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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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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.

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Cover letter to the editors addressing the following points:

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· 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.

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· Informed consent.

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

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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:

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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.

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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).

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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	,500 words (~22 pages)$^{\Psi}$	NA	30
Case report	150 words	,000 words (~8 pages)	4	8
Systematic review	300 words	6,250 words (~25 pages)	4	60
Current commentary	250 words	,000 words (~12 pages)	4	12
Procedure and Instruments	200 words	,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;

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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.

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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.

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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.

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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.

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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.

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

Examples

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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

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TURKISH JOURNAL OF OBSTETRICS AND GYNECOLOGY

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TURKISH JOURNAL OF OBSTETRICS AND GYNECOLOGY

LETTER FROM THE PRESIDENT

Dear Colleagues;

As the Turkish Society of Gynaecology and Obstetrics, I am proud to present to you the September issue of our scientific journal. In this issue, which has been prepared for you as a result of meticulous work, there are a total of 12 articles with high scientific value and up-to-date literature information. I would also like to state that the international acceptance value of our journal is increasing day by day, and its scientific value is increasing with each passing day.

We invite all our colleagues to the 2nd IVF and Infertility Congress (TÜBİD), which will be held in Cyprus between 28 September 2023 - 2 October 2023, where current issues in the field of infertility will be discussed.

Bulent Tiras, Prof. MD
President of TJOD

TURKISH JOURNAL OF OBSTETRICS AND GYNECOLOGY

EDITORIAL

Dear Colleagues;

As the Turkish Journal of Gynaecology and Obstetrics, the Scientific Publishing Organ of the Turkish Society of Gynaecology and Obstetrics (TJOD), we are very pleased to be back with the September issue. In our September issue, which we prepared with intensive efforts as TJOD Family, there are 12 scientific studies in total.

Eleven studies are within the scope of original research, and one article is presented to your information as a review. We think that our articles, which examine the cesarean section rates and characteristics in our country, will be of interest both to the gynecologists and specialists on duty and to the scientific literature.

Ercan Yilmaz, Prof. MD
Fatih Sendag, Prof. MD

Clinical Investigation / Araştırma

DOI: 10.4274/tjod.galenos.2023.62819

Turk J Obstet Gynecol 2023;20:164-73

Clear cell carcinoma of the uterine cervix; an unusual HPV-independent tumor: Clinicopathological features, PD-L1 expression, and mismatch repair protein deficiency status of 16 cases

Uterin serviksin berrak hücreli karsinomu; HPV-ilişkisiz nadir bir tümör: 16 olgunun klinikopatolojik özellikleri, PD-L1 ekspresyon ve MMR protein ekspresyon kaybı durumları

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Abstract

Objective: Endocervical clear cell carcinoma (c-CCC) is a rare and HPV-independent adenocarcinoma type of cervix. Being usually resistant to conventional chemotherapy. Immunotherapy has recently been added as a preferred regimen as a second-line treatment option for programed cell death-ligand 1 (PD-L1)-positive or mismatch repair (MMR) deficient cervical carcinomas. In this study, clinicopathological features, PD-L1 expression, and MMR deficiency status of c-CCCs were investigated.

Materials and Methods: Sixteen c-CCC diagnosed cases were included in this study. PD-L1 expression was evaluated using two different PD-L1 clones (22C3 and SP263). MMR deficiency status of the cases was evaluated using four MMR proteins (MLH1, PMS2, MSH2, and MSH6).

Results: Most of the c-CCC cases were presented as FIGO Stage I (68.75%). PD-L1 expression in either tumoral or tumor-infiltrating immune cells (TILs) was present in 62.5% (10/16) and 69% (11/16) of the 22C3 and SP263 clones, respectively. Most of the cases with high TIL density were also positive for PD-L1. The PD-L1 expression rate was less than 50% in most of the cases and 12.5% of the cases shared extensive PD-L1 staining. Overall, MMR deficiency was observed in 31.25% of the cases. Most of the MMR-deficient cases (80%) were PD-L1 positive.

Conclusion: Although our study cohort is limited, we have shown that PD-L1 expression and MMR deficiency can be found in c-CCCs in variable degrees. These findings suggest that accompanying TIL density and MMR deficiency could be used as candidates for predicting PD-L1 positivity for c-CCCs. However, to indicate the clinical importance of these findings, objective treatment outcomes of cases treated with immunotherapy should be seen.

Keywords: Endocervical clear cell carcinoma, PD-L1 22C3, mismatch repair deficiency

Öz

Amaç: Endoservikal berrak hücreli karsinomlar (s-CCC), genellikle geleneksel kemoterapiye dirençli olan serviksin nadir bir HPV ilişkisiz adenokarsinom tipidir. İmmünoterapi yakın bir zamanda programlanmış hücre ölümü ligandı 1 (PD-L1)-pozitif veya mismatch-onarım (MMR) protein ekspresyon kaybı olan servikal karsinomların ikinci basamak tedavisinde tercih edilen bir rejim olarak eklenmiştir. Bu çalışmada, s-CCC'lerin klinikopatolojik özellikleri, PD-L1 ekspresyonu ve MMR eksikliği durumu araştırıldı.

Gereç ve Yöntemler: S-CCC tanısı koyulmuş olan 16 hasta bu çalışmaya dahil edildi. PD-L1 ekspresyonu iki farklı PD-L1 klonu (22C3 ve SP263) kullanılarak değerlendirildi. MMR eksikliği durumu dört MMR proteini ile (MLH1, PMS2, MSH2, MSH6) değerlendirildi.

PRECIS: The presence of PD-L1 expression and Microsatellite Instability in endocervical clear cell carcinomas predicts the immunotherapy may yield promising results in the treatment of endocervical clear cell carcinomas as well.

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Bulgular: S-CCC olgularının çoğu FIGO Evre I (%68,75) olarak prezente oldu. PD-L1 ekspresyonu, tümörde ya da tümörü infiltre eden lenfositlerde (TIL) 22C3 ve SP263 klonlarıyla sırayla olguların %62,5'inde (10/16) ve %69'unda (11/16) mevcuttu. Yüksek TIL yoğunluğuna sahip olguların çoğu PD-L1 ile de pozitifti. PD-L1 ekspresyon oranı çoğu olguda %50'den azdı ve olguların %12,5'i yaygın PD-L1 boyanması gösteriyordu. Genel olarak, olguların %32,25'inde MMR proteinlerinde ekspresyon kaybı gözlendi. MMR proteinlerinde ekspresyon kaybı olan olguların çoğu (%80) PD-L1 pozitifti.

Sonuç: Çalışma grubumuz sınırlı olmasına rağmen, PD-L1 ekspresyonu ve MMR proteinlerinde ekspresyon kaybının s-CCC'lerde değişen oranlarda bulunabileceğini gösterdik. Bulgular, eşlik eden TIL yoğunluğunun ve MMR protein ekspresyon kaybının, PD-L1 pozitifliğini tahmin etmek için bir aday olarak kullanılabileceğini düşündürmektedir. Ancak bu bulguların klinik öneminin gösterilebilmesi için immünoterapi ile tedavi edilmiş olguların objektif tedavi sonuçlarının görülmesi gerekmektedir.

Anahtar Kelimeler: Endoservikal berrak hücreli karsinom, PD-L1, 22C3, uyumsuzluk onarımı eksikliği

Introduction

Clear cell carcinoma (CCC) of Müllerian origin is a rare tumor with distinct histology that may occur in the ovary, vagina, uterus and cervix. Among carcinomas of the cervix, the most frequent histologic type is squamous cell carcinoma (SCC), which represents 75% of all cases. This is followed by endocervical adenocarcinoma (ECA), which accounts for 20-25% of all cases. ECA in fact represents a heterogeneous group of tumors with various etiologies, molecular drivers, morphologies, responses to treatment, and prognoses. ECA classification has recently been reorganized by the International Endocervical Adenocarcinoma Criteria and Classification and updated by the 2020 World Health Organization with separation into human papillomavirus (HPV)-associated (HPVA) and HPV-independent (HPVI) categories[1]. Cervical clear cell carcinoma (c-CCC) is one of the HPVI ECA types and accounts for only 3.3% of all ECAs[2]. Although the clinicopathological features of other Müllerian system-derived CCCs have been broadly studied, little is known about the clinicopathological features and optimal treatment strategies of c-CCCs due to their rarity.

C-CCCs is morphologically identical to their endometrial and ovarian counterparts, with solid, tubulocystic, and papillary architectures. Tumor cells are typically characterized by clear cytoplasm and hobnail nuclei, prominent cell membranes, hyperchromatic nuclei, and low mitotic rate. Oxyphilic, flat, and signet ring cells can be seen albeit rarely. Clear cells are round or polyhedral and contain abundant glycogen and occasionally hyaline globules[3,4].

The prognosis of c-CCC varies with stage, but the actual risk associated with this histology is unknown. Although most studies on ECA survival rates have not evaluated c-CCC separately, they have shown that HPVI ECAs have a worse prognosis than HPVAs[5].

The treatment approach c-CCC is consistent with the other cervical cancer types. Radical hysterectomy or trachelectomy, pelvic lymphadenectomy, +/- external beam radiotherapy (EBRT), and +/- brachytherapy constitute a standard treatment approach for early-stage cervical carcinoma (FIGO stages IA and IB1), while EBRT and systemic chemotherapy are the standard surgical treatment regimen for further stages (FIGO stages IB2, II, and III). According to the NCNN (National Comprehensive Cancer Network) version 1.2023 cervical cancer guidelines, pembrolizumab has been added as a preferred regimen as a second-line option for treating programed cell death-ligand 1 (PD-L1) positive or microsatellite instability-high (MSI-H)/mismatch-repair deficient (dMMR) tumors[6-8]. However, PD-L1 expression status in c-CCCs has been demonstrated in only a few studies in the English literature, and a successful immunotherapy response has recently been published as a case report[9-11].

As is known, four commercial PD-L1 expression assays linked to different PD-1/PD-L1 checkpoint inhibitors are currently available for the treatment of several cancer types 22C3, 28-8, SP142, and SP263. Among these, the 22C3 assay has received FDA approval as a "companion diagnostics" for the treatment of pembrolizumab in many cancer types, including cervical cancer[12].

Furthermore, MSI-H/dMMR is identified as a biomarker for immunotherapy efficacy and pointed to the potential use of immune checkpoint inhibitors in several cancer types including cervical cancer by the Keynote-158 trial[13]. PD-L1 expression, MSI status, and their correlation with clinicalopathologic features have already been investigated in other HPVA and HPVI-type cervical carcinomas in larger series[14]. However, the data regarding the situation for these biomarkers in c-CCCs are limited. In this study, we investigated the prevalence of PD-L1 expression (by using PD-L1 22C3 and SP263 assays) and MMR (by using MLH1, MSH2, MSH6, and PMS2 proteins) deficiency status and their relationship with the clinicalopathological features in our c-CCC series.

Materials and Methods

Case Selection and Clinicopathological Evaluation

A total of 16 primary c-CCC cases were included in this study. Among them, 14 cases were selected from the 105 ECA cases that were gathered previously for the design of the reproducibility of the new ECA classification study by our team[5]. After adding 2 new cases to expand the cohort, all available hematoxylin and eosin-stained (H&E) slides were reviewed by two pathologists to confirm the diagnosis and determine the optimal tumor-containing tissue block.

Diagnostic confirmation was made by identification of the classic morphologic features of c-CCC, including high-grade tumor cells with hobnail nuclei and prominent nucleoli in solid, papillary, and/or tubulocystic architectures. Napsin-A, p53, ER, PR immunohistochemical stains, and HPV-DNA in

situ hybridization (ISH) techniques were also applied for the confirmation of the morphologic diagnosis The presence of stromal tumor-infiltrating lymphocytes (TILs) was assessed independently on each slide. Stromal TILs are reported as a percentage of tumor stroma occupied by lymphocytes. TILs are classified into three groups using the following cut-off values: 10% (mild), 10-40% (moderate), and >40% (high).

Clinicopathological parameters, including age at diagnosis, presence of lymphovascular space invasion, nodal status, and International Federation of Gynecology and Obstetrics (FIGO) stage, were recorded from electronic medical records. It was also confirmed that the tumors originated from the endocervix by confirming that there was no tumor infiltration either in the ovary or in the endometrium.

Immunohistochemical Assessment

The 4-um-thick whole tissue sections were taken from formalin-fixed paraffin-embedded blocks. PD-L1 immunohistochemistry was conducted with two PD-L1 antibody clones on two different staining platforms. The SP263 antibody clone (Roche Diagnostics, Mannheim, Germany) was used on the Ventana Benchmark Ultra platform, and the 22C3 antibody clone (Agilent Technologies, Waldbronn, Germany) was run on a DAKO Autostainer Link 48 at the Koç University Hospital, Pathology Department (Istanbul, Turkey). All assays are referred to hereafter by the antibody clone used.

The combined positive score (CPS) and tumor proportion score (TPS) were used for evaluating PD-L1 positivity. CPS was calculated by dividing the total number of viable tumor cells by the number of cells stained with PD-L1 (including tumor cells, lymphocytes, and macrophages) and multiplying by 100. Only intra- and peritumoral immune cells were counted for scoring immune cells in the CPS system. Stromal immune cells from outside the tumor were not included. The percentage of viable tumor cells with partial or complete membrane staining at any intensity was used to calculate TPS. Cut-off score 1 was considered positive for CPS, and over 50 was considered as extensive staining[9,15]. For each stain, including PD-L1, appropriate positive and negative controls were included.

MSI status was evaluated by immunohistochemistry using DNA mismatch repair (MMR) proteins; MLH1 (1:200, Abcam, Cambridge, England), MSH2 (1:50, Roche, Mannheim, Germany), MSH6 (1:100, Roche, Mannheim, Germany), and PMS2 (1:100, Abcam, Cambridge, England). Simultaneous expression of four MMR proteins (MLH1, MSH2, MSH6, and PMS2) was considered "proficient DNA mismatch repair (pMMR)". Otherwise, "deficient DNA mismatch repair (dMMR)" is defined as the absence of at least one of the four indexes stated above. The normal expression was defined as nuclear staining within tumor cells, with expression in tumor-infiltrating lymphocytes as the positive internal control. The absence of nuclear staining within tumor cells despite concurrent positive labeling in internal nonneoplastic tissues was described as a loss of expression.

Napsin-A (mouse monoclonal, MSVA-112), estrogen receptor (ER, 1:50, monoclonal rabbit ab, clone SP1), progesterone receptor (PR, 1:50, monoclonal rabbit ab, clone 1E2), p53 (1:200, monoclonal mouse ab, clone DO7), and p16 (prediluted, monoclonal mouse ab, clone E6H4) were stained on an automatic immunostainer [Ventana Benchmark XT (Ventana Medical Systems, Tucson, AZ, USA)]. ER and PR stains were scored on a continuous quantitative scale based on the percentage of nuclear staining in tumor cells (0-100%). Focal or diffuse granular cytoplasmic staining was recorded as positive for Napsin-A. Diffuse, block-like staining of moderate or strong intensity was accepted as positive for p16, while patchy or no staining was interpreted as negative. p53 was noted as mutated either in the complete absence (null pattern) of staining or in the strong staining of >75% of tumor cell nuclei. The other positive staining rates were accepted as wild-type for p53[16]. The non-HPV associated status of the cases was already shown in a previous study using the HPV-DNA ISH technique (Detailed knowledge about the HPV-DNA ISH technique can be found in the referenced study)[5].

Immunohistochemical stains were initially independently assessed by two pathologists (P.B., O.C.E.). In the case of ambiguity, a consensus diagnosis was reached with a gynecopathologist with 20 years of experience in multi-head microscope (N.K.).

Statistical Analysis

To correlate PD-L1 expression with MMR status, clinicopathological features, and different TIL group, evaluations were made via 2-tailed χ^2 tests. For every analysis, statistical significance was set with p-value <0.05. IBM SPSS Statistics for Windows version 28.0 (IBM Corp., Armonk, NY, USA) was used for the analysis.

Results

Clinicopathological characteristics

The clinical characteristics of the cases are summarized in Table 1. The median age at diagnosis of 16 cases was 54 years (range 31-79 years), and 18% of the cases were under 40. There was no history of in utero diethylstilbestrol (DES) exposure in any of the patients. Most cases presented with vaginal bleeding and/or watery discharge. All patients underwent radical hysterectomy with bilateral salpingo-oophorectomy and pelvic-para-aortic lymph node dissection. The mean tumor size was 3.8 cm (1.0-8.0 cm). Three of the tumors invaded the upper two-thirds of the vagina (18.75%, 3/16) and one invaded the left parametrium (6.25%, 1/16). Two penetrates the vaginal surgical margin, one extended to the posterior cervical margin, and one concurrently had a tumor implant in the serosa of the sigmoid colon. The remaining tumors are confined to the cervix with a negative surgical margin (75%, 12/16). The initial FIGO stages for most patients were stages I and III (87.5%), except for one patient with stage IIA1 and one with stage IVA. The initial

Table 1. Clinicopathological characteristics of the cases

Parameters	n (%)
Age	
Average (range)	54 (31-79)
Tumor size (cm)	
Average (range)	3.8 (1-8)
FIGO Stage at diagnosis	
Stage I	11 (68.75)
IA1	2 (12.5)
IA2	0 (0)
IB1	1 (6.25)
IB2	3 (18.75)
IB3	5 (31.25)
Stage II	1 (6.25)
IIA1	1 (6.25)
IIA2	0 (0)
IIB	0 (0)
Stage III	3 (18.75)
IIIA	0 (0)
IIIB	0 (0)
IIIC1	1 (6.25)
IIIC2	2 (12.5)
Stage IV	1 (6.25)
IVA	1 (6.25)
IVB	0 (0)
Silva Pattern invasion	
A	0 (0)
B	1 (6.25)
C	15 (93.75)
TILs	
Mild	6 (37.5)
Moderate	3 (18.75)
High	7 (43.75)
LVI	
Positive	8 (50)
Negative	8 (50)
PNI	
Positive	2 (12.5)
Negative	14 (87.5)
LNM	
Negative	12 (75)
Positive (pelvic)	2 (12.5)
Positive (paraaortic)	2 (12.5)

TILs: Tumor-infiltrating lymphocytes, LVI: Lymph vascular invasion, LNM: Lymph node metastasis, PNI: Perineural invasion

FIGO stages were distributed as follows: stage IA1: 12.5% (2/16), IB1: 6.25% (1/16), IB2: 18.75% (3/16), IB3: 31.25% (5/16), IIA1: 6.25% (1/16), IIIC1: 6.25% (1/16), IIIC2: 12.5% (2/16), and IVA: 6.25% (1/16). Lymphovascular invasion (LVI) was in eight (50%) cases and absent in eight (50%) cases. LVI was not seen in the early-stage tumors (FIGO Stages IA and IB1). Lymph node metastasis (LNM) at the time of diagnosis was in 4 (25%) and absent in 12 (75%). Two of these were pelvics (50%) and two were paraaortic (50%) lymph nodes. Survival data of 14 cases were available. The mean follow-up time of the cases was 36 months. Two of the cases died on the 13th and 18th months after surgery (Cases are numbered 1 and 10, respectively, according to their position in Table 2). Other cases were still alive with no recurrence or metastasis in the following period.

Histologically, 6 (37.5%) tumors had an exophytic polypoid appearance with mostly superficial infiltration of the endocervical mucosa. One case was restricted to the endocervical epithelium, with a polypoid appearance (5 cm in size) and no evident cervical stormal invasion. Fifteen cases showed Silva pattern-C infiltration (93.75%), and one case had a Silva pattern-B infiltration as there was no significant cervical stormal invasion (6.25%)[17].

The histological appearance of the cases was similar to other gynecological system origins, with an ordinary CCC appearance (Figure 1A). The tumor cells were arranged in a tubullocystic, papillary, or solid architecture (often a mix to varying degrees) and were surrounded by cells with clear (intracytoplasmic glycogen), eosinophilic, granular, and sometimes hobnailed cytoplasm with minimal stratification. In some cases, pseudonuclear inclusions and hyaline globules were seen. One case consisted of well-differentiated cysts lined by a single flattened epithelial layer on a hyalinized stroma that could easily be mistaken for benign entities as defined. TILs were seen

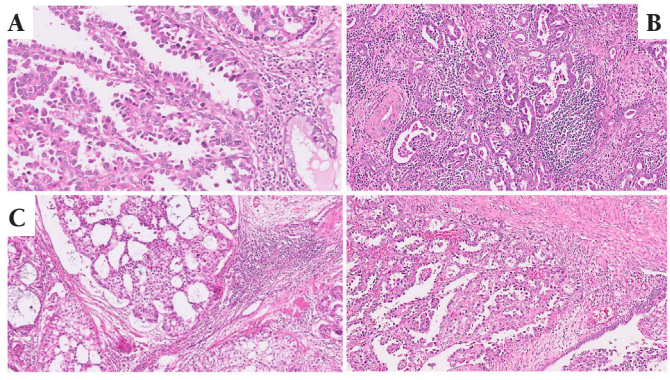

Figure 1. A-D) Histological appearance of clear cell carcinomas (CCC) and accompanying varying amounts of tumor-infiltrating lymphocytes (TILs). **A-** The histologic appearance of classic CCC from the high-power view (H&E x200), **B-** High TIL infiltration (H&E, x100), **C-** Moderate TIL infiltration (H&E, x100), **D-** Mild TIL infiltration. A few TIL clusters can be seen in the right part of the figure (H&E, x100)

in all cases at different rates (Figure 1B-D). However, stromal high TIL was observed in 7 (43.75%) cases. Moderate and mild TIL was observed in 18.75% and 37.5% of cases, respectively. Carcinoma *in situ* foci defined for CCCs in the literature were not observed in any of the cases[18]. Immunohistochemically, all tumors were Napsin-A positive and wild-type with p53. Conveniently, ER, PR, or P16 expression was not seen in any case.

Mismatch Repair Protein Deficiency

Thirty-one percent (5/16) of the c-CCC cases demonstrated MMR deficiency (Table 2). Two of them showed a dual loss of MSH2 and MSH6 (Figure 2), 1 showed a dual loss of MLH1 and PMS2, 1 showed a triple loss of MLH1, PMS2, and MSH6, and 1 showed a single loss of MSH6 proteins. Forty percent (2/5) of dMMR c-CCC cases showed extensive PD-L1 positivity in both TPS and CPS scores. PD-L1 positivity was seen in 80% of dMMR cases. Eighty percent (4/5) of dMMR cases were accompanied by a high rate of TILs. The remaining dMMR case exhibited no PD-L1 expression in tumor cells or TILs. The relationship between PD-L1 expression and MMR status was not statistically significant for either tumor (p=0.3) or combined tumor and inflammatory cells (p=1) (Table 3).

