, vaginal septum, direct contact between urethra and vagina, and the proximal end in the rectum was compatible with atresia. On the second day, colostomy and cystoscopy were performed. Her renal condition continued to be stable. She is now waiting for definitive surgery for cloaca. The patient postpartum period was uncomplicated. At the follow-up visit between 4-6 weeks, the patient was in good health and without symptoms.

Discussion

Persistent cloaca is an uncommon malformation that generally occurs in females, with a reported incidence of 1:50,000[1]. It is a complex anomaly with a confluence of the rectum, vagina, and urethra into a single common channel, and can be associated with female hypospadias, duplex uteri, bladder diverticulum, and double vagina, to more complex anomalies[3].

Cloacal anomalies are considered prenatally in the presence of bilateral hydronephrosis, a pelvic cystic lesion, and a poorly visualized bladder[1]. However, prenatal diagnoses of female urogenital anomalies are usually difficult. Accurate diagnosis and classification of cloacal anomalies during pregnancy are essential to predict perinatal morbidity and mortality. These defects are rare, manifest as varying defects, and particularly in the late stages of pregnancy, lack characteristic ultrasound signs[4].

In our case, the cloacal anomaly presented as a fetal duplication cyst. The fetal pelvic septated cyst that raised clinical suspicion confirmed on the postnatal period as uterine duplication with hydrometrocolpos. Most cases of cloacal anomaly have been reported to be associated with fetal ascites[5]. Fetal urine drains through the fallopian tube to the peritoneal cavity and this process causes a chemical reaction that determines the tubal obstruction, hydrocolpos, and resolution of ascites, but in our case, no ascites was observed. Fetal magnetic resonance imaging (MRI) is an important diagnostic imaging adjunct to ultrasonography, particularly for the initial diagnosis of cloacal malformation[6]. Ultrasonography or fetal MRI can identify large cystic pelvic masses, but their origin cannot be determined in most cases.

Conclusion

Persistent cloaca can be diagnosed prenatally and should be considered in any female fetus presenting with hydronephrosis

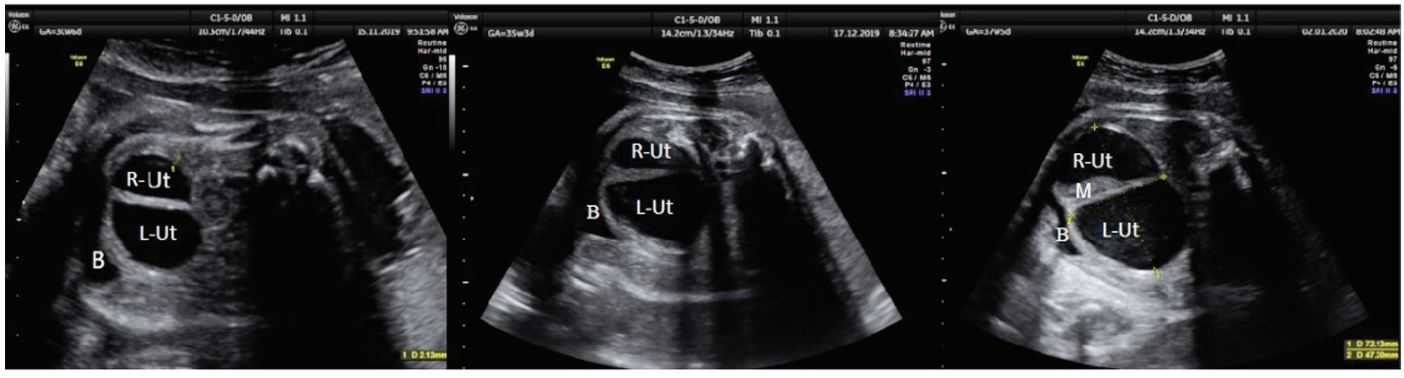

Figure 1. Ultrasound images show the evolution of cloaca accompanied uterus didelphys from 30, 35 and 37 weeks of gestation respectively

R-Ut: The right uterine cavity, L-Ut: The left uterine cavity, B: Bladder, M: Meconium

and a large cystic lesion arising from the pelvis as assessed by ultrasound. Prenatal diagnosis allows time for parental counseling and delivery planning at a tertiary hospital for neonatal intensive care and pediatric surgery. A cystic pelvic mass in any female fetus should be evaluated for cloacal anomalies.

Ethics

Informed Consent: The patient has given their informed consent for the case report to be published.
Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

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