Tumoral and Peritumoral Immune PD-L1 Expression

PD-L1 expression status and their relationship with the MMR results for each case are shown in Table 2. In 69% (11/16) of cases with SP263 clones and 62.5% (10/16) of cases with 22C3 clones, either the tumor (TPS) or immune cells like lymphocytes and macrophages that were infiltrating the tumor (CPS) were stained with PD-L1. PD-L1 expression was seen in 56.25% (9/16) of the cases based on TPS in both clones. Both PD-L1 SP263 and 22C3 clones showed perfect accordance with TPS. For CPS also, concordance was excellent, except for one case that was considered negative for the SP263 clone

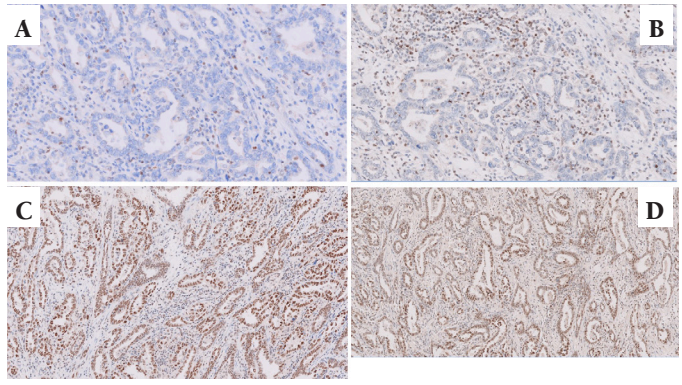

Figure 2. A-D) The microsatellite instability (MSI) status of case no 2. **A-** Concurrent loss of MSH-6 in tumor cells (IHC; x200), **B-** Concurrent loss of MSH-2 in tumor cells (IHC; x200), **C-** Intact MLH-1 expression (IHC; x100), **D-** Intact PMS-2 expression (IHC; x100)

Table 2. Detailed TIL status, PD-L1, and MMR IHC expression results of the cases

Case no	PD-L1/SP263		PD-L1/22C3		TILs	MMR IHC			
	TPS (%)	CPS (%)	TPS (%)	CPS (%)	Score	MLH1	PMS2	MSH2	MSH6
1	90	83	90	70	H	Loss	Loss	p	Loss
2	90	65	90	60	H	p	p	Loss	Loss
3	5	20	10	35	H	p	p	Loss	Loss
4	2	1	2	1	H	p	p	p	Loss
5	-	-	-	-	Mi	Loss	Loss	p	p
6	-	5	-	-	H	p	p	p	p
7	-	-	-	-	Mod	p	p	p	p
8	-	-	-	-	Mi	p	p	p	p
9	-	-	-	-	Mi	p	p	p	p
10	3	5	1	2	Mod	p	p	p	p
11	1	3	5	10	H	p	p	p	p
12	-	-	-	-	Mi	p	p	p	p
13	10	7	2	1	Mod	p	p	p	p
14	5	2	2	5	Mi	p	p	p	p
15	-	30	-	30	H	p	p	p	p
16	10	9	10	9	Mi	p	p	p	p

First 5 cases have a loss of expression (dMMR) with at least one of the MMR: Mismatch repair, IHC: Immunohistochemistry, TPS: Tumor proportion score, CPS: Combined positive score, TILs: Tumor-infiltrating lymphocytes, (-): Negative, p: proficient DNA mismatch repair (pMMR), Loss: deficient DNA mismatch repair (dMMR), H: High, Mod: Moderate, Mi: Mild

Table 3. Correlation between PD-L1 expression (according to the PD-L1 22C3 clone) and the clinicopathological characteristics of the cases

Variable	n (%)	PD-L1 expression TPS			n (%)	PD-L1 expression CPS		
		Positive	Negative	p-value		Positive	Negative	p-value
MMR IHC				0.3				0.58
dMMR	5 (31.3)	4 (25)	1 (6)		6 (37.5)	5 (31.25)	1 (6.25)	
pMMR	11 (68.7)	5 (31)	6 (38)		10 (62.5)	6 (37.5)	4 (25)	
TILs				0.35				0.061
Mild	6 (37.5)	2 (12.5)	4 (25)		6 (37.5)	2 (12.5)	4 (25)	
Moderate	3 (18.7)	2 (12.5)	1 (6.25)		3 (18.7)	2 (12.5)	1 (6.25)	
High	7 (43.8)	5 (31.25)	2 (12.5)		7 (43.8)	6 (37.5)	1 (6.25)	
Age (years)				0.61				0.11
<54	8 (50)	4 (25)	4 (25)		8 (50)	4 (25)	4 (25)	
⩾54	8 (50)	5 (18.8)	3 (31.3)		8 (50)	7 (43.8)	1 (6.3)	
Tumor size (cm)				0.13				0.59
<3.8	8 (50)	3 (18.8)	5 (31.3)		8 (50)	5 (31.3)	3 (18.8)	
⩾3.8	8 (50)	6 (37.5)	2 (12.5)		8 (50)	6 (37.5)	2 (12.5)	
FIGO stage				0.83				0.60
I-II	11 (68.8)	6 (37.5)	5 (31.3)		10 (62.5)	8 (50)	2 (12.5)	
III-IV	5 (31.3)	3 (18.8)	2 (12.5)		6 (37.5)	4 (25)	2 (12.5)	
LVI				0.61				0.60
Positive	8 (50)	5 (31.3)	3 (18.8)		8 (50)	6 (37.5)	2 (12.5)	
Negative	8 (50)	4 (25)	4 (25)		8 (50)	4 (25)	4 (25)	
LNM				0.77				0.60
Positive	4 (25)	2 (12.5)	2 (12.5)		4 (25)	2 (12.5)	2 (12.5)	
Negative	12 (75)	7 (43.8)	5 (31.3)		12 (75)	8 (50)	4 (25)	

MMR: Mismatch repair, IHC: Immunohistochemistry, pMMR: proficient DNA mismatch repair, dMMR: deficient DNA mismatch repair, TILs: Tumor-infiltrating lymphocytes, LVI: Lymph vascular Invasion, LNM: Lymph node metastasis

(which was 5% positive for the 22C3 clone) (Table 2). Two of the cases showed extensive PD-L1 staining in both clones (18%; 2/11) (Figure 3). Most of the remaining PD-L1-positive cases showed tumoral staining in less than 50% of cells (82%; 9/11). The mean PD-L1 expressing rates for CPS were 16% for SP263 and 15.4% for 22C3. PD-L1 positivity was seen only in TILs in 2 cases (18%; 2/11), with one where positivity was seen only on the SP263 clone (5% PD-L1 staining rate).

Relationship Between PD-L1 Expression and Clinicopathological Features

The relationship between PD-L1 expression and clinicopathological parameters of the patients is shown in Table 3.

There was no correlation between CPS/TPS with age (p=0.61/p=0.11), tumor size (p=0.13/p=0.59), lymphovascular invasion (p=0.60/p=0.61) or lymph node metastasis (p=0.60/p=0.77) based on PD-L1 expression. There was no correlation between PD-L1 expression and FIGO stage as well (p=0.60/p=0.83). PD-L1 expression was higher in both TPS and CPS with high amounts of TIL. However, statistical significance was not found (CPS, p=0.061, TPS, p=0.35).

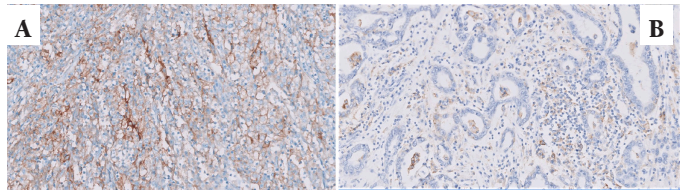

Figure 3. A- Extensive PD-L1 staining (with the 22C3 clone) in tumor and immune cells of case no 2 (TPS 90%, CPS 60%) (IHC; x200); **B-** A lesser degree of PD-L1 staining (with the SP263 clone) in tumor and immune cells of case no 3 (TPS 5%, CPS 20%) (IHC; x200)

Discussion

Immune checkpoints such as programmed cell death 1 (PD-1) and its ligand (PD-L1) are critical in antitumor immunity, and blocking them has been demonstrated to enhance

outcomes in patients with numerous types of malignancies[19]. Pembrolizumab, a PD-1 inhibitor, has been approved for the treatment of patients with PD-L1-positive cervical cancer that is locally progressed, recurrent, or metastatic[20]. In this study, a objective was to evaluate PD-L1 expression in c-CCC.

The CCC of the cervix accounts for approximately 3.3% of ECAs, which is further reduced if SCCs are also included in the cohort. Therefore, there is a paucity of data in the literature regarding the prognostic indicators, clinical outcomes, and treatment strategies of c-CCC. According to recent studies, the prognosis of early-stage c-CCC is similar to that of other types of cervical cancer. Advanced-stage and lymphatic involvement are associated with worse survival for both progression-free survival (PFS) and overall survival (OS) for c-CCC[21-25]. According to Liu et al.[24], the 5-year OS for FIGO stages IB to IIA and stage IIB to IIIC was 95.7% and 46.2%, respectively. Thomaset al.[25] have similarly found that the presence of positive lymph nodes has a negative impact on 5-year PFS (31% vs. 92%, p<0.001) and 5-year OS (80% vs. 100%, p=0.02) in stage I and IIA c-CCC patients. Stolnicu et al.[26] have recently compared the survival outcome between c-CCC and ECA and found a significant difference in 5- and 10-year OS between c-CCC and HPVA ECA, whereas no significant difference was shown between c-CCCs and gastric-type ECAs. Moreover, they emphasize the importance of the stage in OS with their results; They stated that OS in stage I CCC was 85.3% at both 5 and 10 years, while it was 39.7% at 5 years and 0% at 10 years in stages II to IV (p<0.001)[26].

Despite variations in survival and treatment responses between cervical HPVA and HPVI carcinomas, there is currently no therapeutic difference between SCCs and adenocarcinomas, including c-CCCs[27,28]. Radical surgery combined with targeted adjuvant therapy may cure early-stage disease. However, more advanced diseases are treated with EBRT and systemic chemotherapy[6]. According to the NCNN Version 1.2023 guidelines for cervical cancer treatment, concurrent chemoradiation is generally the primary treatment choice for stages of IB3 to IVA disease. In 2020, the FDA approved the addition of the PD-1 inhibitor pembrolizumab to the treatment of PD-L1-positive (CPS1)- or dMMR patients whose disease progresses after chemotherap[29-31]. Keynote-158 (pembrolizumab)[13], Empower-Cervical-1 (cemiplimab)[32], and Keynote-826 (pembrolizumab)[7] were pivotal studies that indicated immunotherapy enhanced overall survival in both post-platinum failure and frontline persistent, recurrent, or metastatic PD-L1 positive cervical cancer patients.

According to the Keynote-158 trial, the overall response rate was 14.4% in PD-L1-positive patients. Median PFS and OS for PD-L1-positive patients were 2.1 and 11 months, respectively. However, no responses were observed in patients with PD-L1-negative tumors. Regarding safety, 4.1% of patients stopped treatment because of treatment-related adverse events (including hepatitis, severe skin reactions, and adrenal insufficiency)[13].

The Empower-Cervical-1 study investigated the therapeutic efficacy of cemiplimab (PD-1 inhibitor) in 608 patients (304 of the patients randomly received cemiplimab and 304 received chemotherapy) with recurrent or metastatic cervical cancer. Although only a small portion of their patients could have their PD-L1 expression assessed, cemiplimab had a longer median overall survival than the chemotherapy group. (13.9 vs. 9.3 months) among the PD-L1 positive patients (≥1%). The median overall survival rates were 7.7 and 6.7 months with cemiplimab and chemotherapy in PD-L1-negative patients, respectively. According to the results, objective responses to cemiplimab were observed in 18% (≥1%) of PD-L1 positive patients and 11% (<1%) of PD-L1 negative patients. In the overall population, an objective response was obtained in 16.4% of patients in the complimab group compared with 6.3% in the chemotherapy group. This means that PD-L1-positive patients generally have an increased overall survival benefit. However, it can be concluded that PD-L1-negative patients also have an overall survival benefit with cemiplimab as or slightly better than patients receiving chemotherapy. Of their complimab and chemotherapy-received cohorts, 45% and 53.4% had grade 3 or higher adverse events, and 15.7% and 0.7% had immune-related adverse events, respectively[32].

The relative benefit of adding pembrolizumab (PD-1 inhibitor) to platinum-based chemotherapy with or without bevacizumab in PD-L1-positive metastatic or unresectable cervical cancer patients was investigated in 548 patients by Keynote-826 (pembrolizumab) study[7]. According to their results, progression-free (10.4 vs. 8.2 months) and overall survival (24-month estimate of patients alive, 53.0% vs. 41.7%) were significantly longer with pembrolizumab than with the placebo group. According to their study's adverse event data, 42.4% of patients in the placebo group and 49.8% of patients who received pembrolizumab experienced major adverse events. Only hypothyroidism (18.2% vs. 9.1%) and a lower white blood cell count (12.1% vs. 7.1%) posed a greater risk in the pembrolizumab group.

PD-L1 protein expression is currently used as a predictive biomarker for checkpoint therapy in cervical cancer. However, the heterogeneous expression tendency of the PD-L1 protein makes this method suboptimal. Therefore, the PD-L1 protein expression rate may not be directly associated with prognostic significance and treatment response. The patient selection according to PD-L1 protein expression excludes potential patients for whom checkpoint therapy could be effective. Rotman et al.[33] investigated the tumoral PD-L1 expression heterogeneity for cervical cancer. According to their results, 27% of cases had heterogeneity between different tumor cores based on the percentage of positive tumor cells. Additionally, for comparison, they also applied the RNAish technique and observed heterogeneity in 11% of the cases. Their results showed that core biopsies can consequently lead to false negative results

and the RNAish technique could serve as a better biomarker than IHC detection.

In this study, the PD-L1 expression status of c-CCCs was investigated using two different PD-L1 clones (SP263 and 22C3). PD-L1 expression scores were almost completely similar between the two clones. According to the results of a meta-analysis of the diagnostic accuracy of the PD-L1 IHC assays, the diagnostic sensitivity of the 22C3 was higher than the SP263 assay[34]. The fact that our results between the two clones were almost similar for both TPS and CPS scores may be due to the small number of patients.

PD-L1 expression in SCC and ECA has been previously reported[35,36]. According to their results, SCC had significantly higher PD-L1 expression positivity in tumor cells than ECAs (5% cut-off). Omenai et al.[35] recently published the PD-L1 expression profiles of 183 cervical cancer patients, irrespective of their histological type. According to their results, PD-L1 positivity was seen in 57.4% of the cases (58.7% in SCCs and 50% in ECAs). Song et al.[9] shared their results on PD-L1 expression and immune stormal features in HPVI cervical adenocarcinomas. According to their results, PD-L1 expression was seen in 58.3% (7 of 12) of c-CCCs. Moreover, they also found that PD-L1-positive cases (CPS ≥1) showed worse PFS and OS than PD-L1-negative cases. However, data in the literature regarding the PD-L1 expression status of CCCs, the prognostic effect of this expression, and their response to treatment are quite limited. Zong et al.[10] investigated the expression of different immune checkpoint proteins in c-CCCs. They found that 22% of cases had PD-L1-positive tumor cells (CPS ≥1).

Diffuse PD-L1 expression was observed in 18% of our cohort, and the mean PD-L1 expression rate in our c-CCC series was 16%.

Even not statistically significant high TIL density was seen more frequently in PD-L1-positive cases. PD-L1 positivity has been shown to be associated with the number of TILs in many tumor types, including cervical carcinoma[37]. As is known, TIL density is likely related to immunotherapy response[38]. Similarly, Song et al.[9] showed a significant association between high TIL percentage and CPS or TPS-based PD-L1 expression in their c-CCC cohorts. Additionally, PFS and OS were significantly poorer for PD-L1-positive subjects in their group than for PD-L1-negative cases. Unfortunately, we could not perform a survival analysis due to the short follow-up period in our series and the low number of cases.

Another aim of our study was to investigate the MMR deficiency status in c-CCCs, as the presence of MSI is one of the predictors of anti-PD1/PD-L1 immunotherapy response[39,40]. Anti-PD-L1 drugs can enhance survival, particularly in dMMR malignancies[41].

According to our results, 80% of the dMMR cases were also PD-L1 positive and 80% of the dMMR cases had high TILs. In a recent study involving 39 ECA cases, 2 of which were c-CCC, 15% of the cases were dMMR (all usual type), and 1 dMMR case was PD-L1 positive[42]. Song et al.[9] reported an MMR deficiency status of 16% in their c-CCC cohort (2/12 cases). Both were PD-L1-positive as well. Since the number of studies on the MSI status of c-CCCs is very limited, further studies with larger cohorts will be needed to validate these findings.

Study Limitations

This study has inherent limitations due to the limited sample size owing to the rarity of the disease. As a result, the generalizability of the current findings is limited.

Conclusion

However, our results are valuable in showing that c-CCCs can have PD-L1 expression and MMR deficiency. This shows that c-CCC cases, which are tumor types resistant to conventional chemotherapy, are candidates for immunotherapy similar to other cervical cancer types.

Ethics

Ethics Committee Approval: This study was approved by the ethics review board of Koç University (approval number: 2023.120.IRB2.028, date: 06.04.2023).

Informed Consent: Informed consent was obtained from all participants.

Peer-review: Internally peer-reviewed.

Authorship Contributions

Conceht: P.B., N.K., Design: P.B., N.K., Data Collection or Processing: P.B., Ö.C.E., Ö.Ö., A.N.H., N.K., Analysis or Interpretation: P.B., N.K., Literature Search: P.B., Ö.C.E., Writing: P.B.

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Role of acetyl-CoA acetyltransferase 1 expression in the molecular mechanism of adenomyosis

Asetil-KoA asetiltransferaz 1 ekspresyonunun adenomyozis moleküler mekanizmasınaki rolü

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Abstract

Objective: Adenomyosis is a benign uterine illness characterized by endometrial gland and stromal invasion into the myometrium. Acetyl-CoA acetyltransferase 1 (ACAT1) is an enzyme localized in mitochondria that is involved in ketogenesis and ketolysis processes by reversibly catalyzing the formation of acetoacetyl-CoA from two acetyl-CoA molecules. The current study investigated the expression of the ACAT1 molecule in tissue samples of patients diagnosed with adenomyosis and healthy endometrial tissues. It is aimed to determine the differences in *ACAT1* gene expression and in this way to discover the first information about the role of ACAT1 in the development and molecular mechanism of adenomyosis.

Materials and Methods: In the current retrospective study, formalin-fixed paraffin-embedded archival tissues were employed. A total of 76 patient samples were included in the study. Of these samples, 28 are adenomyotic tissue (Group I), 30 are eutopic endometrial tissue (Group II), and 18 are the Control Group. In these groups, the expression levels of the *ACAT1* gene were determined by the reverse transcription-polymerase chain reaction method.

Results: When the expression results of the *ACAT1* gene were evaluated, statistically significant differences were found between the groups ($p<0.05$). There was a difference between Group I-Group II and Group I-Control Group regarding the *ACAT1* gene. No statistically significant change was observed between Group II and Control Group. It is a remarkable finding that the expression of ACAT1 in adenomyosis tissue is decreased compared with both eutopic endometrium and control groups tissues.

Conclusion: The results suggest that ACAT1 may be associated with the molecular pathogenesis of adenomyosis.

Keywords: Adenomyosis, ketogenesis, gene expression

Öz

Amaç: Adenomyozis, endometriyal bez ve stromanın miyometriyuma invazyonu ile karakterize iyi huylu bir rahim hastalığıdır. Asetil-KoA asetiltransferaz 1 (ACAT1) iki asetil-KoA molekülünden asetoasetil-KoA oluşumunu geri dönüşümlü katalize ederek ketogenez ve ketoliz süreçlerinde yer alan mitokondride lokalize bir enzimdir. Mevcut çalışmada, adenomyozis tanısı konmuş hastaların doku örnekleri ve sağlıklı endometrial dokularda ACAT1 molekülünün ekspresyonu incelenmiştir. *ACAT1* gen ekspresyonundaki farklılıklarının belirlenmesi ve buna bağlı olarak adenomyozis gelişimi ve moleküler mekanizmasında ACAT1'in rolü ile ilgili ilk bilgilerin keşfedilmesi amaçlanmıştır.

Gereç ve Yöntemler: Gerçekleştirdiğimiz retrospektif çalışmada, formalinle fikse edilmiş parafine gömülü arşiv dokuları kullanılmıştır. Çalışmaya toplam 76 hasta örneği dahil edildi. Bunların 28'i adenomyotik doku (Group I), 30'u ötopik endometrium dokusu (Group II) ve 18'i Kontrol Grubundan oluşmaktadır. Bu gruplarda, *ACAT1* geninin ekspresyon düzeyi reverse transkripsiyon-polimeraz zincir reaksiyonu yöntemiyle belirlenmiştir.

PRECIS: Low expression of Acetyl-CoA Acetyltransferase 1 (ACAT1) was observed in adenomyotic tissues. Decreased expression of ACAT1 may be involved in the molecular mechanism of adenomyosis development.

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Bulgular: *ACAT1* geninin ekspresyon sonuçları değerlendirildiğinde gruplar arasında istatistiksel olarak anlamlı farklılıklar tespit edilmiştir (p<0,05). Grup I-Grup II ile Grup I-Kontrol Grubu arasında *ACAT1* geni açısından farklılık bulunmuştur. Grup II ve Kontrol Grubu arasında yapılan incelemelerde ise istatistiksel açıdan anlamlı bir değişim gözlenmemiştir. Adenmyozis dokusunda ACAT1 ekspresyonunun hem ötopik endometriyum hem de kontrol grubu dokularına göre azalmış olması dikkat çekici bir bulgudur.

Sonuç: Elde edilen bulgular, ACAT1'in adenomyozisin moleküler patogenezi ile ilişkili olabileceğini düşündürmektedir.

Anahtar Kelimeler: Adenomyozis, ACAT1, ketogenez, gen ekspresyonu

Introduction

Adenomyosis is described as the aberrant implantation of endometrial tissue into the myometrium associated with uterine enlargement[1].

It is a common gynecological disorder that affects the reproductive period of women. Menorrhagia, dysmenorrhea, pelvic pain, dyspareunia, and abnormal uterine bleeding are the most common symptoms in adenomyosis patients and may also be asymptomatic in some women with adenomyotic lesions[2]. Endometriosis, leiomyomas, endometrial hyperplasia, and endometrial polyps are frequently related to adenomyosis[3]. Molecular studies on adenomyosis have revealed differences in the expression of genes involved in different metabolic pathways. In recent years, evidence has been reported showing that genetic mutations, gene expression, and epigenetic differences are associated with clinical findings. However, it is stated that more molecular studies are needed[4]. Although it is a disease with a high prevalence, its molecular pathogenesis remains unclear.

Acetyl-CoA acetyltransferase (ACAT) refers to two enzymes called ACAT1 and ACAT2 located in the mitochondria and cytoplasm, respectively[5]. ACAT1 encodes a mitochondrial enzyme that catalyzes the reversible synthesis of acetoacetyl-CoA from two acetyl-CoA molecules. Cells can use acetyl-CoA to produce the energy needed. The ACAT1 enzyme is responsible for the final step in ketolysis (convert acetoacetyl-CoA into two molecules of acetyl-CoA) during fat metabolism. The enzyme also performs the reverse reaction of this step called ketogenesis. Ketogenesis is a biochemical process in the liver that produces ketone bodies by breaking down fatty acids[6,7]. Studies have shown that ACAT1 and ACAT2 are potential markers and therapeutic targets in neoplastic tissues and may be associated with prognosis in cancer[8].

The molecular development mechanism of adenomyosis has not been fully explained. This indicates that different cellular metabolic pathways and several genes involved in these pathways are effective in the formation of the disease. Based on this, we determined expression levels of ACAT1 in different experimental groups to define the role of ACAT1 and ketone metabolism in the molecular mechanism of the disease, which has not been studied before in adenomyosis. In this way, we aimed to present a marker that can be diagnostic, prognostic, and therapeutic for adenomyosis patients whose diagnostic methods and pathognomonic molecular markers are limited.

Materials and Methods

Collection of Tissue Samples

In the current study, 76 paraffin-embedded archival tissues were used. The tissues were collected in three groups as follows: Group I (n=28); adenomyotic tissues (ectopic endometrial tissues) and Group II (n=30); normally located endometrial tissues (eutopic endometrial tissues) of adenomyosis patients. Control group: Endometrial tissues of individuals without adenomyosis (n=18). Tissues were collected surgically from 30 adenomyosis patients. The patients diagnosed with adenomyosis were selected carefully after clinical and histopathological examinations. Tissue collection was carried out in the Department of Obstetrics and Gynecology and the Department of Pathology, Hospital of Mersin University (Mersin/Turkey). The women in groups I and II were of reproductive age and had been diagnosed with adenomyosis. The control group was made up of women without adenomyosis and were likewise of reproductive age.

This study was approved by the ethics review board of Mersin University (approval number: 450, date: 24.06.2020).

Reagents

To isolate RNA from FFPE tissues, the innuPREP FFPE total RNA kit (Analytikjena PN: 845-KS-2050050) was utilized. RT-PCR was used to generate cDNA from the acquired RNAs. For cDNA synthesis, High-Capacity cDNA Reverse Transcription Kit (Thermo Cat. No. 4368814) was used. For the gene expression procedure, TaqMan® Gene Expression Master Mix (Appliedbiosystems PN: 4371135) was used.

RNA Extraction, cDNA Synthesis, and the Rt-qPCR Expression Method

The cDNA synthesis step was performed after the RNA extraction stage which employed the innuPREP FFPE total RNA kit. High-Capacity cDNA Reverse Transcription Kit methodology was used to create cDNA from RNA extracted from FFPE tissue samples. The following is the Thermal Cycler technique for converting RNA to cDNA: 10 min at 25°C, 120 min at 37°C, 5 s at 85°C, and 1 min at 4°C. Following this, the gene expression method was used. The gene expression method employed TaqMan® Gene Expression Master Mix, cDNAs, and primers (forward and reverse) designed for the genes under investigation.

The following stages were carried out with the Roche LightCycler 480 II: 50°C for 2 minutes (incubation phase), 95°C for 10 min (activation phase), amplification step (95°C for 15 seconds then 60°C for 60 seconds, 40 cycles), and 40°C for 30 seconds (cooling phase). The housekeeping gene ACTB (beta-actin) was employed as the control gene in this investigation. Following the completion of the experimental stages, Delta Ct (ΔCt) and $2^{-\Delta\Delta Ct}$ values were computed and employed in statistical analysis.

Statistical Analysis

Statistical analysis was performed using the IBM SPSS Statistics program. Data were expressed as the mean and standard deviation. In the comparison of the expression levels of the genes for the three groups in the study, the ANOVA and the Kruskal-Wallis tests were used for the normal and non-normal data distributions, respectively. P value of <0.05 was considered statistically significant.

Results

Although 90 paraffin-embedded archival tissues were collected, some samples were not used in statistical analysis due to RNA quantity and quality. After removing these samples, 76 patient samples were used. The samples were divided into three groups: adenomyotic tissues (Group I, n=28), endometrial tissues of adenomyosis patients (Group II, n=30), and endometrial tissues of individuals without adenomyosis (Control Group, n=18).

Ectopic and eutopic endometrial tissues of patients diagnosed with adenomyosis were used in Group I and Group II, respectively. Ectopic endometrial tissue samples include stroma and glands found in the myometrium of patients with adenomyosis. Eutopic endometrial tissue is a noninvasive, typically situated endometrial tissue of adenomyosis patients. The control group was formed from the endometrial tissues of individuals who were not diagnosed with adenomyosis and related gynecological diseases such as endometriosis, leiomyomas, endometrial polyps, and endometrial cancer. Samples of women in the reproductive period were used in all three groups.

In the evaluation made between the three experimental groups, a statistically significant difference was found in terms of the *ACAT1* gene (p<0.05) (Table 1).

Expression of ACAT1

Regarding *ACAT1* gene expression levels, significant differences were found between Group I-Group II (p=0.0001) and Group I-Control Group (p=0.025). No significant difference was found between the Group II and Control groups (p=0.261) (Figure 1).

These results show a statistically significant decrease in ACAT1 expression in adenomyotic tissues (Grup I) compared with eutopic endometrial tissues of adenomyosis patients (Grup II) and endometrial tissues of individuals without adenomyosis (Control Group).

Discussion

Tumor biusedy be utilized to investigate the metabolic processes of adenomyosis, which is also characterized as a tumor-like lesion in various studies, although it is a non-neoplastic lesion. Changes in energy metabolism due to glucose and fatty acids used by cells are highly determinative in tumor development. Therefore, similar metabolic changes may be effective in the molecular mechanism of adenomyosis, which is a benign lesion. Based on this, ACAT1, one of the enzymes involved in the ketogenic pathway, was examined in our study.

Decreased ACAT1 expression was observed in adenomyotic lesions compared with the eutopic endometrium and control groups. In contrast, no significant difference in the enzyme expression was found between the eutopic endometrium and control group. Our results suggest that the decrease in ACAT1 expression in adenomyotic tissue may be effective in the molecular mechanism of adenomyosis.

It has been shown that ACAT1 is associated with many aggressive cancer types, is expressed higher in malignant tissues compared to normal tissues and benign lesions, and is associated with poor prognosis[9,10]. One study with enzymes involved in the ketogenic pathway found that ACAT1 exhibited a higher expression profile in high-grade prostate cancer tissues compared to low-grade tissues[11]. In addition,

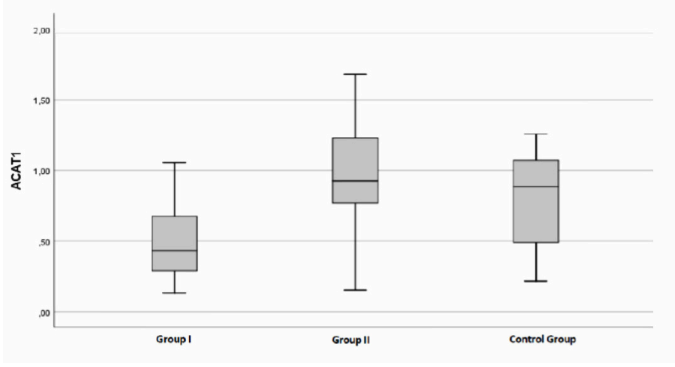

Figure 1. Comparison of $2^{-\Delta\Delta Ct}$ values of ACAT1 and 95% confidence intervals

ACAT1: Acetyl-CoA acetyltransferase 1

Table 1. Mean value and p-value of *ACAT1* gene for each group

Gene	Group I (n=28) Mean ± SD	Group II (n=30) Mean ± SD	Control (n=18) Mean ± SD	p-value[a]
ACAT1	0.535±0.361	1.044±0.504	0.809±0.350	0.0001*

[a]P-value shows the result of the ANOVA test for the normal distribution and the Kruskal-Wallis test for the non-normal distribution of the data.
SD: Standard deviation, ACAT1: Acetyl-CoA acetyltransferase 1, *p<0.05, statistically significant

the overexpression of ACAT1 in breast cancer cells has been noted to increase tumor growth and metastasis[12]. The decrease in ACAT1 activity by various inhibitors has a reducing effect on cell proliferation and tumor growth, and ACAT1 has been proposed as a pharmacologically potential anticancer target[13,14]. Similar results have been reported in many studies. It is stated that tumor cells use ketone bodies as an energy source and the increase in the expression of enzymes in the ketogenesis pathway, such as the ACAT1 contributes to carcinogenesis.

Contrary to the hypothesis supported by the previously mentioned research, some studies indicate that the ketogenesis pathway is limited in tumor cells with access to glucose because fatty acid degradation is restricted[15]. Fatty acids and cholesterol are needed for the production of membranes of new cells in uncontrolled proliferation. Therefore, it is expected that ketogenesis is suppressed in tumor cells, considering that anabolic pathways are more active in terms of lipid metabolism. According to this idea, a decrease in the expression of enzymes related to ketogenesis can be detected in tumor tissues. In a study that can provide proof of this idea, it has been reported that suppressing ketogenesis via ACAT1 increases the proliferation and metastasis of cancer cells, and overexpression of ACAT1 reduces tumor growth[16]. In another study with triplenegative breast cancer, an aggressive, malignant, and poor prognostic cancer type, it was reported that ACAT1 inhibited cell migration and invasion and suppressed cancer progression through different molecules[17]. Although different results regarding ACAT1 have been detected in different cancer types, it is emphasized that ACAT1 is a marker with therapeutic, diagnostic, and prognostic potential as a common opinion in studies. It is also pointed out that more molecular and pharmacological studies are needed.

Various investigations specify that adenomyosis is similar to tumor tissues in several metabolic pathways[18]. However, the role of ACAT1 and ketone metabolism in the development of the disease, which is the subject of our study, is unknown. The results of our study are similar to those of articles reporting lower ACAT1 expression in normal tissues and benign lesions. Considering that adenomyosis is a benign disease with low malignant transformation, it can be thought that ACAT1 may play a role in the molecular pathogenesis of the disease. In addition, it is noteworthy that we detected lower levels of ACAT1 expression in adenomyosis samples than in normal tissues. For this reason, it is another important finding for the disease that ketogenesis may also be suppressed.

This study is the first to investigate ACAT1 in adenomyosis samples. Since the molecular pathogenesis of adenomyosis has not been fully understood, each of the molecular studies will play an important role in illuminating the molecular mechanism of the disease. Although we have detected low ACAT1 expression in adenomyosis tissue suggesting that ketogenesis may be suppressed, investigation of different genes involved in ketone metabolism will provide a better understanding of this issue.

Study Limitations

Although more patient samples were examined, 76 samples whose RNA quantity and quality were suitable for the study could be used. More samples could not be included in the study due to the loss of quality and quantity of nucleic acids in formalin-fixed paraffin-embedded archive tissues.

Conclusion

In conclusion, the fact that there are still many unanswered questions about the development of the disease and the limited preoperative diagnosis and treatment options increase the importance of research on this subject. Investigating the genes involved in different metabolic pathways will provide a better understanding of the mechanism of adenomyosis development. We think that our research is a reasonable start to explain how ACAT1 and ketogenesis play a role in the pathogenesis of adenomyosis.

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Ethics

Ethics Committee Approval: This study was approved by the ethics review board of Mersin University (approval number: 450, date: 24.06.2020).
Informed Consent: Retrospective study.
Peer-review: Internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: H.A., Concept: C.Y., E.A., Design: C.Y., Data Collection or Processing: E.A., F.T.D., Analysis or Interpretation: N.C., S.E., Literature Search: C.Y., H.Ö., Writing: C.Y.
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The NEWS2 score predicts prolonged hospitalization in the intensive care unit in major surgery patients

NEWS2 skoru, majör cerrahi hastalarında uzun süreli yoğun bakım yatışını öngörür

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Abstract

Objective: Gynecological malignancies are significant causes of mortality and morbidity in women worldwide. Although surgery is an important treatment method, both the extent of the surgery and the factors related to the patient affect postoperative processes. The National Early Warning Score 2 (NEWS2) is a simple, inexpensive, and safe early warning score developed in 2012 and updated in 2017. Although it is not commonly used in surgical patients, its use in patients who will undergo major surgery may provide insights about the postoperative process. This study investigates the importance of NEWS2 and its relationship in patients with for major gynecologic oncology surgery.

Materials and Methods: Forty-four patients with gynecologic malignancies scheduled for major abdominal surgery were included in this study. Patients with a NEWS-2 score of <3 were included in group 1, and patients with a NEWS-2 score of more than 3 were included in groups 2. NEWS2 Score, Sequential Organ Failure Assessment (SOFA), and Acute Physiology and Chronic Health Evaluation 2 scores (APACHE 2) were calculated. In addition, postoperative routine clinical and laboratory parameters were evaluated. Operation time, duration of intubation in the intensive care unit (ICU), the length of the intensive care stay, and length of hospitalization were recorded.

Results: Duration of intubation in the ICU in group 1 with a NEWS2 <3 [8.2 (0-18) vs 16.2 (3-39), respectively; p<0.01], ICU length of stay [21.6 (4-27) vs 47.3 (4-113), respectively; p<0.01], length of hospitalization [11.6 (5-56) vs 18.6 (8-67), respectively; p<0.01]. NEWS2 >3 was significantly higher compared to group 2. The SOFA score was significantly higher in group 2 compared with group 1 [1.2±0.5 vs 4.1±1.9; respectively; p<0.01]. In the correlation analysis, the NEWS2 score level was positively correlated with the SOFA score (p<0.001, r=0.81) and hospitalization time (p<0.001, r=0.60) and neutrophil lymphocyte ratio (NLR) (p<0.001, r=0.47).

Conclusion: These findings suggest that the NEWS2 score may be correlated with the length of intensive care intubation, length of intensive care stay, and length of hospitalization. NEWS2 is an effective and simple scoring system that provides information about postoperative outcomes in gynecologic oncology patients scheduled for major surgery.

Keywords: NEWS2 score, gynecologic oncology, major surgery

Öz

Amaç: Jinekolojik maligniteler tüm dünyada kadınlarda mortalite ve morbiditenin önemli nedenleridir. Cerrahi tedavi önemli bir tedavi yöntemi olsa da gerek cerrahinin büyüklüğü, gerek hastaya bağlı faktörler postoperatif süreçleri etkilemektedir. The National Early Warning Score 2 (NEWS2), basit, ucuz ve güvenli bir erken uyarı skorudur. Cerrahi hastalarda kullanımı çok yaygın olmasa da major cerrahi geçirecek hastalarda kullanımı postoperatif süreç hakkında fikir verebilir. Bu çalışmada jinekolojik onkolojik majör cerrahi geçiren hastalarda NEWS2 skorunun önemi ve ilişkisi araştırılmıştır.

Gereç ve Yöntemler: Bu çalışmaya major cerrahi planlanan 44 jinekoloji hastası dahil edilmiştir. Grup 1'e NEWS2 <3 olan hastalar, grup 2'ye ise NEWS-2 >3 olan hastalar kabul edildi. NEWS2, Sequential Organ Failure Assessment (SOFA) and Acute Physiology and Chronic Health Evaluation 2 skoru

PRECIS: We found that in gynecologic oncology patients undergoing major abdominal surgery, the duration of ICU stay, intubation time, and hospitalization were significantly shorter in patients with a NEWS2 <3 than in patients with a NEWS2 >3.

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hesaplandı. Ayrıca ameliyat sonrası rutin klinik ve laboratuvar parametreleri kaydedildi. Operasyon süresi, yoğun bakımda entübasyon süresi, yoğun bakım kalış süresi ve hastane kalış süresi kaydedildi.

Bulgular: NEWS2 <3 olan Grup 1'de yoğun bakımda entübasyon süresi [8,2 (0-18) vs 16,2 (3-39), sırasıyla; p<0,01], yoğun bakımda kalış süresi [21,6 (4-27) vs 47,3 (4-113), sırasıyla; p<0,01], hastane kalış süresi [11,6 (5-56) vs 18,6 (8-67), sırasıyla; p<0,01], NEWS2 >3 olan grup 2 ile karşılaştırıldığında anlamlı olarak daha yüksekti. SOFA Grup 1 ile karşılaştırıldığında anlamlı olarak Grup 2'de daha yüksekti [1,2±0,5 vs 4,1±1,9; sırasıyla; p<0,01]. Spearman korelasyon analizinde, NEWS2, SOFA skoru (p<0,001, r=0,81) ve hastane kalış süresi (p<0,001, r=0,60) ve nötrofil lenfosit oranı (p<0,001, r=0,47) ile pozitif korele idi.

Sonuç: Bu bulgular NEWS2 skorunun yoğun bakım entübasyon süresi, yoğun bakım kalış süresi ve hastane kalış süresi ile korele olabileceğini göstermektedir. Majör cerrahi geçirmesi planlanan jinekolojik onkoloji hastalarında NEWS2 postoperatif sonuçlar hakkında bilgi veren etkin ve basit bir skorlama sistemidir.

Anahtar Kelimeler: NEWS2 skor, jinekolojik onkoloji, majör cerrahi

Introduction

Gynecological cancers are the fourth most common type of cancer among women worldwide. Survival has increased with effective treatment. While there is ongoing interest in developing new treatments, surgical procedures remain the most popular option[1,2]. Complications resulting from major surgeries can impact both morbidity and survival. For this reason, it is crucial to identify these patients beforehand and take preventive measures against potential complications[2].

Early warning scores are typically scoring systems that are calculated based on patient's vital signs. These systems, first described in 1997, are now commonly used to predict unfavorable patient outcomes, such as intensive care unit (ICU) stays[3]. The National Early Warning Score (NEWS), associated with clinical outcomes in surgical patients, including hospital mortality and admission to the ICU, was developed by the Royal College of Physicians[4,5]. In this scoring system, clinical patient data such as respiratory rate, saturation, systolic blood pressure, heart rate, consciousness, and temperature are recorded preoperatively. This system, which does not require a laboratory parameter, is also considered to be very cost effective. Gynecological cancer surgery is a complex procedure associated with several prognostic complications such as prolonged hospitalization and delay in treatment[6].

Materials and Methods

This study was conducted prospectively at the Süleyman Demirel University Faculty of Medicine Hospital. Necessary permissions for the study and ethics committee approval were obtained from the Süleyman Demirel University Ethics Committee (date: 11.02.2023, approval number: 58). Informed consent was obtained from all patients or their relatives. The study included 44 patients scheduled for major abdominal surgery (44 females). In addition to standard preoperative evaluation, the NEWS2 score was calculated on the day of the operation, and patients with a NEWS2 Score of <3 were defined as group 1 (n=19) and patients with a NEWS2 score of >3 as group 2 (n=25) (Table 1). The preoperative hemogram and biochemistry parameters of the patients were recorded (Table 2). Twenty-six patients were operated on for ovarian cancer, 5 for cervical cancer, and 13 for endometrial cancer. All patients were operated under general anesthesia. A combined epidural spinal catheter was applied for postoperative analgesia. Catheterization was not performed in 3 patients because they rejected the procedure. All catheterized patients (n=41) received intrathecal morphine before surgery. Surgical time, complication time, and intubation time were recorded. Postoperative intubation time, ICU length of stay, hospitalization, and complications were recorded for patients whose postoperative follow-up continued in the ICU. The first 24-hour Sequential Organ Failure Assessment (SOFA) and Acute Physiology and Chronic Health Evaluation (APACHE) 2 scores were calculated and recorded (Table 1). Patients with an operation duration of 2 h, no malignancy, and patients who were not admitted to the postoperative ICU were excluded from the study.

Statistical Analysis

Statistical analysis was performed using SPSS software version 26 (SPSS, Chicago, IL). The distribution of continuous variables was tested by the Kolmogorov-Smirnov test. Continuous variables were expressed as mean ± standard deviation or median and 25^{th}-75^{th} percentile values (interquartile range) (normally and not normally distributed, respectively). Categorical variables were expressed as percentages. Statistical differences among groups were tested by Mann-Whitney U. Spearman correlation coefficients were calculated to evaluate the relationships between variables. Thereafter, binary logistic regression analyzes were performed stepwise to identify the possible association of NEWS2 levels as a dependent variable with potential confounding factors. These independent confounders were ICU and hospitalization time, operation time, neutrophil to lymphocyte ratio (NLR), and white blood cell. P value less than 0.05 was considered.

Results

Demographic and clinic data for the groups are presented in Table 1. The mean age of patients was 61±10 years in Group 1, and 65±9 years in Group 2. No significant difference was found between the groups. The presence of diabetes mellitus was similar between the groups (p=0.50). However, the presence of hypertension was significantly higher in group 2 (p=0.02).

The operation time was comparable between the two groups (p= 0.89). At the end of the operation, 20 patients (80%) were transferred to the ICU as intubated in group 1 and 19 patients (100%) were transferred as intubated in group 2 (Table 1). Statistical differences among groups were tested by Mann-Whitney U.

In group 2, the duration of a mechanical ventilator in the ICU [8.2 (0-18) vs 16.2 (3-39) days, respectively, p<0.01], the total duration of the ICU stay [21.6 (4-27) vs 47.3 (4-113) hours, respectively, p<0.01], and the duration of hospitalization [11.6 (5-56) vs 18.6 (8-67) days, respectively, p<0.01] were significantly higher than group 1. Similarly, the SOFA Score (1.2±0.5 vs 4.1±1.9, respectively, p<0.01) was also higher in groups 2. However, the APACHE 2 score was similar between the groups (p=077, Table 1).

The neutrophil count [4.8 (1.8-13.6) vs 7.2 (3.4-20.8) $x10^3$/mL, respectively, p<0.01] and NLR [4.4 (1.4-42.5) vs 5.6 (1.4-20.6), respectively; p<0.01] were significantly higher in group 2. Similarly, the glucose (131±52 vs 177±62 mg/dL, respectively; p<0.01) and the aspartate transaminase level [13 (8-23) vs 38 (8-148) U/L, respectively; p<0.01] were also prominently higher in group 2 than in group 1 (Table 2).

Table III shows the results of binary logistic regression. High values of the NEWS2 score (>3) were significantly associated with the length of stay in the ICU (p=0.042). The final model was found to fit the data adequately (Hoshmer-Lemeshow χ^2 = 14.65, sig = 0.01). In the Spearman correlation analysis, the NEWS2 score was positively correlated with the SOFA score (p<0.001, r=0.81) and the hospitalization time (p<0.001, r=0.60) and NLR (p<0.001, r=0.47) (Table 3).

Table 1. Comparison of demographic and clinical parameters between the groups

NEWS2 score	<3 n=25	>3 n=19	p
Mean age, year	61±10	65±9	0.18
Diabetes Melllitus, n (%)	9 (36)	6 (31)	0.50
Hypertension, n (%)	7 (28)	12 (63)	0.02
Mechanical Ventilator, n (%)	20 (80%)	19 (100%)	0.06
Mechanical Ventilator duration, day	8.2 (0-18)	16.2 (3-39)	**<0.01**
Hospitalized time, day	11.6 (5-56)	18.6 (8-67)	**<0.01**
ICU time, hour	21.6 (4-27)	47.3 (4-113)	**<0.01**
Operation time, hour	515±90	534±120	0.89
SOFA score, n	1.2±0.5	4.1±1.9	**<0.01**
APACHE score, n	14.7±.3.9	15.1±4.4	0.77

APACHE: Acute Physiology and Chronic Health Evaluation, ICU: Intensive Care Unit, NEWS2: The National Early Warning Score 2, SOFA: Sequential Organ Failure Assessment Score

Table 2. Change in laboratory characteristics

NEWS2 score	<3 n=25	>3 n=19	p
Glucose, mg/dL	131±52	177±62	<0.01
Creatinine, mg/dL	0.64 (0.4-1.7)	0.75 (0.3-1.6)	0.09
GFR	95±16	85±24	0.06
Sodium, mg/dL	141±20	135±22	0.18
Potassium, mg/dL	4.5±0.6	4.4±1.1	0.12
Calcium, mg/dL	9.1±0.9	8.6±1.3	0.19
Magnesium, mg/dL	1.9±0.3	1.8±0.2	0.79
AST, U/L	23 (11-50)	58 (12-282)	0.10
ALT, U/L	13 (8-23)	38 (8-148)	**<0.01**
Hemoglobin, g/dL	12.2±1.2	11.8±1.8	0.44
Platelet, $x10^3/mm^3$	293±69	300±90	0.77
Mean Platelet Volume, fl	8.2±0.8	8.5±1.1	0.54
Eosinophyl, $x10^3$/mL	0.11 (0.01-0.60)	0.08 (0.01-0.20)	0.65
Neutrophyl, $x10^3$/mL	4.8 (1.8-13.6)	7.2 (3.4-20.8)	**<0.01**
Lymphocyt, $x10^3$/mL	1.94 (0.20-6.10)	1.52 (0.70-3.10)	0.13
NLR	4.4 (1.4-42.5)	5.6 (1.4-20.6)	**<0.01**
WBC, $x10^3$/mL	7.4±3.3	9.6±4.3	0.04

ALT: Alanine transaminase, AST: Aspartate transaminase, NEWS2: The National Early Warning Score 2, NLR: Neutrophil lymphocyte ratio, WBC: White blood cells

Discussion

In this study, NEWS2 was used to predict postoperative complications in patients with gynecologic oncology who underwent major abdominal surgery. In patients undergoing major abdominal surgery for gynecologic oncology, those with a NEWS Score of <3 had significantly shorter durations of of ICU stay, intubation time, and hospitalization compared to those with a NEWS Score >3. Gynecological oncology cases that undergo major open abdominal surgery should be carefully evaluated in terms of perioperative complications. Factors such as age, type of malignancy, patient weight, and length of hospitalization are associated with increased complications[7]. Enhanced recovery after surgery protocols, which have been actively implemented in our clinic in major gynecologic oncology cases, are a set of recommendations that reduce the length of stay, complications, and cost without increasing readmission or mortality rates in gynecologic oncology surgery[8].

In major abdominal surgeries, early and efficient pain management prevents atelectasis, facilitates early mobilization, and shortens the length of hospitalization[9,10]. In our study, all

Table 3. The results of the binary logistic regression. The dependent variable was the NEWS2 score, independent variables were the ICU time, hospitalization time, operation time, MV time, and NLR. High values of the NEWS2 score (>3) were significantly associated with the length of stay in the ICU

	B	S.E.	Wald	df	Sig.	Exp (B)	Lower	Upper
The ICU time, hour	0.156	0.077	4.142	1	0.042	1.169	1.006	1.358
Hospitalization time, day	0.057	0.053	1.151	1	0.283	1.059	0.954	1.175
Operation time, hour	-0.003	0.005	0.364	1	0.564	0.997	0.987	1.007
MV time, hour	0.086	0.082	1.096	1	0.295	1.090	0.928	1.281
NLR	-0.008	0.070	0.014	1	0.906	0.992	0.864	1.138
Constant	-1.758	2.526	0.485	1	0.486	0.172		

ICU: Intensive care unit, MV: Mechanical ventilation, NLR: Neutrophil lymphocyte ratio

eligible patients received a combined epidural catheter during the pre-operative period; intrathecal morphine was administered during the pre-operative period; and analgesia was provided with an epidural catheter during the follow-up. There was no need to administer parenteral rescue analgesics during or after the intensive care period. The severity of surgery is associated with increased major postoperative complications after gynecologic procedures[7,11]. There are a limited number of studies with scoring systems for the early detection of patients with high probability of complications. In studies involving gynecologic oncology patients, 24% of all unplanned readmissions were attributed to uncontrolled symptoms or minor complications that were potentially preventable[11,12]. The identification of these patients with simple scoring systems may also prevent complications such as rehospitalization. It is evident that new studies are needed in this regard. Early warning scoring systems are scoring systems calculated on the vital signs of patients and are widely used to identify patients who are ill or whose condition is deteriorating, including those admitted to surgical areas of hospitals[13]. NEWS2 has been shown to be very useful for predicting conditions such as ICU admission, death, and cardiopulmonary resuscitation. There have been very limited studies in patients before or after surgery. Although NEWS2 is a score developed for general hospitalization, it is beneficial for scoring for surgery[14]. There are very limited studies predicting patient outcomes in gynecologic oncology patients undergoing major surgery. The Surgical Apgar score, APACHE 2, and the surgical complexity score have been used and recommended for this purpose. The Surgical Apgar Score predicts postoperative complications in patients undergoing cytoreductive surgical procedures for stage III and IV ovarian cancer. It is an easy-to-apply scoring system as simple parameters are used, similar to our study[15]. A surgical complexity score is a scoring system produced for this purpose, similar to the surgical Apgar score. It can be predicted and quality of care can be improved with this score calculated[16].

High APACHE 2 scores were associated with mortality in gynecologic oncology patients[17]. In contrast to this study, we did not find a significant association between the APACHE 2 score and mortality.

The SOFA score is a scoring system that indicates the severity of the disease in the ICU. It is frequently used to indicate mortality, especially in sepsis patients. This scoring system, which evaluated six different systems, is difficult to implement outside the ICU due to the necessity of blood collection from the patient and its cost. In one study, it was used to predict mortality in cardiovascular surgery patients[18]. In our study, the SOFA score correlated with NEWS2 and with the duration of intensive care and hospitalization. Multiple blood draws and laboratory dependency limit its use, especially in surgical patients. We found no use for major gynecologic oncology surgery in the literature.

Concluison

We suggest that the NEWS scoring system, which is more commonly used in nonsurgical patients, can predict postoperative patient parameters in gynecologic oncology patients undergoing major abdominal surgery, and further studies are warranted in this regard.

Ethics

Ethics Committee Approval: This study was conducted prospectively at the Süleyman Demirel University Faculty of Medicine Hospital. Necessary permissions for the study and ethics committee approval were obtained from the local ethics committee (date: 11.02.2023, approval number: 58).

Informed Consent: Informed consent was obtained from all patients or their relatives.

Peer-review: Internally and externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: P.K., İ.T., E.E., Concept: P.K., A.B., B.G.C., Design: P.K., E.E., B.G.C., Data Collection or Processing: P.K., A.B., İ.T., Analysis or Interpretation: P.K., B.G.C., Literature Search: P.K., A.B., İ.T., Writing: P.K., A.B., B.G.C.

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When a caesarean section is necessary: Analysis of cesarean sections performed in the Republic of Turkey in 2022 in accordance with the World Health Organization Multi-Country Research Guidelines

Ne zaman sezaryen: Türkiye Cumhuriyeti'nde 2022 yılında uygulanan sezaryen operasyonlarının Dünya Sağlık Örgütü Çok-Ülkeli Araştırma Rehberi doğrultusunda incelenmesi

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Abstract

Objective: The aim of our study, in light of the World Health Organization Multi-Country Survey (WHO-MCS) data examining the data of the Ministry of Health for the year 2022, comparing the cesarean sections (C/S) performed in the Republic of Turkey (TR) with the WHO-MCS data, and comparing the number of cesarean sections applied more than the reference values.

Materials and Methods: According to the database of the Turkish Ministry of Health, in 2022, 1166175 deliveries took place in the Republic of Turkey, and 706370 (60.5%) cesarean section deliveries were recorded as 365764 (51%) primary C/S. Using the Ministry of Health registration system based on the Robson classification.

Results: The number and rate of C/S operations performed per birth in 2022 in TR (n=706370; 60.50%) were found to be significantly higher when compared to the number and rate of C/S on a global scale (n=246062; 21.10%), (p<0.001). When cesarean section operations performed in the Ministry of Health hospitals, private institutions, foundation universities, public universities and other public unit hospitals were compared with WHO MCS reference values and C/S ratios, 44.2% versus 24.7% (p=0.05), versus 77.4%, versus 34.2% (p<0.001), 74.3% versus 29.5% (p<0.001), 75% versus 35.8% (p<0.001), 69.3% versus 35.9% (p<0.001).

Conclusion: The amount of cesarean sections performed according to the total number of births in the Turkish Republic is relatively high and its cost nearly 1 billion 750 million TL.

Keywords: Republic of Turkey, cesarean section, normal birth, the year 2022, World Health Organization Multi-Country Survey (WHO-MCS) data guide

Öz

Amaç: Çalışmamızın amacı, Dünya Sağlık Örgütü Çok Ülkeli Araştırma (WHO-MCS) verileri ışığında, Sağlık Bakanlığı'nın 2022 yılı verilerini inceleyerek, Türkiye Cumhuriyeti'nde (TR) yapılan sezaryenleri (C/S) WHO-MCS verileri ile karşılaştırmak ve referans değerlerden daha fazla uygulanan sezaryen sayısını karşılaştırmaktır.

Gereç ve Yöntemler: T.C. Sağlık Bakanlığı veri tabanına göre, 2022 yılında Türkiye Cumhuriyeti'nde 1166175 doğum gerçekleşmiş ve 706370 (%60,5) sezaryen doğum 365764 (%51) primer C/S olarak kaydedilmiştir. Robson sınıflandırmasına dayalı Sağlık Bakanlığı kayıt sistemi kullanılmıştır.

Bulgular: TR'de 2022 yılında doğum başına gerçekleştirilen sezaryen operasyon sayısı ve oranı (n=706370; %60,50), küresel ölçekteki sezaryen operasyon sayısı ve oranı (n=246062; %21,10) ile karşılaştırıldığında anlamlı derecede yüksek bulunmuştur (p<0,001). Sağlık Bakanlığı hastaneleri, özel kurumlar,

PRECIS: In this article, caesarean section rates in Turkey are compared with international Robson standards. Accordingly, statistical tests of the rates in Turkey were performed. According to these results, some inferences have been made in the light of the striking statistics.

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vakıf üniversiteleri, devlet üniversiteleri ve diğer kamu birim hastanelerinde gerçekleştirilen sezaryen operasyonları DSÖ MCS referans değerleri ve C/S oranları ile karşılaştırıldığında, %44,2'ye karşı %24,7 (p=0,05), %77,4'e karşı %34,2 (p<0,001), %74,3'e karşı %29,5 (p<0,001), %75'e karşı %35,8 (p<0,001), %69,3'e karşı %35,9 (p<0,001).

Sonuç: Türkiye Cumhuriyeti'nde toplam doğum sayısına göre yapılan sezaryen miktarı nispeten yüksektir ve maliyeti yaklaşık 1 milyar 750 milyon TL'dir.

Anahtar Kelimeler: Türkiye Cumhuriyeti, sezaryen, normal doğum, 2022 yılı, Dünya Sağlık Örgütü Çok Ülkeli Araştırma (WHO-MCS) veri kılavuzu

Introduction

In recent years, there has been a significant increase in cesarean section delivery (C/S) practices in many countries worldwide. Not applying cesarean delivery when necessary or applying it unnecessarily brings with it many problems[1]. The Robson Classification System is a universal reference for evaluating and tracking cesarean section rates in healthcare facilities. This reference system is accepted by the International Federation of Gynecology and Obstetrics and the European Board of Obstetrics and Gynecology. The World Health Organization has developed a global reference for C/S ratios from the Multi-Country Survey (WHO-MCS) C-Model[1-3]. In this classification system, a 50% C/S ratio is accepted as the threshold value, and Robson groups are formed by maternal obstetric clinical evaluation. The Robson classification is a perinatal classification that covers all delivery methods consisting of 10 subgroups[4]. The advantages of the Robson test are that it is reproducible, simple, clearly articulated, and prospective.

Cesarean delivery has many risk factors in terms of anesthesia and gynecology compared with vaginal delivery[5]. Complications that may develop due to cesarean delivery and anesthesia may cause severe consequences for the mother and the baby[6-8]. The economic cost of a standard C/S operation exponentially creates a considerable burden on the country's economy in the event of an unexpected complication.

Our study, using the Turkish Ministry of Health data for the year 2022, is planned to examine the C/S application according to the months during the year, the provinces throughout the country, the Robson classification, and between hospitals. We aim to detect off-label cesarean section operations in our country. The aim of calculating the economic cost of off-label C/S operations is to show the negative effects it creates on the effective functioning of health services in seizure conditions and the workload of the anesthesiology department doctors.

Materials and Methods

The Turkish Ministry of Health has started to record birth analysis in the country with the registration system established in 2012. In line with family planning and demographic analysis, an electronic registration system was initiated in 2014. All health units and institutions providing obstetrics and gynecology services upload patient data to the automation system with an electronic signature. These data, including obstetric evaluation, Robson classification, and birth information, are then transferred to the automation system of the Ministry of Health. The Robson-10 group classification comprises 10 evidence-based, comprehensive, mutually exclusive subgroups. The obstetric evaluation criteria used were parity, gestational age, previous cesarean section, fetal presentationlabor onset, and the number of fetuses. This study was initiated after the necessary approvals were obtained with the decision of the Ministry of Health dated 05.29.2023 with the numbers E-76244415-000-216532095. According to the Turkish Ministry of Health database, 1166175 deliveries occurred in Turkey in 2022, and 706370 (60.5%) cesarean deliveries were examined. Hospitals where cesarean delivery was performed were recorded as Ministry of Health hospitals, university hospitals, foundation university hospitals, private hospitals, and other health-related public institutions.

Statistical Analysis

The inspected and recorded data in the study were analyzed using the IBM SPSS 20.0 (Chicago, IL, USA) statistical program. Data are presented as n (number) and percentage (%). The chi-square test was used to compare two ratios. P<0.05 were considered statistically significant.

Results

When the data were analyzed, 1166175 births were recorded nationwide in 2022. The rate of cesarean section performed in labor was 60.5% (n=706370). 2022 Turkey total Caesarean section numberdistribution rates in Ministry of Health, private institutions, foundation universities, state universities and other public unit hospitals are rates 36.69%, 54.89%, 1.91%, 6.23% and 0.28%, (total 100%) respectively (Figure 1). The number and rate of C/S operations per birth in the Republic of Turkey (n=706370; 60.50%) were found to be significantly higher when compared to the global number and rate of C/S (n=246062; 21.10%) (p<0.001).

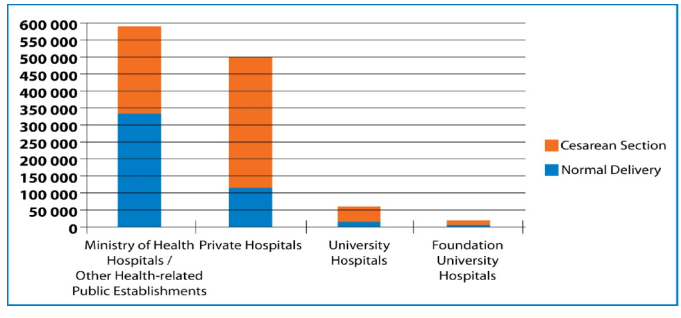

Figure 1. Distribution of births in 2022 across Turkey by institutions

On a global scale, when C/S ratios calculated with reference values are compared between hospitals, cesarean delivery was mainly performed in private hospitals; C/S delivery rates performed in private hospitals in Turkey were statistically significantly higher than reference values (77.4% versus 34.2%; p<0.001). Similarly, in university hospitals (75% versus 35.8%; p<0.001), foundation university hospitals (74.3% versus 29.5%; p<0.001), Ministry of Health hospitals (44.2% versus statistical results were found to be significantly higher in 24.7%; p=0.05) and other public units (69.3% versus 35.9%; p<0.001) (Figure 2).

A statistically significant difference was observed when non-reference cesarean delivery rates were compared between hospitals (p=0.001) (Table 1). The rate of non-reference cesarean section was statistically the least determined in the hospitals of the Ministry of Health (19.5%). There was no statistically significant difference in non-reference cesarean section rates between private hospitals, state universities, and foundation university hospitals (p=0.750).

When labor rates were analyzed between Robson subgroups and hospitals, the highest rates of labor in Robson-2, 3, 4, 5, and 10 groups were in the Ministry of Health hospitals, respectively, with a rate of 48.9%, 72.4%, 60.2%, 47.3%, and 50.8%. In private hospitalslabor occurred at rates of 55.7%, 70.9%, 60.2%, 50.7%, and 67.2% in Robson-1, 6, 7, 8, and 9 groups, respectively. Table 2 shows the values of total births by hospitals according to Robson classes.

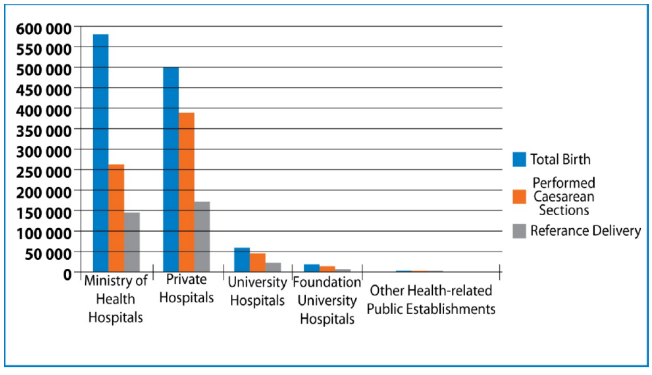

Figure 2. Total, cesarean and reference cesarean section numbers by hospitals

C/S ratios performed in Robson groups compared with reference values (Table 3), Group 6 (99.8% versus 97.6%, p=0.155), Group 7 (98.4% versus 95.5%, p=0.254), respectively and Group 9 (98.2% vs. 95.7%, p=0.407), there was no statistically significant difference. When reference values and actual C/S ratios in other groups are compared, respectively, Group 1 (9.4% versus 59.6%; p<0.001), Group 2 (31.3% versus 54.3%; p=0.001), Group 3 (1.6% versus 15.8%; p<0.001), Group 4 (11.1% versus 22.3%; p=0.036), Group 5 (61.2% versus 98.4%; p<0.001), Group 8 (54.0 versus 94.4%; p<0.001) and there was a statistically significant difference in Group 10 (29.7% versus 68.8%; p<0.001).

When cesarean deliveries were analyzed by months in 2022, C/S deliveries were significantly higher in each month compared to reference values (p<0.001) (Table 4). There was no statistically significant difference between the months.

When the data were analyzed among the provinces, the three provinces with the highest number of C/S births were Karabük, Kırklareli, and Zonguldak. The C/S ratios of these provinces against reference values were statistically significantly higher (p<0.05) (Table 5). Şırnak, Ardahan, and Kilis were determined as the three provinces where the cesarean section was the least. There was no statistically significant difference between the C/S ratios of these provinces against reference values (p=0.067, p=0.115, p=0.098) (Table 5). When the first three big cities of Turkey (Ankara, Istanbul, Izmir) were examined, the cesarean section rates were statistically higher than the reference values (p<0.05) (Table 5).

Discussion

Worldwide, between 1990 and 2018, C/S applications increased by 19%. This rate has increased by more than 50% in TR. Although the latest data show that C/S implementation is 21% worldwide, this rate is predicted to approach 30% between 2021 and 2030[9]. According to the Organization for Economic Co-operation and Development (OECD) 2020 data, TR has the highest rate after Mexico (58%), with a C/S rate of 57% (573/1000 live births)[10]. In our study, data for 2022 show that C/S application is 60.5% in TR. In the study of Molina et al.[11], the optimum C/S ratio was reported as 19% in terms of maternal and neonatal mortality, and the WHO

Table 1. Non-reference cesarean section rates between hospitals

	Ministry of Health Hospitals/Other Health-related Public Establishments	Private Hospitals	Public University Hospitals	Foundation University Hospitals	p-value
Total Birth	588600	500800	58697	18230	
Performed Caesarean Sections	261200	387600	44034	13441	
Referance Delivery (%)	115200 (19.5%)	216277 (43.1%)	23133 (39.4%)	8089 (44.3%)	**0.001**

recommendation is 10-15% in some countries where perinatal mortality is below 10%. The results of our study clearly show that cesarean section rates in TR were found to be remarkably higher than the reference values.

C/S indication is an approach that needs special attention to prevent maternal and perinatal mortality[4]. In a 2015 study covering 169 countries, it was determined that approximately 29.7 million pregnant women had cesarean sections. This shows that cesarean delivery has increased exponentially in the last 20 years[4]. Indications for cesarean section include maternal pelvic deformity, eclampsia and HELLP syndrome, fetal stress, cord prolapse, placenta previa, uterine rupture, previous cesarean delivery history, prolonged delivery, fetal presentation, and major antepartum hemorrhage[3-13]. Studies in the literature also show that cesarean section operations performed within indications are lifesaving. Surgical complications of cesarean delivery include; postpartum infection (surgical area), hemorrhage and blood product transfusion, hysterectomy, prolongation of hospital and intensive care unit length of stay, maternal mortality, neonatal respiratory complications, and fetal mortality can be listed[14-16]. Long-term complications such as abnormal adherent placenta, uterine rupture, and adhesions may also be seen[17]. In Canada, 308755 C/S applications were examined, and it was stated that although the risk of uterine rupture is higher in vaginal delivery, maternal mortality may increase with C/S application[18]. In C/S, it was stated that the applications performed within the indication can reduce maternal mortality and morbidity by 1% to 5%[19]. In the study conducted in Ireland, cesarean section and vaginal delivery were compared; although the number of maternal mortality was higher in cesarean section, no statistically significant difference was found[20]. Inference from these studies shows that maternal mortality in cesarean delivery can be associated with nonsurgical practices. Cesarean section delivery also brings with it complications of anaesthesiaanesthesia Among the complications of anesthesia application; are failed intubation, failed regional anesthesia, high-level anesthesia, headache, chemical meningitis, epidural hematoma, and extradural abscess[21]. C/S indications under general anesthesia are hematological neurological, infectious, congestive heart failure, severe preeclampsia, local anesthesia allergy, spinal cord arteriovenous malformation, placenta areata and fetal factors[22]. In the study of Bloom et al.[21], in which 37142 cesarean deliveries were examined, neonatal complications were compared with the type of anesthesia applied, and low Apgar score and umbilical artery pH values were found. These complications were

Table 3. Comparison of actual and reference cesarean section rates between Robson groups

Robson	Actual Cesarean Rate (%)	Cesarean Rate Calculated with Reference Values (%)	p-value
1	59.67%	9.43%	**<0.001**
2	54.37%	31.34%	0.001
3	15.84%	1.69%	**<0.001**
4	22.33%	11.12%	0.036
5	98.48%	61.20%	**<0.001**
6	97.61%	99.84%	0.155
7	95.54%	98.48%	0.254
8	94.47%	54.04%	**<0.001**
9	95.72%	98.23%	0.407
10	68.84%	29.72%	**<0.001**

Table 2. Values of total births by hospitals according to Robson classes

	Institutions				
Robson	Private	Foundation University	Ministry of health and other public institutions	University	Number of births, n/%
1	**55.7%**	2.2%	38.2%	3.9%	299600/25.7%
2	43.3%	3.1%	**48.9%**	4.7%	35800/3.1%
3	23.4%	0.8%	**72.4%**	3.4%	**302948/26.0%**
4	35.7%	1.7%	**60.2%**	2.4%	31051/2.7%
5	45.5%	1.5%	**47.3%**	5.7%	269300/23.1%
6	**70.9%**	1.8%	22.2%	5.1%	31049/2.6%
7	**60.2%**	0.9%	31.5%	7.4%	28241/2.4%
8	**50.7%**	1.9%	36.4%	11%	38439/3.3%
9	**67.2%**	1.6%	27.1%	4.1%	18447/1.6%
10	38.7%	1.4%	**50.8%**	9.1%	111300/9.5%
Total Birth Number					1166175

primarily associated with cesarean indication, gestational age, and emergency cesarean section. One maternal death recorded in the study was directly related to anaesthesiaanesthesia In C/S, more studies are needed on intensive care and prolonged hospitalizations due to anaesthesia/surgical application. These studies show that especially off-label cesarean section practices pose severe risks in terms of fetal and maternal aspects. As seen in our study, off-label cesarean section rates were high in TR. We think that more stringent measures should be taken in this regard. More studies are needed on the complications of off-label C/S operations.

C/S application is applied in line with clinical and nonclinical evaluations[23]. Studies show that nonclinical factors play an essential role in the decision of off-label C/S operations[23]. These factors include sociocultural situations, economic factors, the health systemmalpractise and fear of professional lawsuits caused by complications[24]. In particular, obstetricianshaving to deal with lawsuits and forensic investigations is a critically important etiology[3]. The WHO 2020 reports made recommendations to prevent C/S application with nonclinical indications[25]. This recommendation and the points to be considered are the dissemination of vaginal birth training, effective application of relaxation techniques such as deep breathing under the control of midwives and nurses, including couples in a psychosocial program, and psychological rehabilitation of pregnant women against the fear of pain[23]. We think that there is a need for detailed studies in TR on these issues as well.

In our study, dystocia with cephalic presentation may increase the risk of cesarean delivery, especially in groups 1 and 2 with nulliparity in Robson Group 1-2-3-4-5-8-10, which exceeded the reference values. In Robson Group 6-7-9, the actual C/S action was calculated below the reference values. Robson Group-1 represents the least risky pregnant women, and the hospitals most applied to are private hospitals, with a rate of 55.7%. In 2022, C/S was applied to 387600 pregnant women out of 500800 applications for labor in private hospitals. Sociocultural factors and psychosocial conditions of pregnant individuals may have provided this orientation. In Robson Group 10 consisting of preterm actions, 50.8% of the pregnant women applied to the hospitals of the Ministry of Health. In addition, clinical evaluations and classifications should not put psychosocial factors into the background[26,27]. According to the results of our study, the rate of cesarean section is very high in the Robson group 1 and 2 pregnant groups, which is the most preventable cesarean section group, compared to the reference values throughout the country, and we think that private hospitals serving in Turkey should be informed and investigated on this issue.

C/S operations constitute a significant part of surgical operations performed under emergency conditions. According to the 2022 TR Ministry of Health data, 209,623 (41%) of 502,692 out-of-hours/emergency operation reports were recorded as emergency

Table 4. Comparison of cesarean rates according to months with reference cesarean rates

Time (month)	Actual Cesarean Rate (%)	Cesarean Rate Calculated with Reference Values (%)	p-value
2022-01	59.92%	29.59%	<0.001
2022-02	60.17%	29.80%	<0.001
2022-03	60.31%	29.75%	<0.001
2022-04	60.55%	29.34%	<0.001
2022-05	60.58%	29.51%	<0.001
2022-06	61.79%	29.84%	<0.001
2022-07	60.07%	29.14%	<0.001
2022-08	60.31%	29.42%	<0.001
2022-09	60.67%	29.49%	<0.001
2022-10	60.11%	29.01%	<0.001
2022-11	61.30%	29.55%	<0.001
2022-12	61.04%	29.21%	<0.001

Table 5. Comparison of the rates of cesarean delivery according to the reference value, with the highest / lowest rates and three big cities

City	Provincial ranking in Turkey according to the cesarean section rate	Actual Cesarean Rate	Cesarean Rate Calculated with Reference Values	p-values
KARABÜK	1	82.69%	32.71%	**<0.001**
KIRKLARELİ	2	79.93%	31.95%	**<0.001**
ZONGULDAK	3	79.84%	33.34%	**<0.001**
İZMİR	20	68.08%	30.53%	**<0.001**
İSTANBUL	34	63.10%	27.65%	**<0.001**
ANKARA	40	61.65%	27.45%	**<0.001**
ŞIRNAK	79	37.33%	25.22%	0.067
ARDAHAN	80	33.13%	22.61%	0.115
KİLİS	81	28.61%	19.40%	0.098

SCs. In the Health Implementation Communiqué (SUT) decree, the cost of SC operations in 2022 is stated as 3,692 Turkish liras per birth[28]. In our study, while the World Health Organization reference value was n=246062 (21.1%) in 2022, n=460308 (difference 39.4%) cesarean delivery difference was calculated in the TR. When the cost is calculated, 1 billion 750 million Turkish Liras burdens the country's economy due to preventable cesarean section practices. The current assessment was performed without considering the complications and additional costs incurred. The report prepared by WHO emphasized that off-label C/S applications should be considered, especially in middle and low-income regions, in terms of consumption of country resources[29]. Cesarean section operation is performed by an efficient team of anesthesiology and reanimation and gynecology and obstetrics units. It should not be forgotten that the process directly concerns many units and allied health teams within the health institution. Moreover, C/S applied off-label negatively affects the working motivation of the anesthesia and surgical teams.

Study Limitations

The limitations of our study were that our data were related to system logs. The electronic recording system and the data transfer process cannot ignore possible missing records. This study did not have data on maternal and neonatal short- and long-term complications. The type of anesthesia applied in C/S was not recorded. Robson grouping was not performed in the C/Ss that were made with the decision of emergency operation.

Conclusion

Statistical studies show that the C/S ratio will approach 30% worldwide in 2030. In 2022, this rate was 60.5% in TR. If preventable C/Ss were implemented, 1 billion 750 million Turkish liras could only be brought into the country's economy in 2022.

The Robson classification in C/S application is the accepted reference guide today. The fact that nonclinical factors do not constitute an indication for C/S operation is an issue that requires effort. Off-label C/S adversely affects the motivation of anesthesia and obstetrics units and makes the mother and newborn vulnerable to many complications.

Ethics

Ethics Committee Approval: Not necessary.
Informed Consent: Not necessary.
Peer-review: Internally peer-reviewed.

Authorship Contributions

Design: Ş.B., Ü.M.P., Data Collection or Processing: Ş.B., Ü.M.P., Analysis or Interpretation: Ş.B., Ü.M.P., Literature Search: Ş.B., Ü.M.P., Writing: Ş.B., Ü.M.P.

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Cesarean section rates in Turkey 2018-2023: Overview of national data by using Robson ten group classification system

Türkiye'de sezaryen oranları 2018-2023; Robson on grup sınıflandırma sistemini kullanarak ulusal verilere genel bakış

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Abstract

Objective: Cesarean section (CS) rates continue to rise globally because of various factors. Medically unnecessary cesarean operations have no benefit to the mother or child's health. Since the World Health Organization (WHO) has determined that the acceptable CS rate should not be more than 10-15%, it also stated the use of a classification system to compare different patient groups and facilities. Turkey has the highest CS rates globally and has been rising over the years. This study aims to assess CS rates between 2018 and 2023 using National Health Data and to analyze them according to the Robson classification system and WHO reference values to discuss possible measures against increasing rates.

Materials and Methods: In this study, we assessed the rates of CSs between 2018 and 2023; the CS rate including all 5-years and analysis of CS rates for each Robson group as advocated by WHO. Also, another assessment was done of the facilities where the CSs were applied (Public, private, or university hospitals).

Results: The total number of births recorded between 2018 and2023 was 6.161.976. The overall CS rate was 57.55%. The number of total CS operations was 3.546.049. The primary CS rate was 28.83% (N:1.776.503). Significant differences were observed between the public and private centers for each Robson group.

Conclusion: The CS rates of Groups 1-4 are obviously higher than expected. The CSs of these groups cumulatively affect the rates of other groups. Nulliparous women have CSs mostly in private hospitals. There is a need for improvements in the health system in this regard for better maternal and child health.

Keywords: Turkey, cesarean section rate, delivery, Robson, WHO

Öz

Amaç: Sezaryen oranları, çeşitli faktörler nedeniyle tüm dünyada artmaya devam etmektedir. Tıbbi açıdan gerekli olmayan sezaryen operasyonlarının anne ve yenidoğan sağlığına anlamlı bir faydası olmadığı bilinmektedir. Dünya Sağlık Örgütü (DSÖ) kabul edilebilir sezaryen oranı oranının %10-15'i geçmemesi gerektiğini belirlediğinden, farklı hasta gruplarını ve sağlık tesislerini karşılaştırmak için bir sınıflandırma sisteminin kullanılması gerektiğini de vurgulamıştır. Türkiye, dünya çapında en yüksek sezaryen oranlarına sahiptir ve bu oranlar yıllar içinde artmaya devam etmektedir. Bu çalışma, Ulusal Sağlık verilerini kullanarak 2018-2023 yılları arasındaki sezaryen oranlarını değerlendirmeyi ve artan oranlara karşı olası önlemleri tartışmak için Robson sınıflandırma sistemi ve DSÖ referans değerlerine göre analiz etmeyi amaçlamaktadır.

PRECIS: Total and primary CS rates between 2018-2023 were 57.55, 28.83% respectively and Robson Groups 1-4 groups constituted 58% of cesarean sections.

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Gereç ve Yöntemler: Bu çalışmada 2018 ile 2023 yılları arasındaki sezaryen oranlarını değerlendirdik; 5 yılın tamamını içeren sezaryen oranı ve DSÖ tarafından önerilmiş olan On Gruplu Robson sınıflama sistemi kullanılarak her bir grup için sezaryen oranlarının analizi ve ayrıca, sezaryenlerin uygulandığı tesislerin (Kamu, özel veya üniversite hastaneleri) oranlarının da değerlendirmesi ve analizi yapılmıştır.

Bulgular: 2018-2023 yılları arasında kaydedilen toplam doğum sayısı 6.161.976 olarak gerçekleşmiştir. Genel olarak sezaryen oranı %57,55 idi. Toplam sezaryen operasyon sayısı 3.546.049 olarak gerçekleşmiştir. Primer sezaryen oranı %28,83 (N:1,776,503) idi. Her bir Robson grubu için kamu ve özel merkezler arasında önemli farklılıklar olduğu ve özel hastanelerde sezaryen oranlarının kamu hastanelerinden belirgin olarak yüksek olduğu gözlemlendi.

Sonuç: Grup 1-4'ün sezaryen oranları açıkça beklenenden daha yüksektir. Bu grupların sezaryen olmas kümülatif olarak diğer grupların oranlarını da belirgin olarak etkilemektedir. Nullipar kadınların sezaryenleri çoğunlukla özel hastanelerde ogerçekleşmektedir. Anne ve çocuk sağlığının geliştirilmesi için sağlık sisteminde bu konuda iyileştirmelere ihtiyaç vardır.

Anahtar Kelimeler: Türkiye, sezaryen oranı, doğum, Robson, DSÖ

Introduction

The cesarean section (CS) is an operative mode of delivery that can be lifesaving for the mother and fetus under certain circumstances. However, as it is an operation itself; contains various risks and possible complications that may be encountered. CS rates continue to rise globally, especially in middle- and high-income countries during the last few decades[1]. This increase has been attributed to various factors that may vary across most countries[1-3]. When medically necessary, CS effectively prevents perinatal mortality and morbidity. However, in cases where CS is not necessary, there is no evidence showing the benefit of CS for the mother or child.

In recent years, governments and clinicians have expressed concerns about the increasing number of cesarean deliveries and the potential negative consequences of CS for maternal and child health[4]. In addition, the cost is an important factor for equitable access to resources in improving maternal and newborn health, and cesarean deliveries pose a serious financial burden, especially on overburdened and often weak health systems, as they require more expenditure.

Since the World Health Organization (WHO) has determined the acceptable CS rate should not be more than 10-15% there has been a necessity for a classification system of cesareans to analyze and make proper comparisons between countries or even different hospitals or healthcare systems[5]. The most challenging part in defining the optimum cesarean rate is the lack of a reliable and internationally accepted classification system to produce standardized data in providing a tool that can be used to compare populations at any level and to investigate the increasing trend of cesarean rates. In 2001, the system proposed by Robson[6] stratified women according to their obstetric characteristics so that comparisons can be made with fewer confounding factors. This system classifies women independently into ten different groups based on five basic birth characteristics. This classification is simple, reliable, reproducible, clinically significant, and prospective in every woman presenting for delivery. Allows the comparison and analysis of CS rates within and between groups[7]. To control and provide acceptable rates for CS globally, the WHO made a statement regarding the use of the Robson classification as a global reporting tool for CS rates[7,8]. Moreover, in 2013, a cross-sectional study; "The WHO Multicountry Survey on Maternal and Newborn Health" was published, implementing data from 29 countries to settle reference values for CS rates that are globally applicable. Based on WHO MCS, another study was conducted to create mathematical modeling for reference value generation for the health facilities of each country[9,10].

Unfortunately, Turkey has the highest CS rates globally and has been rising over the years since the last decade[11]. This study aims to assess CS rates between 2018 and 2023 using National Health Data and to analyze them according to the Robson classification system and WHO reference values to discuss possible measures against increasing rates.

Materials and Methods

The Ministry of Health in Turkey has been analyzing CS rates since 2014 through the electronic registration system. The system takes information from the hospitals' electronic automation system, which includes data on obstetric outcomes, Robson classification, and birth certificates from the state (public), private, and university hospitals all over Turkey. Robson's woman-based totally inclusive and mutually exclusive 10-group classification employs simple clinical obstetrical parameters (parity, previous CS, gestational age, the onset of labor, fetal presentation, and number of fetuses). In the present study, we assessed the rates of CS delivery for each year between 2018 and 2023; the CS rate including all 5-years, and analysis of CS rates for each Robson group as advocated by WHO. We aimed to evaluate the contribution of each Robson group to the CS rate and calculate how much deviation there is from the global reference values prescribed by the WHO. Also, another assessment was done of the facilities where the CSs were applied (public, private, or university hospitals); therefore, it would be important to reveal the contribution of different kinds of stakeholders and to shed light on solution suggestions for reducing the CS rates. This cohort has been undertaken by the Ministry of Health through a specific circular. Data collection permission was granted from the Ministry of Health.

Statistical Analysis

We performed the statistical analysis with the Statistical Package for Social Sciences (SPSS 26.0 IBM SPSS Inc., Chicago, IL) program. Demographic data are presented as numbers with percentages (%). To determine the statistical difference between

categorical data (Robson group cesarean rates among facilities), we used the Pearson chi-square test and presented the crude odds ratio. Risk ratio/relative risk was also calculated for a more detailed presentation. Statistical significance of the p-value accepted as $p<0.05$ at 95% confidence interval.

Results

The total number of births recorded between May 2018 and June 2023 was 6.161.976. The overall CS rate between 2018 and June 2023 was 57.55 %. The number of total CS operations performed was 3.546.049. The primary CS rate was 28.83%, which means that 1.776.503 primary CSs were performed during this period. According to the WHO MCS population reference calculation, the number of CSs and primary CS operations were expected as 1.833.116 and 754.039 respectively[10]. However, in our country, the number of applied CSs was approximately twice the value.

Robson Classification of the CSs

The classification of the CS numbers according to the Robson Classification System is shown in Table 1. Robson groups 1,2,3 and 4 comprised 58.4% of all CSs. Another analysis of the CSs shows the rates disaggregated according to the health facilities (public, university, and private hospitals) where CSs are performed (Table 2). Also, the risk ratios and odds ratios for having a CS based on the health facility are detailed in Table 2. This analysis revealed the risk of having CS in a group of patients with similar characteristics when applied to a different kind of health facility. Table 3 shows pairwise comparisons of centers for each Robson group in terms of CS rates (Public vs. University, public vs private, and private vs university). When the centers were compared with each other, significant differences were observed in each Robson group, especially between the public and private centers.

When Robson group 1 was examined from this viewpoint, it was seen that the CS rate was significantly higher than the reference values according to WHO. The total risk ratio was calculated as 5.80. Among this group, admission to a private hospital was associated with significantly higher cesarean rates. Examination of Robson group 2 revealed that the CS rate was found to be higher compared to the reference values of WHO. In this group, the highest risk rate for CS was found in university hospitals. It was observed that the total risk ratio was 8.18. The highest risk of having CS was found to be 33.43 in private hospitals. In university-based hospitals, the risk ratio is 23.23; and the odds ratio was calculated as 33.15.

In the Robson 4 group, while public hospitals showed compatible results with the reference values, a 6.34 risk ratio was calculated in university hospitals (odds ratio: 7.27). In the Robson 5 group, the CS rate was again significantly higher than the reference values. 97.9% of the women who had a previous CS had a repeat CS operation.

CS rates in the Robson 6,7,8,9 groups were consistent with the expected reference values. Women with a breach presentation (Groups 6 and 7); underwent CS with percentages of 97.6% and 95.2%, respectively. CS rates of Robson group 10 in all three kinds of health facilities were also significantly higher than the WHO reference values.

Table 3 shows pairwise comparisons of centers for each Robson group in terms of CS rates (Public vs University, public vs private, and private vs university). When the centers were compared with each other, significant differences were

Table 1. Number of cesarean (CS) deliveries according to Robson Classification

Robson Group		Number of Cesarean Sections	WHO MCS*	Total Number of Deliveries
1	Nulliparous, single, cephalic, 37 weeks, spontaneous labor	823.300	143.500	1.500.000
2	Nulliparous, single, cephalic, 37 weeks, induction, or CS before labor	107.600	68.500	217.000
3	Multiparous, (exclude previous CS), single, cephalic 25 032/224 300 Public 32.5 Public 6.9 2.8 37-week spontaneous labor	244.700	29.900	1.700.000
4	Multiparous, (exclude previous CS), single, cephalic, 24 720/67 088 Public 7.9 Public 26.3 2.8 37 weeks, induction or CS before labor	37.000	23.400	200.900
5	Previous CS single cephalic 37 weeks	1.400.000	867.300	1.400.000
6	All nulliparous breeches	151.200	154.700	154.900
7	All multiparous breeches (including previous CS)	146.600	151.300	153.800
8	All multiple pregnancies (including previous CS)	183.900	109.500	199.400
9	All abnormal lies ((including previous CS)	96.900	98.500	100.400
10	All single, cephalic, <36 weeks (including previous CS)	353.500	159.200	534.100
	Total	3.546049	1.833.116	6.161.976

* Global reference for CS rates from WHO Multi-Country Survey (MCS)

Table 2. The number of CS operations/number of women delivered, size of the group (%), CS rate (%), and contribution of each group to CS (%) according to Robson and distribution according to different types of Health Facilities

Robson group	Number of cs/ number of women delivered	Healthcare Facilities	CS rate (%[a]) of each institution in each Robson level	RDG CS rate of each institution	Risk ratio (CS rate/RDG CS Rate) [b]	Odds ratio with CI (CS rate/ RDG CS rate)	Patient percentage (%[c]) among Robson score	Patient percentage (%[c]) in all CS patients
1	191763/601438	Public	31.88	9.77	3.26*	4.32 (4.27-4.36)	39.82	24.51
	33446/56413	University	59.29	9.22	6.43*	14.93 (14.45-15.42)	3.74	
	614209/863852	Private	71.10	9.35	7.60*	23.85 (23.65-24.05)	57.19	
	832281/1510372	Total	55.10	9.50	5.80*	11.69 (11.62-11.76)		
2	35224/105959	Public	33.24	31.90	1.04*	1.06 (1.04-1.08)	48.33	3.52
	6468/7961	University	81.25	31.67	2.57*	9.34 (8.68-10.06)	3.67	
	66701/104560	Private	63.79	31.21	2.04*	3.88 (3.81-3.95)	48.18	
	107575/217012	Total	49.57	31.55	1.57*	2.13 (2.10-2.15)		
3	117822/1206909	Public	9.76	2.15	4.54*	4.92 (4.86-4.99)	72.27	27.10
	16021/51860	University	30.89	1.33	23.23*	33,15 (30,68-35,82)	3.11	
	112505/420736	Private	26.74	0.80	33.43*	4.20 (4.14-4.25)	25.19	
	244659/1669923	Total	14.65	1.79	8.18*	9.41 (9.30-9.53)		
4	15853/128474	Public	12.34	12.34	1.00**	-	63.96	3.26
	2211/4062	University	54.43	8.59	6.34*	7.27 (6.53-8.10)	2.02	
	19100/69290	Private	27.57	10.50	2.63*	3.24 (3.15-3.34)	34.49	
	36969/200877	Total	18.40	11.63	1.58*	1.71 (1.68-1.74)		
5	655305/673265	Public	97.33	60.52	1.61*	23.80 (23.43-24.20)	47.37	23.06
	74827/75773	University	98.75	60.71	1.63*	51.19 (47.93-54.67)	5.33	
	671757/681699	Private	98.54	61.54	1.60*	42.22 (41.37-43.01)	47.97	
	1392478/1421180	Total	97.98	61.03	1.61*	30.98 (30.60-31.36)		
6	32240/34177	Public	94.33	99.92	0.94*	0.013 (0.009-0.002)	22.07	2.51
	6144/6524	University	94.18	100	0.94*	0.001 (0.0001-0.0198)	4.21	
	114117/115470	Private	98.83	99.86	0.99*	0.118 (0.100-0.139)	74.55	
	151235/154889	Total	97.64	99.88	0.98*	0.050 (0.043-0.058)		

Table 2. continued

Robson group	Number of cs/ number of women delivered	Healthcare Facilities	CS rate (%[a]) of each institution in each Robson level	RDG CS rate of each institution	Risk ratio (CS rate/RDG CS Rate) [b]	Odds ratio with CI (CS rate/ RDG CS rate)	Patient percentage (%[c]) among Robson score	Patient percentage (%[c]) in all CS patients
7	47014/52142	Public	90.17	99.12	0.91*	0.081 (0.074-0.090)	33.90	2.50
	8498/9059	University	93.81	99.28	0.94*	0.109 (0.084-0.142)	5.89	
	91946/93567	Private	98.27	97.82	1.00*	1.26 (1.18-1.35)	60.82	
	146557/153832	Total	95.27	98.34	0.97*	0.340 (0.325-0.356)		
8	64342/74666	Public	86.17	55.21	1.56*	5.05 (4.92-5.18)	37.45	3.24
	183886/199394	University	93.82	57.52	1.63*	8.75 (8.59-8.92)	10.36	
	101535/105517	Private	96.23	54.19	1.78*	21.55 (20.83-22.30)	52.92	
	183886/199394	Total	92.22	54.91	1.68*	9.74 (9.56-9.92)		
9	26853/29878	Public	89.88	97.96	0.92*	0.185 (0.169-0.202)	29.75	1.63
	3472/3528	University	98.41	99.77	0.99*	0.141 (0.067-0.296)	3.51	
	67193/67609	Private	99.38	98.05	1.01*	3.21 (2.87-3.59)	67.33	
	96948/100416	Total	96.55	98.08	0.98	0.547 (0.517-0.579)		
10	142593/271070	Public	52.60	30.36	1.73	2.54 (2.51-2.57)	50.75	8.67
	353461/534081	University	78.46	30.43	2.58	4.47 (4.44-4.51)	9.30	
	174909/217547	Private	80.40	28.99	2.77	10,05 (9.90-10.19)	40,73	
	353461/534081	Total	66.18	29.80	2.22	4.61 (4.57-4.65)		
All groups	1329009/3177978	Public	41.82	25.99	1.61	2.05 (2.04-2.05)	21.6	100
	209438/285509	University	73.36	35.18	2.09	5.07 (5.02-5.13)	3.4	
	2033972/2739847	Private	74.23	34.20	2.17	5.54 (5.52-5.56)	33.0	
	3546049/6161976	Total	57.55	27.75	2.07	35.29 (35.22-35.37)	57.5	

CI: Confidence Interval, [a]: Row percentage, [b]: Pearson chi-square test was used, [c]: Column percentage, *Significant at level <0.001, **p=0

observed in each Robson group, especially between the public and private centers.

Discussion

This study is the largest cohort study conducted in Turkey to date, which constitutes the National Data of the 5-year period. Data from more than 6 million deliveries and more than hundreds of healthcare facilities are analyzed and categorized according to the Robson classification system. The overall and primary CS rates between 2018 and June 2023 were 57.55% and 28.83%, respectively. When analyzed according to the ten groups of Robson, it was found that Groups 1-4 constituted

Table 3. Analysis of differences in CS rates between healthcare facilities

	Public vs University[a]		Public vs Private[a]		Private[a] vs University	
	CS Rates					
Robson group	Risk ratio	Odds ratio[b] (CI)	Risk ratio	Odds ratio[b] (CI)	Risk ratio	Odds ratio[b] (CI)
1	1.86	**3.11 (3.06-3.17)***	2.23	**5.26 (5.22-5.29)***	1.20	**1.69 (1.66-1.72)***
2	2.44	**8.70 (8.21-9.22)***	1.92	**3.54 (3.47-3.60)***	0.79	**0.407 (0.384-0.431)***
3	3.16	**4.13 (4.05-4.21)***	2.74	**3.37 (3.34-3.40)***	0.87	**0.816 (0.800-0.833)***
4	4.41	**8.49 (7.96-9.05)***	2.23	**2.70 (2.64-2.77)***	0.51	**0.319 (0.299-0.340)***
5	1.01	**2.17 (2.03-2.31)***	1.01	**1.85 (1.80-1.90)***	1.00	**0.854 (0.799-0.913)***
6	1.00	**0.971 (0.867-1.09)***	1.05	**5.06 (4.72-5.44)***	1.05	**5.22 (4.64-5.86)***
7	1.04	**1.65 (1.51-1.80)***	1.09	**6.19 (5.84-6.55)***	1.05	**3.74 (3.39-4.13)***
8	1.09	**2.43 (2.29-2.59)***	1.12	**2.44 (2.29-2.59)***	1.03	**4.09 (3.94-4.25)***
9	1.09	**1.68 (1.58-1.79)***	1.11	**10.64 (9.52-11.90)***	1.01	**1.68 (1.58-1.79)***
10	1.49	**3.28 (3.21-3.36)***	1.53	**3.70 (3.65-3.74)***	1.02	**1.13 (1.10-1.15)***
Total	1.75	**3.83 (3.80-3.86)***	1.78	**4.01 (4.00-4.02)***	1.01	**1.05 (1.04-1.06)***

CI: Confidence interval, [a]: Reference category, [b]: Pearson chi-square test was used, *Significant at level <0.001

58.4% of all CSs. These four groups represent women who are more than 37 weeks pregnant without any previous CSs and have no presentation anomalies that make them proper candidates for vaginal delivery. However, more than half of this population has had primary CS with various indications. According to WHO reference values, almost two-thirds of these women should not have had a CS[10]. According to the available records, it is not possible to determine whether the cause of these CSs is due to an indicative situation that occurred during labor or the preference of the patient or the physician.

Our analysis determined significant differences between different healthcare facilities regarding CS rates. We presented this difference as risk ratio and odds ratio, which clearly revealed that admission to a private hospital is associated with the possibility of a CS more than either university or public hospitals on its own. This finding is compatible with the previous study by Eyi et al.[12]. In their study, they analyzed CS rates for 2017 over the National Health Record System and found overall CS rates as 51.2%. The CS rate in private hospitals was 70.6%, which was higher than that in university and public hospitals. This finding may actually be related to some CS cases applied upon maternal request. As previously mentioned, increasing childbearing age, fear of childbirth, and sexual concerns may affect women's preferences on the mode of delivery[13,14]. The debate continues about whether the mother has the right to request CS. Several guidelines still advocate that CS should be applied under certain medical circumstances related directly to the health of the mother or fetus, but some support the woman's right to decide[14,15].

Perhaps the most predictable of these findings is the increasing number and so the rate of women with previous CSs (Group 5). It is inevitable that the increase in the numbers in groups 1-4 will create serious accumulation in group 5 over the years. Women who had at least one CS (group 5) accounted for more than a quarter of all CSs. These women seem to be inevitably going through CS as the rate among them is 97.9%. Vaginal birth after CS seems to have a very low rate of 2.1%, which is clearly lower than in other countries as the rate of VBAC is approximately 10% in the USA[16], rising to 45-55% in Finland, Sweeden and Netherlands[17]. In the literature, evidence-based data support VBAC as a safe procedure that is applicable to many women under certain circumstances. However, there are

still several concerns on both the patient and clinician sides[18]. Knowing that encouraging VBAC may be one of the important strategies on the way to reducing CS rates; it is not so easy to achieve the goal until all the question marks are clear[18]. The decision process of women should be held professionally, including proper guidance and clarity about safety information. For the clinician, healthcare organizational support, teamwork ability, and clarification of the issue in the legislation system for medicolegal concerns are all truly important in reducing anxiety[19,20].

As mentioned before, Turkey is a country with the highest CS rates. South American countries are the others with almost similar rates. In Brazil the overall CS rate was reported as 55.8%, and Group 5 had the highest contribution to the total numbers[21]. The same group in another paper stated that the high number of lawsuits and medicolegal issues prompted doctors to adopt a defensive approach while managing obstetric patients and as a result do more CSs[22]. This may be attributable to our country as well. In the USA, the overall CS rate was 31.6%, having the highest contribution from Group 5[23].

The main strength of this study is being conducted on the largest population to date regarding overall CS rates and their Robson classification in Turkey. The previous reviews of National Data contained one-year projections[12]. Besides, of course, it is not free from some limitations. Working on big data has several challenges, as it is hard to get every single detail needed. National data come from more than one thousand facilities, and it is sometimes hard to standardize the recording ability and clarity. Despite all the difficulties, high numbers of patients allow researchers to reach better conclusions and make better comments on the big picture of a certain issue[24].

Conclusion

The use of the Robson classification system while recording and reporting the CSs provides a global tool to improve perspectives for reducing the rates. Based on the National Data from Turkey, there are groups that deserve to act. Groups 1-2 represent (Nulliparous, single, cephalic, 37 weeks, spontaneous labor/induction) favorable candidates for vaginal delivery, and probably are the population that will get the easiest response to various initiatives. Moreover, there were significant differences between healthcare facilities, especially private hospitals. This issue is of concern because of the financial burden of unnecessary CSs on the healthcare system. Several implementations have been investigated by different publications, these are worth discussing and can be adapted to the social and demographic characteristics of each country and inserted into the system.

Ethics

Ethics Committee Approval: Not necessary.
Informed Consent: Informed consent was obtained from all participants.

Peer-review: Internally peer-reviewed.

Authorship Contributions

Concept: M.M.U., S.B., M.G.G., Design: S.B., T.A.E., Data Collection or Processing: M.M.U., T.A.E., M.G.G., Analysis or Interpretation: S.B., M.G.G., Literature Search: S.B., T.A.E., M.G.G., Writing: M.M.U., T.A.E.

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Does the decrease in E2 levels between the trigger of ovulation and embryo transfer affect the reproductive outcome in IVF-ICSI cycles?

HCG enjeksiyonu ve embriyo transferi arasındaki E2 seviyelerindeki düşüş, IVF-ICSI döngülerinde üreme sonucunu etkiler mi?

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Abstract

Objective: This study aimed to evaluate the effect of the rate of decline in serum estradiol (E2) levels between hCG injection and the day of embryo transfer (ET) on the success of assisted reproductive technology (ART) in women with infertility of different etiologies.

Materials and Methods: Women 20-45 years of age who underwent a standard GnRH antagonist or long agonist protocol and fresh ET during day 3 of their first ART cycle were included. Group 1 was diagnosed with low ovarian reserve, group 2 comprised high ovarian responders, and group 3 consisted of normal responders. Both groups were divided into four subgroups according to the decrease in E2 levels between the day of hCG injection and the day of ET. Subgroup A patients had a decrease of <20%, subgroup B a decrease of 20-40%, subgroup C a decrease of 41-60%, and subgroup D a decrease >60%. The primary outcome measure was the effect of an E2 decline, based on the measurement of E2 on the day of hCG administration and day of ET, on the implantation rate. The secondary outcome was the change in E2 values in these three groups.

Results: The study was conducted on 1.928 women. Of these, 639 were poor responders (group 1), 502 were high responders (group 2), and 787 women had a normal ovarian response (group 3). Patients with a 60% decrease in their E2 levels on the ET day after hCG had a lower live birth rate (LBR) and higher miscarriage rate (MCR), except normoresponders, in whom a similar decline was significant only with respect to MCR.

Conclusion: We indicate that high ovarian responders who underwent fresh ET cycles with a 60% decrease in their E2 levels on the ET day after human chorionic gonadotropin had lower LBRs and higher miscarriage. However, in normoresponder women, this decline was only significant in miscarriage.

Keywords: E2 decline, high ovarian response, live birth rate, low ovarian reserve, miscarriage rate

Öz

Amaç: Bu çalışma, farklı etiyolojilere bağlı infertilitesi olan kadınlarda embriyo transfer günü olan hCG enjeksiyonu arasında serum E2 düzeylerindeki düşüş hızının yardımcı üreme teknolojisi (ART) başarısına etkisini değerlendirmek amacıyla yapılmıştır.

Gereç ve Yöntemler: Ocak 2011-Aralık 2018 tarihleri arasındaki veriler Tüp Bebek Kliniği hastane kayıtlarından alınmıştır. İlk ART sikluslarının 3. gününde standart bir GnRH antagonisti veya uzun agonist protokolü ve taze embriyo transferi uygulanan 20-45 yaş arası kadınlar dahil edildi. Grup-1 yumurtalık rezervi düşük olanlardan, Grup-2 yüksek yumurtalık yanıtı verenlerden, Grup-3 normal yanıt verenlerden oluştu. Her iki grup hCG enjeksiyon

PRECIS: High ovarian responders who underwent fresh ET cycles with a 60% decrease in their E2 levels on the ET day after HCG had lower live birth rates and higher abortion rates. However, in normoresponder women, this decrease was significant only in abortion rates. In patients with low ovarian reserve, the change in E2 between HCG and the third day ET had no clinical effect.

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günü ile embriyo transfer günü arasında E2 düzeyindeki azalmaya göre dört alt gruba ayrıldı. Alt grup A hastalarında <%20, alt grup B'de %20-40, alt grup C'de %41-60 ve alt grup D'de >%60 azalma vardı. Hastaların demografik özellikleri ve tüp bebek sonuçları çıkarıldı.

Bulgular: Çalışma 1.928 hasta ile yapıldı. Bunlardan 639'u düşük over yanıtı veren (grup 1), 502'si yüksek over yanıtı veren (grup 2) ve 787 kadının normal yumurtalık yanıtı vardı (grup 3). hCG'den sonraki embriyo transfer gününde E2 düzeylerinde %60 azalma olan hastaların düşük doğum ağırlığı daha düşük ve düşük yapma oranı daha yüksekti, ancak normo-yanıt verenler hariç benzer bir düşüşün sadece düşük yapma oranında anlamlı olduğu gösterilmiştir.

Sonuç: İnsan koryonik gonadotropinden sonraki embriyo transfer gününde E2 seviyelerinde %60'lık bir düşüşle taze embriyo transfer siklusları uygulanan yüksek over yanıtlı kişilerin, daha düşük canlı doğum oranlarına ve daha yüksek düşük yapma oranlarına sahip olduğunu belirtiyoruz. Ancak, normo yanıt veren kadınlarda bu düşüş yalnızca düşük yapmada anlamlıydı.

Anahtar Kelimeler: E2 düşüşü, yüksek over yanıtı, canlı doğum oranı, düşük over rezervi, düşük yapma oranı

Introduction

Implantation is the most important step in assisted reproductive technologies (ARTs), but the process is still poorly understood. However, it has been shown that implantation is strongly related to endometrial receptivity, which in turn is affected by serum estradiol (E2) and progesterone (P) levels[1-3]. The results clearly showed that very low or supraphysiological E2 levels have a negative impact on the reproductive outcome[4,5].

E2 levels that become supraphysiological following ovarian hyperstimulation during gonadotropin therapy may decline promptly after therapy is stopped prior to the hCG injection. This response is due to the withdrawal of the injected gonadotropins and aspiration of the granulosa cells during the oocyte retrieval process. The decrease is more severe in high-responder patients and may lead to low CPRs due to deteriorating endometrial receptivity. Accordingly, patients with early and rapid declines in P levels receive luteal support during the early stages of In vitro fertilization (IVF)[6]. However, whether the decrease in E2 levels affects endometrial receptivity or the success of treatment on the day of hCG injection and ET is unclear.

The aim of this study was to evaluate the effect of the rate of decline in serum E2 levels between hCG injection and the day of ET on the success of ART in women with infertility of different etiologies. In a secondary analysis, these findings were evaluated in three groups of patients with a poor, normal, or high response to gonadotropin therapy.

Materials and Methods

This study was a retrospective, single-center cohort trial at the IVF clinic of the University of Health Sciences School of Medicine, Etlik Zubeyde Hanım Research and Training Hospital (Turkey). The study period was from January 2011 to December 2018. The study protocol was approved by the hospital ethics committee (Etlik Zubeyde Hanım Research and Training Hospital; no: 90057706-799, date: 19.02.2019). Signed informed consent was obtained from all patients.

Women 20-45 years of age who underwent a standard gonadotropin-releasing hormone (GnRH) antagonist or long agonist protocol and fresh ET during day 3 of their first ART cycle were included in the study. Patients who had an organic pathology involving the uterus and/or endometrium or whose treatment protocol differed from antagonist or long luteal agonist protocols for ovarian hyperstimulation were excluded, as were those with previous IVF-ICSI cycles, who currently had a freeze-thaw cycle, or who underwent day 5 ET. To take advantage of the participants, we did not exclude patients we could not follow up until birth.

In our study population, patients who underwent ET on day 3 and had an antral follicle count (AFC) <11, a serum anti-Müllerian hormone (AMH) level <1.1 ng/mL, and <4 collected oocytes[7,8] were diagnosed with low ovarian reserve and assigned to group 1. Group 2 comprised high ovarian responders who met the inclusion criterion and had a peak E2 level >3000 pg/mL on the day of hCG administration, >15 retrieved oocytes, or a basal AFC >10[9]. Patients with E2 values above 4000[10] were given an agonist trigger for OHSS prophylaxis. Group 3 consisted of normal responders with a peak E2 level of 500-3000 pg/mL on the day of hCG administration, 5-15 retrieved oocytes, and a basal AFC of 7-10[11].

Each group was divided into four subgroups according to the decrease in E2 levels between the day of hCG injection and the day of ET. Subgroup A patients had a decrease of <20%, subgroup B a decrease of 20-40%, subgroup C a decrease of 41-60%, and subgroup D a decrease >60%[12]. Differences in the implantation rate (IR), CPR, miscarriage rate (MCR), and live birth rate (LBR) among subgroups were assessed.

Age, body mass index (BMI) [weight (kg)/height × height (m^2)], follicle stimulating hormone (FSH), and E2 values on the third day of menstruation, total AFC, duration of infertility (months), E2 levels on the days of hCG administration and ET, number of retrieved oocytes, and number of mature oocytes were recorded.

In conventional protocols, recombinant FSH (Gonal-F, Merck Serono, Germany; Puregon, Organon, the Netherlands) with or without human menopausal gonadotropin (Menogon, Ferring Pharmaceuticals, Germany; Merional, IBSA, Switzerland) was used at doses ranging from 150 IU/day to 450 IU/day in accordance with body mass index, patient age, and the number of antral follicles. Patients underwent pituitary downregulation using the luteal long protocol with a GnRH agonist (Lucrin, Abbott, France) or the GnRH antagonist protocol (Cetrotide, 0.25 mg/day, Serono, Germany). During controlled ovarian hyperstimulation (COH), we monitored serum hormone levels, the size and count of follicles, and endometrial thickness. Recombinant hCG (250 mg Ovidrel; Serono) was administered to trigger ovulation when at least two leading follicles reached ≥18 mm in diameter. Transvaginal ovum pick-up was

performed 34-36 h after ovulatory induction. Oocyte pick-up (OPU) was conducted under general anesthesia, followed by ET on day 3 post-retrieval. Serum E2 levels on the day of hCG administration and on the day of ET were measured using an electrochemiluminescence immunoassay kit (Roche Diagnostics GmbH, Mannheim, Germany). Patients who underwent ET received luteal phase support with a P-containing vaginal gel (90 mg/d twice daily, Crinone 8% vaginal gel, Merck-Serono, Switzerland) after oocyte collection and continued during pregnancy until approximately 12 weeks of gestation.

The primary outcome measure was the effect of an E2 decline, based on the measurement of E2 on the day of hCG administration and day of embryo transfer, on IR (positive β-hCG test ≥10 IU, 10 days after embryo transfer), CPR (presence of an intrauterine gestational sac detected on transvaginal USG), MCR (spontaneous pregnancy loss before 20 weeks of gestation) and LBR (the delivery of a viable infant any time after 24 weeks gestation) in the three groups of patients (normal, high, and poor responders). The secondary outcome was the change in E2 values in these three groups. The decline was graded as <20%, 20-40%, 40-60% and >60%.

Statistical Analysis

SPSS 20 (IBM Corp. released 2011. IBM SPSS Statistics for Windows, version 20.0, Armonk, NY: IBM Corp.) was used to evaluate the data. The data were investigated using visual (histograms, probability plots) and analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk's tests) to determine their normal distribution. A One-Way ANOVA and Kruskal-Wallis test were used to compare continuous variables with a normal and non-normal distribution, respectively. Differences between groups were evaluated using Student's t-test for parametric data and the Mann-Whitney U-test for non-parametric data. Relationships between categorical variables were analyzed using a chi-square test. A p-value <0.05 was considered to indicate statistical significance.

Results

The study was conducted on 1.928 women. Of these, 639 were poor responders (group 1), 502 were high responders (group 2), and 787 women had a normal ovarian response (group 3). The age range of the patients was 20-45 years, and the duration of infertility ranged from 12 to 112 months.

The clinical characteristics of the three groups are presented in Table 1. Patient age, basal FSH and E2 levels were significantly higher in group 1 than in groups 2 and 3. AFC, duration of infertility, E2 levels on the day of hCG injection and day of ET, number of retrieved embryos, number of mature oocytes, and IR, CR, and LBR were significantly lower in group 1 than in groups 2 and 3. Group 2 had the highest IR and CPR, and group 3 the highest LBR. The total number of miscarriages

Table 1. Clinical characteristics of subjects

	Group 1 (n=639)	Group 2 (n=502)	Group 3 (n=787)	p
Age (years)	36.7±7.5[b,c]	28.8±4.6	29.5±4.7	<0.001
BMI (kg/m²)	26.9±5.1	27.4±5.2[c]	25.9±4.9[a,b]	<0.001
Basal FSH (IU/L)	10.8±6.2[b,c]	5.9±2.5	6.9±1.6	<0.001
Basal E2 (pmol/L)	55.8±47.5[b,c]	44.3±18.6	47.5±27.2	0.001
AFC	5.7±3.4[b,c]	22.7±7.1[c]	12.9±6.1	<0.001
Infertility duration (month)	65.0±64.7[b,c]	81.1±50.2[c]	69.0±53.3	<0.001
E2 value on HCG day (pmol/L)	1354.6±1070.3[b,c]	3113.8±1841.0[c]	2209.3±1103.6	<0.001
E2 value on ET day (pmol/L)	803.9±639.1[b,c]	2452.4±1588.1[c]	1463.1±859.5	<0.001
No. of retrieved oocytes	3.7±3.0[b,c]	16.6±8.6[c]	10.0±2.9	<0.001
No. of mature oocytes	3.2±2.4[b,c]	11.8±6.6[c]	7.5±3.0	<0.001
Implantation rate (%)	184 (28.7)[b,c]	246 (49.0)	325 (41.2)	<0.001
Clinic pregnancy rate (%)	155 (24.2)[b,c]	201 (40.0)	289 (36.8)	<0.001
Miscarriage rate (%)	53 (28.8)	60 (24.3)	85 (26.2)	0.588
Live birh rate (%)	101 (15.8)[b,c]	127 (25.4)	185 (23.5)	<0.001

Data presented as mean ± SD or n (%). BMI: Body mass index, AFC: Antral follicle count, FSH: Follicle stimulating hormone, E2: Estradiol, P: Progesterone, HCG: Human chorionic gonadotropin, ET: Embryo transfer

**The total number of miscarriage and live births did not add up to the number of pregnancies as the results of the patients with clinical pregnancy was not obtained.

[a]There was a significant difference with compared group 1 in post-hoc comparison.

[b]There was a significant difference with compared group 2 in post-hoc comparison.

[c]There was a significant difference with compared group 3 in post-hoc comparison.

and live births did not add up to the number of pregnancies because the results of all patients with clinical pregnancy were not obtained.

Table 2 shows the LBR, MCR, IR, and CPR following fresh ET in poor responders (group 1), high ovarian responders (group 2), and normoresponders (group 3) with different E2 declines. In poor responders (group 1), the rate of decrease in the E2 level had no effect on LBR or MCR. In addition, the highest CPR and LBR were in subgroup 1A, which interestingly also had the highest MCR. However, these differences relative to the other subgroups were not statistically significant. In group 2, MCR was highest and LBR lowest in subgroup 2D, whereas IR was lowest in subgroup 2C. In normoresponders (group 3), MCR was significantly higher and LBR significantly lower in subgroup 3D ($p<0.05$) than in subgroups 3A and 3B.

Table 3 shows the clinical features and cycle outcomes of women with high ovarian responses who had a rate of serum estradiol decline of more or less than 60%. High responders were further analyzed according to the ratio of E2 decline in Table 3 (<60% and >60%). The comparison showed that patient age, duration of infertility, basal FSH, P level on hCG day, number of retrieved oocytes, ET, mature oocytes, IR, and CPR were similar ($p>0.05$). However, BMI, AFC, E2 on the day of hCG injection, and MCR were higher in high responders with an E2 decline >60%, whereas LBR was lower. In normoresponders, age, E2 level on hCG injection day, and MR were significantly higher, whereas LBR was significantly lowerin women with an E2 decline >60% (Figure 1).

Table 4 shows the clinical features and cycle outcomes of women with normoresponders (Group 3) who had a rate of serum estradiol decline of more or less than 60%. Patients with a 60% decrease in their E2 levels on the ET day after hCG had a lower LBR and higher MCR, except normoresponders, in whom a similar decline was significant only with respect to MCR (Table 4, Figure 1). In patients with low ovarian reserves, the change in E2 between hCG and ET days had no effect on any clinical variable.

Table 2. Live birth, abortion, implantation and clinical pregnancy rates of fresh embryo transfer cycles in poor responders (Group 1), high ovarian responders (Group 2) and women with normal ovarian responses (Group 3) in subgroups with different E2 declines on the embryo transfer day 3

Groups	No. of patients (%)	Implantation (IPR %)	Clinical pregnancy (CPR %)	Miscarriage (MCR %)	Live birth (LBR %)
Group 1 (n=639)					
Subgroup A (E2%<20)	38 (8.9)	15 (39.4)	15 (39.4)	6 (40)	9 (23.6)
Subgroup B (E2%20-40)	125 (19.5)	33 (26.4)	30 (24.0)	7 (21.2)	23 (18.4)
Subgroup C(E2%41-60)	244 (38.1)	75 (30.7)	62 (25.4)	19 (25.3)	43 (17.6)
Subgroup D (E2%>60)	232 (36.3)	61 (26.2)	48 (20.6)	21 (34.4)	26 (11.2)
P value		0.115	0.086	0.358	0.082
Group 2 (n=502)					
Subgroup A (E2%<20)	119 (23.7)	61 (51.2)	50 (42.0)	8 (13.1)	38 (31.9)
Subgroup B (E2%20-40)	162 (32.3)	94 (58.0)	73 (45.0)	12 (12.7)	52 (32.0)
Subgroup C (E2%41-60)	140 (27.9)	54 (38.5)	45 (32.1)	15 (27.7)	30 (22.4)
Subgroup D (E2%>60)	81 (16.1)	37 (45.6)[$]	33 (40.7)	25 (67.5)[#]	7 (8.6)[*]
P value	0.007[*]	0.135	<0.001[*]		<0.001[*]
Group 3 (n=787)					
Subgroup A (E2%<20)	150 (19.1)	65 (43.3)	60 (40.0)	15 (23.0)	43 (28.6)
Subgroup B (E2%20-40)	228 (29)	90 (39.4)	83 (36.4)	16 (17.7)	64 (28.0)
Subgroup C (E2%41-60)	306 (39)	135 (44.1)	116 (37.9)	37 (27.4)	65 (21.2)
Subgroup D (E2%>60)	103 (12.8)	35 (35)	30 (30)	17 (17)[&]	13 (13)[€]
p		0.362	0.419	0.006*	0.034*

E2: estradiol. Data presented as n (%).
[*]: Significant difference between Subgroup-2-D and Subgroup 2-A and 2-B ($p<0.001$).
[#]: Significant difference between Subgroup 2-D and Subgroup 2-A,2-B and 2-C ($p<0.001$).
[$]: Significant difference between Subgroup 2-D and Subgroup 2-B and 2-C (p:0.007)
[&]: Significant difference between Subgroup 3-D and Subgroup 3-A and 3-B (p:0.011).
[€]: Significant difference between Subgroup 3-D and Subgroup 3-A and 3-B (p:0.014)

Table 3. Clinical features and cycle outcomes of women with high ovarian responses who had a rate of serum estradiol decline of more or less than 60%

	<%60 (n=421)	>%60 (n=81)	p-value
Age (years)	28.7±4.7	30.1±3.8	0.072
*BMI (kg/m^2)	27.4±5.4	29.2±5.0	0.040
Infertilityduration (month)	82.0±52.2	79.6±39.9	0.708
Basal FSH (IU/L)	5.9±1.7	5.5±1.5	0.407
Basal E2 (pmol/L)	43.6±18.5	48.9±15.3	0.006
AFC	22.4±7.2	26.5±5.5	<0.001
E2 value on HCG day (pmol/L)	3013.4±1699.8	4494.3±2256.2	<0.001
P value on HCG day (ng/mL)	1.3±1.3	1.5±1.0	0.391
No. of retrieved oocytes	16.5±7.8	18.7±9.1	0.115
No. of mature oocytes	12.2±6.3	11.8±6.4	0.688
Live birth	121 (28.7)	6 (7.4)	<0.001
Miscarriage	35 (16.7)	25 (67.5)	<0.001
Implantation	209 (49.6)	37 (45.6)	0.513
Clinical pregnancy	168 (39.9)	33 (40.7)	0.888

Data presented as mean ± SD or n (%). BMI: Body mass index, AFC: Antral follicle count, FSH: Follicle stimulating hormone, E2: Estradiol, P: Progesterone, HCG: Human chorionic gonadotropin, ET: Embryo transfer

Table 4. Clinical features and cycle outcomes of women with normo-responders (Group-3) who had a rate of serum estradiol decline of more or less than 60%

	<%60 (n=687)	>%60 (n=100)	p-value
Age (years)	29.2±4.8	31.2±4.5	<0.001
*BMI (kg/m^2)	26.1±4.9	25.6±5.2	0.120
Duration of infertility (month)	66.5±53.6	77.2±57.5	0.106
Basal FSH (IU/L)	6.9±1.6	7.2±1.7	0.060
Basal E2 (pmol/L)	46.8±30.6	50.5±25.9	0.210
AFC	13.0±6.1	12.8±6.3	0.646
E2 on HCG day (pmol/L)	2158.5±1029.4	2627.8±1400.5	<0.001
p on HCG day (ng/mL)	1.0±0.5	1.1±0.6	0.106
No. of retrieved oocytes	10.1±2.9	10.0±3.0	0.744
No.of mature oocytes	7.8±2.9	7.8±3.0	0.971
Live birth	172 (25.1)	13 (13)	0.025
Miscarriage	68 (23.4)	17 (17)	<0.001
Implantation	290 (42.3)	35 (35)	0.105
Clinical pregnancy	259 (37.8)	30 (30)	0.052

Data presented as mean ± SD or n (%). BMI: Body mass index, AFC: Antral follicle count, FSH: Follicle stimulating hormone, E2: Estradiol, P: Progesterone, HCG: Human chorionic gonadotropin, ET: Embryo transfer

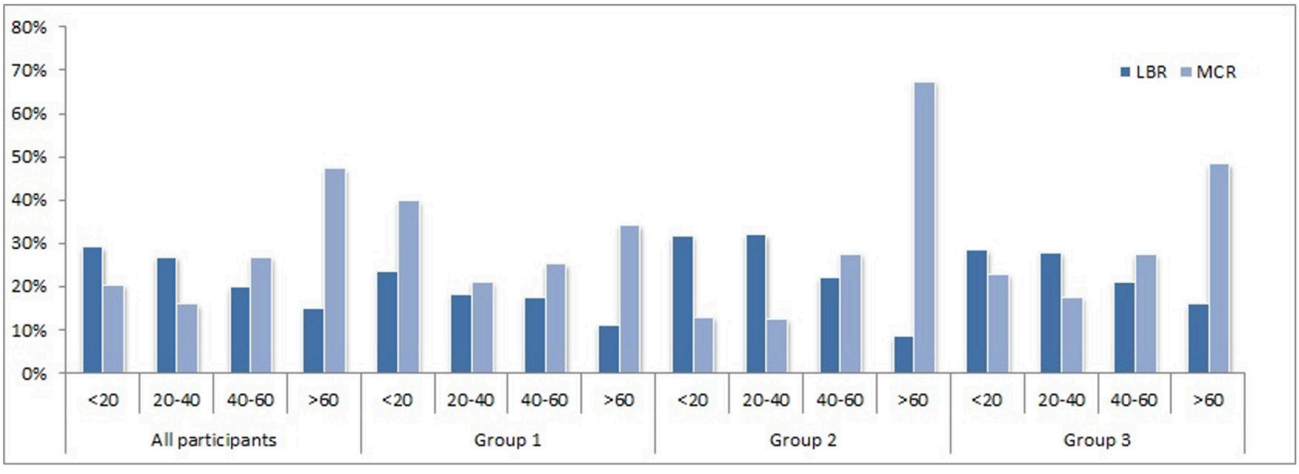

Figure 1. Live birth and miscarriage rates in poor responders (Group 1), high ovarian responders (Group 2) and women with normal ovarian responses (Group 3) in subgroups with different E2 declines on the embryo transfer day 3

Discussion

We found that high ovarian responders who underwent fresh ET cycles with a 60% decrease in their E2 levels on the ET day after HCG had lower live birth rates and higher abortion rates. However, in normoresponder women, this decrease was significant only in abortion rates. In patients with low ovarian reserve, the change in E2 between HCG and the third day ET had no clinical effect.

In our study, the decrease in E2 after the cessation of gonadotropin injection was more prominent in high responders than in low or normal responders. Although this decline in high-responder patients reduces the risk of OHSS, its effect on CPR and LBR is unclear. E2 plays a role in the expression of E2-induced growth factor and E2 receptors[13], and the induction of sufficient P receptors is needed for subsequent P stimulation[12]. Thus, a sudden decrease in E2 may negatively affect implantation[14].

The results of early studies of IVF cycles showed that E2 values measured 24 h after hCG administration are not predictive of reproductive outcome[15,16]. Ozdegirmenci et al.[15] reported a 38% CPR in women with a 30% E2 decline, but in Huang's study[16], the number of women with a 30% decrease was too small to allow a statistical comparison.

The number of patients with an E2 decline >60% was higher in poor responders than in high and normoresponders (36.3% vs. 16.1% and 12.8%, respectively). The subgroup analysis showed that, despite different increments in the E2 decline (subgroup A <20%, subgroup B 20-40%, subgroup C 41-60%, subgroup D >60%), there was no statistically significant difference among the three groups of responders in terms of IR and CPR. However, MCR was significantly higher and LBR significantly lower in subgroup D of the high responders.

An analysis of our patient groups according to the decline in E2 (>60% vs. <60%) showed that high responders with an E2 decline >60% had a lower LBR and higher MCR, whereas IR and CPR did not differ from the corresponding rates in normal and poor responders. In normoresponders with an E2 decline >60%, the E2 level on the day of hCG injection, as well as LBR and MCR, were higher, and the patients were older. While CPR was lower in this group, the difference compared to the other two groups was not statistically significant. We think that the differences in age and BMI may have been a good explanation for the E2 reduction (E2% <20 ~>60%) within the same group. Moreover, the patients' individual cellular response to this mechanical destruction after OPU may also have played a role in this situation.

We also found that the ovarian response and the number of mature oocytes were positively correlated with a favorable IVF outcome. Poor responders with the highest basal E2 level, lowest AFC, and lowest E2 level on the day of hCG injection had the lowest LBR. The main source of post-hCG E2 is the pre-ovulatory E2 produced by oocytes during gonadotropin stimulation. The post-hCG E2 level has been studied for its ability to predict IVF-ET outcome. For example, in an early study by Huang et al.[17], an increase or decrease in the E2 level one day after hCG administration had no effect on the cleavage and fertilization rates of high and low responders.

Few studies have evaluated E2 levels at the beginning of the luteal period. Diluigi et al.[18] divided their patients into two groups according to ovarian responses and then measured E2 values on the day of hCG and 2 days after OPU. After calculating the rates of E2 decline, they found that IR and CPR were lower in high responders with an E2 decline of ≥80%. In our study, we grouped the patients according to their response to ovulation induction and then calculated the percent reduction in E2 levels between the hCG and ET days in each group to analyze the effect of E2 decline rates on the response to gonadotropin therapy. The results showed a significant decline in LBR and an increase in MCR, especially in high and normoresponders, when the E2 decline was >60% (subgroup D). Thus, although the risk of OHSS in these patients is lower due to the sharp decline in serum E2 levels, there is no favorable effect on pregnancy outcome. Whether subgroup D-type patients will benefit from E2 supplementation remains to be investigated.

In our study, we focused on particular groups of patients (poor responders, high responders, and normal responders) in contrast to similar studies on the effect of E2 on ART[12,13]. In addition, our study is one of the few that evaluated E2 levels on the day of ET rather than during the mid-luteal phase. Our results included LBR, which is not reported in most ART studies.

Study Limitations

Our study also has several limitations, especially its retrospective nature and the limited number of patients.

Conclusion

Nonetheless, our findings indicate that the E2 decline on the day of ET may negatively affect ART results in high and normal ovarian responders. Routine measurement of this decline may indicate the need for E2 support alongside P supplementation during the early luteal period. However, larger multicentric studies with prospective designs are needed to definitively determine the relationship of E2 decline with LBR and MCR.

Ethics

Ethics Committee Approval: The study protocol was approved by the hospital ethics committee (Etlik Zubeyde Hanım Research and Training Hospital; no: 90057706-799, date: 19.02.2019).

Informed Consent: Signed informed consent was obtained from all patients.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Editing assistance: O.A., Technical assistance: B.D., Design: N.N.Y., Data Collection or Processing: R.Ö., Analysis or Interpretation: E.B., Literature Search: N.N.Y., S.D., Writing: N.N.Y., Ö.M.T.

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Comparison of classic single-layer uterin suture and double-layer purse-string suture techniques for uterus closure in terms of postoperative short-term uterine isthmocele: A prospective randomized controlled trial

Uterus kapatmada klasik tek kat kapatma ve çift kat kese ağzı uterus kapatma tekniklerinin postoperatif kısa dönem uterin istmosel oluşumu açısından karşılaştırılması: Prospektif randomize kontrollü çalışma

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Abstract

Objective: To compare the short-term results of classic single-layer uterine closure and double-layer purse-string uterine closure (Turan technique) techniques in cesarean section in terms of the incidence of ischiocele formation.

Materials and Methods: This was a prospective randomized controlled trial study. Participants undergoing first-time cesarean delivery were randomized into two groups. Fifty-eight participants were included in the double-layered uterine closure group (study group), while 53 participants were randomized into the classical single-layered uterine closure group (control group). For comparison of isthmocele formation, transvaginal ultrasound examination was planned in all patients 6 weeks after surgery. The operation data,the formation of isthmocele, its dimensions and volume were recorded.

Results: A total of 111 women were included in the study. The incidence of ischiocele at 6 weeks after birth was not significantly different between the groups (p=0.128). Isthmosel was detected in 20.8% of single-layer closures, and this rate was determined as 10.3% in the purse technique. In the Kerr incision made during surgery, the uterine incision size did not differ in either group, but the uterine incision length after suturing was significantly smaller in the purse technique compared with the other group (p<0.001).

Conclusion: The incidence of ischiocele formation after cesarean section and the depth of the ischiocele was independent of the uterotomy closure technique.

Keywords: Cesarean section, isthmocele, residual myometrium, suture technics, uterine scar

Öz

Amaç: Bu çalışmanın amacı sezaryen doğumda lasik tek kat rahmi mahatma ve gift kat kese-string rahmi mahatma (Turan tekniği) tekniklerinin istmosel oluşum insidansı açısından kısa dönem sonuçlarını karşılaştırmaktır.

Gereç ve Yöntemler: Bu çalışma prospektif randomize kontrollü bir çalışmadır. İlk kez sezeryan doğum yapacak olan hastalar iki ayrı gruba randomize edildiler. Elli sekiz katılımcı çift katmanlı kese ağzı uterus kapatma grubuna (çalışma grubu) alınırken, 53 katılımcı lasik tek katmanl uterus mahatma grubyia (kontrol grubu) randomize edildi. Ameliyat sonrasında karşılaştırma için tüm hastalara ameliyattan 6 hafta sonra transvajinal ultrason muayenesi planlandı. Operasyon bilgileri, istmosel oluşup oluşmadığı, varsa boyutlar, hacmi kaydedildi.

PRECIS: Is uterus closure stur technique important in the formation of istmocele?

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Bulgular: Araştırmaya toplam 111 kadın dahil edildi. Doğumdan 6 hafta sonra istmosel insidans gruplar arasındaki fark anlamlı değildi (p=0,128). Tek katlı uterus sapma grubunda %20,8 istmosel oran saptanırken, bu oran lese azo uterus mahatma uygulanan grupta %10,3 olarak saptandı. Ameliyat sırasında yapılan Kerr kerisinde uterine keri bout her iki grupta da farklılık göstermezken, sütur sonrası uterin insizyon uzunluğu kese ağzı kapama tekniğinde diğer gruba göre olarak daha küçüktür (p<0,001).

Sonuç: Sezeryan sonrası istmosel oluşum insidans ve istmosel derinliği uterus mahatma tekniğinden bağımsızdır.

Anahtar Kelimeler: Sezaryen kesisi, istmosel, rezidüel miyometriyum, sütur teknikleri, uterin star

Introduction

The cesarean section is the most frequently performed surgery in the world and its frequency is increasing[1,2]. This increase brings with it an increase in complications. The formation of an isthmocele after cesarean section is one such complication. "Isthmocele" was first described by Hugh Morris in 1995[3]. Uterine ischiocele is defined as a noncontinuous area of the hypoechoic myometrium found in the uterine incision due to a previous cesarean section[4]. When the literature is examined, the precise prevalence of niche formation is unknown. It varies widely from approximately 19%[5] to 100%[4]. Many studies emphasize that this difference in prevalence may be because the standardization of the diagnosis and definition of ischiocele has yet to be clarified, or it may be related to the surgical technique during cesarean section[6]. The cesarean section technique has undergone many changes since it was first defined. With these continuous revisions, the aim is to prevent negative effects that may occur. The way to prevent a complication is to determine its etiology.

The optimal method for the closure of the uterus during cesarean section is still a matter of debate. Different suture materials[7], different closure techniques[8,9], and closures with different incisions[10,11] have also been studied. Complications are attempted to be reduced by trying different methods and conducting studies on them. In the literature, the uterine closure technique is mostly blamed for the formation of ischiocele, and studies have focused on the technique. Accordingly, we evaluated the effects of ischiocele formation by comparing the two uterine closure techniques in the short term.

Materials and Methods

This prospective study was conducted between September 2022 and December 2022 at a training and research hospital. The study was approved by the institutional review board and ethics committee (approval number: 120, date:21/09//2022/) and complied with the Declaration of Helsinki. All patients were informed about the study, and written informed consent was obtained from all participants. In power analysis, it was found appropriate to include 28 people in each group with 80% power and 5% Type I error to detect a difference of at least 0.25 (medium level) effect size between the groups. The calculation was made in the MedCalc program.

A total of 111 women who underwent cesarean section were included in our study. The inclusion criteria were being older than 18 years, younger than 40 years, having been given an elective cesarean section date, and having a history of no previous cesarean sections. All participants had previously decided to undergo cesarean section and were prepared under elective conditions. Women who had to have an emergency cesarean section for any reason, those who had a previous cesarean delivery, those with an early pregnancy below 37 weeks, multiple pregnancies, those who had a cesarean delivery while in active labor, and women who had a history of uterine surgery such as myomectomy, with diseases such as malnutrition, connective tissue disease, and diabetes that might impair wound healing were excluded from the study. All patients underwent their first cesarean section regardless of the number of births. The pregnant women were randomized using a simple random sampling method to one of the groups by a physician during their admission to the delivery room. There were two groups in the study. All operations were performed using Pfannenstiel for abdominal incisions and Kerr techniques for uterine incisions. During the operation, after the delivery of the baby, the size of the kerr incision in the uterus was measured with a sterile ruler just before the uterus was closed. Uterine closure was then performed according to the technique randomized to the patient. The length of the incision area before and after uterine closure is shown in table as "Uterotomy incision length before-after suturing (cm)". To close the uterus during cesarean section, classic single-layer unlocked uterus closure was applied to one of the groups, and uterus closure was performed on the other group using the purse technique, a technique developed by Turan et al.[12] The purse-string technique used in one group can be summarized as follows: starting in one corner, and then the incision is closed using no. 1 Vycryl suture. The first layer is transversely passed through the inner myometrium-decidua line. The second layer passes parallely and transversely through the outer myometrium-visceral peritoneum line continuously in the form of a purse-string closure. With this method, the string starting from the first corner is returned to the starting point and knotted. After the string is tied, the opening in the middle of the uterine incision is closed with a separate figure-eight suture.With the purse suture technique, the uterine closure area is reduced to approximately 3-4 cm (Figure 1). All surgeries were performed by the same surgeon (EY). Polyglycolic Vicryl number: 1 (Johnson & Johnson, Somerville, NJ, USA) was used for uterine closure. Additional hemostatic sutures were placed in the case of bleeding. Preoperative antibiotics (cefuroxime 1 g) were given to all patients. Two grams were given to patients with obesity. The time for collecting data after surgery was determined as 6 weeks. In many studies in the literature, it is reported that uterine healing becomes reflective of whether an

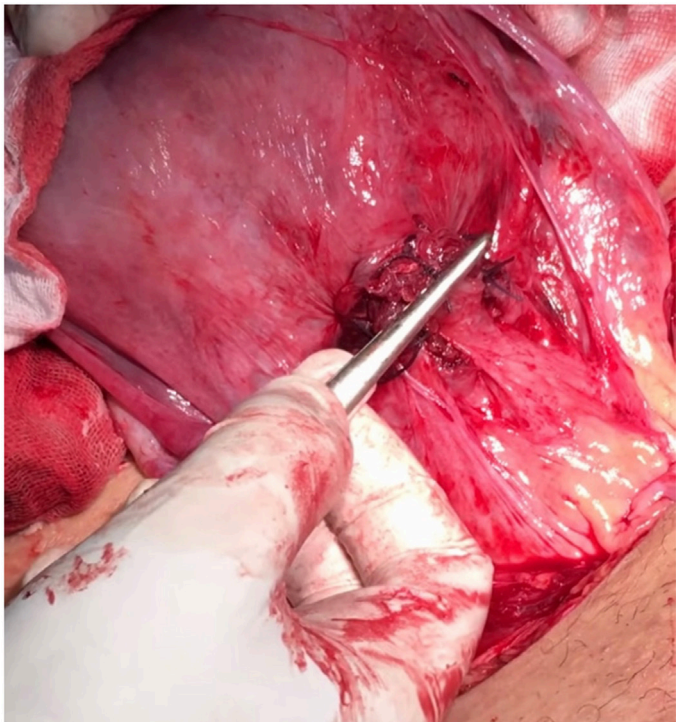

Figure 1. The intraoperative wiew of uterine closure with double-layer purse-string suture techique

ischiocele will occur after 1 month, and ultrasonography (USG) evaluations at the end of 1 month reflect long-term results[13]. All participants were called to the clinic 6 weeks after the surgery. There is no defined gold standard method yet, butthe most frequently used diagnostic method is transvaginal sonography (TVS), although sonohysterography has proven to be at least an equally viable alternative method[14]. In our study, all 6th-week evaluations were performed by the same physician (EY) with using a transvaginal ultrasound who was blinded to the surgical technique to provide standardization and to facilitate objective evaluations. All USG was examined using a 5-9-MHz transvaginal transducer (Mindray DC 8 Expert, Wauwatosa). To achieve standardization, measurements were made and recorded as defined by Naji et al.[15]. All examinations were observed in two dimensions, independent of the menstrual cycle, with the bladder empty, on the gynecologic examination table in the dorsal lithotomy position, with the uterus total, endometrium, and cervico displayed. Scar tissue was measured in 3D in both the sagittal and transverse planes. The uterus was examined for ismocele, defined as anechoic areas at the site of the scar with a depth of at least 1 mm[16]. The length of the scar in the uterotomy area, scar thickness,the presence of an isthmocele, the 3D volume in the presence of an isthmocele, myometrial thickness at cesarean scar site (X) myometrial thickness of the uterus at the level of the internal cervical os (Z), and the myometrial thickness of final neighborhood of scar with interval of scar-isthmus distance (Y). The measured parameters are shown in Figure 2. The data collected for both groups were compared.

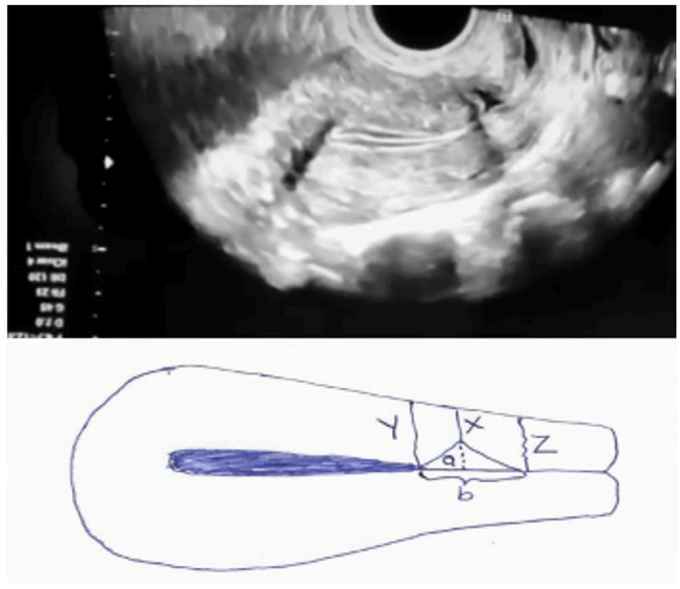

Figure 2. The measured parameters with ultrasound

Ethical Statement

The study protocol was approved by the Ethics Committee of Gaziosmanpaşa Training and Research Hospital (no: 120, date: 21/09/2022), and was conducted according to the principles of the Helsinki Declaration. Written informed consent was obtained from all participants. The clinical trial registration number is NCT05517018.

Statistical Analysis

All statical analyzes were performed using IBM SPSS 21 and MedCalc Version 20.104 programs. The normality control of continuous variables was evaluated using the Shapiro-Wilk test. Quantitative variables are expressed as mean ± standard deviation and qualitative variables as percentages. The Mann-Whitney U test and Student's t-test were used to compare two independent groups. Student's t-test was used for variables with normal distribution and quantitative variables with nonnormal distribution were compared using the Mann-Whitney U test. Categorical data were analyzed using the chi-square and Fisher's exact tests. A p-value of ≤0.05 was considered statistically significant.

Results

A total of 111 participants were included in our study, 53 of whom underwent classic single-layer unlocked closure as the control group, and 58 participants received uterine closure using the purse technique as the case group. There were no significant differences between the two groups in terms of age, gravidity, parity, abortion, body mass index (BMI), gestational week during cesarean section, baby weight, and baby height at birth. The demographic characteristics of the study participants are shown in Table 1.

The indications of the patients for a cesarean section are shown in Table 2.

There was no significant difference between the two groups in terms of the anesthesia method performed during surgery, the need for additional suturing for hemostasis during surgery, whether to make a brace for contraception, the sex of the babies, and breastfeeding status in the postoperative period (Tables 3 and 4).

In the Kerr incision made before surgery, the uterine incision size did not differ between the two groups. In the postoperative purse technique, the uterotomy area was significantly smaller than that in the other group (Table 4).

In the 6th-week follow-ups of the patients, 11 (20.8%) niche formations were found among 53 women who had classic closures and 6 (10.3%) of 58 women who had closed using the purse technique. The incidence of niche formation was not statistically different between the two groups (p=0.128 and p≤0.001, respectively). TV USG findings at the 6th-week follow-up are shown in Table 5.

Discussion

The main results of the present study indicate that the two techniques used showed no differences in the formation of ischiocele in the short postoperative 6-week period, and neither was superior to the other. The purse technique had a significantly smaller wound site in the postoperative period and a significantly smaller scar was associated with the defect at the 6th-week follow-up (p<0.001).

In a cohort study by Hosseini et al.[7], women were divided into two groups and two different suture materials were usedto affect the formation of ischiocele. The authors found ischiocele formation was statistically significantly higher in the group in which they used catgut and defined it as a risk factor. In addition, the residual myometrial tissue thickness, which is thought to be associated with uterine rupture, was found to be higher, and it was argued that using Vicryl was more advantageous as a result. However, there was no standardization for patients in the study. The fact that factors other than suture materials were not excluded may have affected the results.

In the study performed by Sisti et al.[17] to define patient-related risk factors for the development of isthmocele, it was found that the patient's age and the stage of birth at which the patient was

Table 2. Distribution of cesarean section indications according to the groups

		Uterine closure technique		Total
		Classic single-layer uterine suture	Double-layer purse-string suture	
Indications	Brow presentation	2	1	3
	Aneurysm	1	0	1
	Scoliosis	2	1	3
	Breech (Footling)	1	1	2
	Failed induction	2	1	3
	Cephalopelvic disproportion	9	9	18
	Gestational diabetes	2	1	3
	Patient prompt	4	4	8
	HPV	3	6	9
	Slow progress	1	3	4
	Fetal macrosomia	9	6	15
	Cholestasis	3	5	8
	Lumbar hernia	0	2	2
	Breech (Frank)	9	13	22
	Overdue pregnancy	0	1	1
	Occiput posterior	2	2	4
	Shoulder/transverse	3	2	5
Total		53	58	111

Table 1. Demographic characteristics of study participants

	Classic single-layer uterine suture			Double-layer purse-string suture			
	Mean ± SD	Median (IQR)	Min-Max	Mean ± SD	Median (IQR)	Min-Max	p
Age (years)	29±6.2	28 (24-33)	19-47	27.3±5.9	27.5 (23-30.3)	18-47	0.128
Height (cm)	159.8±5.4	160 (157-163.5)	150-170	160.3±5.7	160 (157.3-165)	150-172	0.637
Weight (kg)	75.3±10.6	75 (68-83)	56-95	75.4±11.5	75.5 (67-82.3)	53-99	0.958
BMI (kg/m^2)	29.5±4.2	29.3 (26.7-32)	20,3-41,3	29.4±4.9	28.5 (25.4-33.3)	21.1-40.6	0.929
Gravidity (n)	2.6±2	2 (1-3.5)	1-11	2.4±1.9	2 (1-3)	1-11	0.479
Parity (n)	1.2±1.5	1 (0-2)	0-6	1.1±1.5	0.5 (0-2)	0-7	0.563
Abortion (n)	0.4±0.9	0 (0-0)	0-5	0.3±0.8	0 (0-0)	0-5	0.451
Gestational age (weeks)	39.2±1.1	39.3 (38.6-40)	37-41	39.2±1.1	39.3 (38-40)	37-42	0.940

p: Independent Sample t-test *Mann-Whitney U test, BMI: Body mass index (kg/m^2), IQR: Interquartile range, Min-Max: Minimum-maximum, SD: Standard deviation

Table 3. Operative data group[1]

		Uterine closure technique				Total		
		Classic single-layer uterine suture		Double-layer purse-string suture				
		n	%	n	%	n	%	p
Presentation	Normal	40	75.5	41	70.7	81	73.0	0.768
	Other	5	9.43	4	6.89	9	8.1	
	Frank	8	15.1	13	22.4	21	18.9	
Anesthesia	General	7	13.2	6	10.3	13	11.7	0.639
	Spinal	46	86.8	52	89.7	98	88.3	
Needed additional hemostasis sutures	No	41	77.4	40	69.0	81	73.0	0.320
	Yes	12	22.6	18	31.0	30	27.0	
Tubal ligation	No	48	90.6	50	86.2	98	88.3	0.562*
	Yes	5	9.4	8	13.8	13	11.7	
Baby sex	Boy	30	56.6	33	56.9	63	56.8	0.975
	Girl	23	43.4	25	43.1	48	43.2	
Breast-feeding	No	11	20.8	6	10.3	17	15.3	0.128
	Yes	42	79.2	52	89.7	94	84.7	

p: Chi-square test, *Fisher's exact test

Table 4. Operative data of group[2]

	Classic single-layer uterine suture			Double-layer purse-string suture			
	Mean ± SD	Median (IQR)	Min-Max	Mean ± SD	Median (IQR)	Min-Max	p
Uterotomy incision length before suturing (cm)	10.8±1.4	11 (9.5-12)	8.1-12.9	11.1±1.3	11.5 (10-12)	8.1-12.9	0.334
Uterine incision length after suturing (cm)	8.4±1.1	8 (7.7-9)	7-11	4.2±0.7	4.3 (4-4.5)	2.5-5.7	<0.001
Preop Hgb (g/dL)	11.2±1.1	11.4 (10.4-11.8)	8.2-13.9	11±1.1	11.1 (10.5-11.7)	8.2-13.5	0.548
Postop 24th hour Hgb (g/dL)	10.4±1	11 (10-11)	7.3-13	10.3±1	11 (9-11)	7.3-13	0.805
Baby weight (g)	3405.3±574.1	3350 (3010-3675)	2380-5140	3312.4±550.6	3245 (2990-3655)	2380-4650	0.386
Baby height (cm)	49.4±2	49 (48-51)	46-55	49.2±2.7	49.5 (47-51)	44-57	0.645
Hospital stay (days)	2±0.3	2 (2-2)	1-3	2±0.3	2 (2-2)	1-3	0.779

P: Independent Sample t-test *Mann-Whitney U test, IQR: Interquartile range, Min-Max: Minimum-maximum, SD: Standard deviation

given the decision for cesarean section were risk factors. These were very important findings, but it is not always easy to exclude these factors in practice. Knowing risk factors is important, but categorizing them as controllable and uncontrollable and focusing on modifiable risk factors allows us to go further in terms of preventing isthmocele. Factors such as older age, the number of cesarean sections, the stage at which the cesarean section decision is made, surgery performed under emergency or elective conditions, and the presence of additional diseases have been emphasized in the literature as negative factors on ischiocele and wound healing; however, these factors cannot be controlled. This has led to the need to focus on the technique and improve the surgical technique. Accordingly, different uterine closure techniques have been described in the literature. Although Sisti et al.[17] found residual myometrial tissue to be significantly thick in their retrospective study in which they closed the uterus as a single layer and a double layer, they found the results to be similar in terms of ischiocele formation. Although it seems to be protective in terms of uterine rupture potential in pregnancies after double-layer closure, there seems to be no difference in ischiocele formation. While closing the uterus, the locked or unlocked method may affect wound healing as much as the closure coefficient.

Table 5. Comparison of postoperative 6th-week ultrasonographic results

	Classic single-layer uterine suture			Double-layer purse-string suture			
	Mean ± SD	Median (IQR)	Min-Max	Mean ± SD	Median (IQR)	Min-Max	p
Uterine incision length (mm)	6.4±1.1	6 (5.5-7)	5-9	3.2±0.7	3.3 (4-4.5)	2.5-4.7	<0.001
Distance from internal os to uterine serosal surface (Z) (mm)	9.4±1.1	9 (8.5-10.5)	8-11.3	10.8±1.5	11 (9.9-12)	8-12	0.053
Myometrial thickness adjacent to scar (Y) (mm)	10.3±1.3	10 (9-11)	8.6-13.3	11.7±1.5	12.2 (10.7-12.9)	8.9-13	0.059
Myometrial thickness at cesarean scar site (X) (mm)	5.4±0.6	5.2 (4.9-6)	4.6-6.2	6±0.5	6.1 (5.7-6.3)	5-6.5	0.066
Length of uterine incision defect (c) (mm)	6.9±1.7	7 (5.5-8.5)	4.4-9	7.8±0.9	7.8 (7-8.7)	6.8-9	0.252
Height of uterine incision defect (a) (mm)	3.7±0.5	4 (3.1-4)	2.9-4.5	3.7±0.7	3.8 (3-4.3)	2.9-4.5	0.924
Weight of uterine incision defect (b) (mm)	4.2±0.7	4 (3.6-4.9)	3-5.2	4.5±1	4.3 (3.6-5.3)	3.4-6.2	0.594
Niche volume (cm^3)	104.7±24.4	100.8 (91.1-129.6)	70.9-144	128±35.6	131.2 (95.1-156.8)	80.9-173.6	0.131

p: Independent Sample t-test *Mann-Whitney U test, IQR: Interquartile range, Min-Max: Minimum-maximum, SD: Standard deviation

In a study by Turan et al.[1] in which locked and unlocked single-layer closures were compared, it was found that unlocked uterine closure caused less damage to the myometrium and therefore might be associated with better wound healing and less ithmocele formation. However, in a meta-analysis conducted in 2011, single-layer closure was associated with twice as many uterine ruptures in a postpartum trial[18].

The comprehensive study results of Bamberg et al.[19], in which both the locked and unlocked methods and the single- and double-layer methods were compared in the same study, showed that there was no significant difference in the formation of ischiocele between these three techniques. The results of this study are consistent with our results. However, after the study, Sciosa published a letter to the editor mentioning this study, suggesting that this might be due to the difference in standardization in the evaluation[20].

The purse-string closure technique, which is the subject of our study, described by Turan et al.[12] and known in the literature as the Turan technique, was introduced in a comparative study. In their study, classic double-layer uterine closure and double-layer purse-string uterine closure were compared and short-term 6-week results were reported. The incidence of ischiocele was found to be significantly lower in the study group than in the control group. We compared the single-layer unlocked method and the Turan technique in our study, and to the best of our knowledge, ours is the first study to compare these two techniques. Some strengths of our study are that all participants underwent their first cesarean section and the standardization we provided through the strict exclusion criteria. In our study, there was no significant difference between isthmocele development between the two techniques. This may be due to the superiority of single-layer over double-layer closure and the fact that our sample group consisted of highly selected cases. Further studies are needed to clarify this distinction.

The study has several other strengths: the randomized trial design, location in a single tertiary care center, all examinations were performed by an experienced sonographer who was blinded to the uterine closure technique, the absence of emergency surgeries, and the fact that all surgeries were performed under elective conditions by a single experienced surgeon. Another strength is that all postoperative evaluations were performed standard for women using the same TVS method.

Study Limitations

This study also has some limitations. Because the patients were included in our study when they were pregnant, diseases such as adenomyosis in the uterus that have the potential to affect the formation of ischiocele were not recognized or excluded. It would be more accurate to evaluate surgical techniques in patients who were evaluated in detail before and after pregnancy. In USG follow-up examinations, the study population was not evaluated for gynecologic symptoms such as postmenstrual spotting, which may be associated with cesarean scar defects. Some of the patients had not yet returned to their normal menstrual cycles, and symptoms such as postmenstrual spotting, postcoital spotting, and menstrual irregularity were not questioned in our study. Although ischiocele was not visualized on USG in patients, clinical symptoms may have occurred because a gold standard method for the diagnosis of ischiocele has not yet been defined. There is no consensus on the advantages and disadvantages of hysterography, sonography, or USG imaging methods. Our results may have provided a limited evaluation because we used only one diagnostic method. This may be

the subject of other studies where the diagnosis is confirmed and supported using several methods. In addition, the patients could not be standardized in terms of breastfeeding, and their breastfeeding patterns were not recorded. At the beginning of the study, we calculated our sample size in line with article[12] in the literature and concluded that 28 people in each group would be sufficient. When we were calculating we aimed to compare the incidence and first occurrence of isthmocele, not the percentage decrease in the difference between the two groups, and we used our statistical analysis in this direction. We calculated our sample size in line with the information in the literature and the statistical methods we should use. At the beginning of our research, we did not know exactly what the ishomocele ratio of the data set we were going to collect would result in, and therefore, the sample width we calculated using the literature was sufficient at first. We completed exceeding the number calculated. According to the rates we calculated, we concluded that the incidence of ischiocele is 2 times higher in the classical single-layer uterine closure group than in the purse string closure group. This ratio is clinically important for us.

However, when we looked at our results when we completed our study, we did not find any statistically significant difference, although the 50.48% decrease was clinically significant. This result may be due to the fact that we did not have enough sample size to see this difference statistically. The insignificance of this difference seems to be due to the limited number of our sample. However, we could not detect a statistically significant difference between the rates we obtained, although we have more samples than calculated. This is a limitation of our study. This situation may be the subject of further studies.

The fact that the ischiocele was higher in the control group than in the study group may not only be related to the closure technique (classical vs purse string). The number of sutures (single-double layer) may have been as effective as the closure technique (classical vs purse string). In future studies, a comparison of case groups with classical single fold, clasical double fold, and purse-string double fold may provide more accurate information.

Estimation of surgical techniques should include the evaluation of the long-term effects on the functional integrity of the uterine scar. We do not know the subsequent pregnancy history of the patients and the clinical course after the 6th week. Therefore, our study needs to be confirmed with studies involving longer durations.

Conclusion

As a result of our study, we determined that there was no difference in the short-term results of classical single-layer closure and purse suture techniques in terms of ischiocele formation. The fact that the ischiocele was higher in the control group compared to the study group may not only be related to the closure technique (classical vs. bag string). The number of stitches (single-double fold) may have been as effective as the closure technique (classic and purse string). In future studies, the comparison of classic single-ply, classical double-ply and purse-string double-ply case groups may provide more accurate information. In addition, the limited number of participants may have caused the results to not be statistically different.

However,our results suggest that the technique used does not affect ischiocele formation when the patient population is standardized. In the success of surgery, the perfect application of the technique is as important as the choice of the technique. We believe that the choice of surgical procedure should be decided by discussing the risks associated with the patient and the surgeon's experience.

Ethics

Ethics Committee Approval: The study protocol was approved by the Ethics Committee of Gaziosmanpaşa Training and Research Hospital (no: 120, date: 21/09/2022), and was conducted according to the principles of the Helsinki Declaration.

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

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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

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The impact of maternal electrolyte and albumin levels on the efficacy of single-dose methotrexate treatment for ectopic pregnancies

Ektopik gebeliklerde tek doz metotreksat tedavisi başarısına maternal elektrolit ve albümin düzeylerinin etkisi

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Abstract

Objective: This study aims to investigate the impact of maternal albumin and serum electrolyte levels on the efficacy of single-dose methotrexate (SD-Mtx) therapy for ectopic pregnancies. Building on previous research, recommendations are provided to enhance the success of SD-Mtx therapy in the management of ectopic pregnancy.

Materials and Methods: Conducted at a tertiary center gynecology clinic, the study included 353 patients diagnosed with ectopic pregnancy and treated with SD-Mtx from 2012 to 2023. Patients who responded positively to SD-Mtx treatment comprised Group 1 (n=313), while those requiring surgical intervention due to failed SD-Mtx therapy constituted Group 2 (n=40). Through the hospital's digital database, patient data including complete blood count, biochemistry, and hormone test results were retrospectively examined.

Results: The mean β-hCG value was 1996 IU/mL for Group 1 in contrast to 2058 IU/mL for Group 2. There was no statistically significant difference in β-hCG levels between the two groups. Notably, Group 1 patients exhibited lower serum magnesium levels but higher potassium levels compared to Group 2 patients, with statistically significant differences. Furthermore, Group 1 patients had higher albumin levels than those in Group 2, with a statistically significant difference.

Conclusion: Successful SD-Mtx treatment was associated with lower maternal serum magnesium levels and higher potassium and albumin levels. Considering electrolyte levels before administering SD-Mtx and addressing any imbalances could potentially enhance treatment success. Additionally, restoring low albumin levels might improve the efficacy of SD-Mtx treatment for ectopic pregnancies. While this study suggests these trends, further extensive studies with a larger sample size are necessary to establish more definitive evidence.

Keywords: Ectopic pregnancy, methotrexate, inflammation, β-hcg, serum electrolytes, albumin

Öz

Amaç: Bu çalışmanın amacı maternal albümin ve serum elektrolit düzeylerinin, ektopik gebelik tedavisinde tek doz metotreksat (TD-Mtx) tedavisi üzerine etkisini incelemektir. Daha önce yapılan araştırmalar eşliğinde ektopik gebelik tedavisinde TD-Mtx tedavisinin başarısını artıracak önerilerde bulunmaktır.

PRECIS: Continuous studies have been made to make medical treatment more successful in treating ectopic pregnancy, but sufficient results have not been obtained. In our research, we think that the levels of magnesium and potassium from maternal electrolytes and the plasma protein albumin level may help predict the success of medical treatment in the treatment of ectopic pregnancy in addition to β-hCG, which is the most used parameter in the literature. In addition, we believe that correcting blood electrolyte and albumin values and adjusting them to reference values will increase the success of TD-Mtx treatment in ectopic pregnancy treatment.

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Gereç ve Yöntemler: Çalışma tersiyer merkez olan kadın doğum kliniğinde yapıldı. Çalışmaya 2012-2023 yılları arasında ektopik gebelik tanısı almış ve TD-Mtx tedavisi uygulanmış 353 hasta dahil edildi. TD-Mtx tedavisine yanıt vermiş hastalar Grup 1 (n=313) ve TD-Mtx tedavisi başarısız olup cerrahi tedavi uygulanan hastalar Grup 2 (n=40) olarak belirlendi. Hastaların tam kan, biokimya ve hormon tetkik sonuçları hastane dijital veri tabanı üzerinden retrospektif olarak incelendi.

Bulgular: Grup 1'e dahil edilen hastaların β-hCG ortalama değeri 1996 IU/mL idi. Grup 2'ye dahil edilen hastaların β-hCG ortalama değeri 2058 IU/mL idi. İki grup arasında β-hCG düzeyleri açısından istatistiki olarak anlamlı fark yoktu. Grup 1 hastalarının serum magnezyum değerleri, Grup 2 hastaların serum magnezyum değerlerinden bariz düşük, potasyum değerleri ise daha yüksek bulundu. İki grup arasındaki magnezyum ve potasyum değerlerinin farkı istatistiki olarak da anlamlı idi. Ayrıca albümin değerleri Grup 1 hastalarda, Grup 2 hastalara göre daha yüksek ve aradaki fark istatistiki olarak anlamlı idi.

Sonuç: TD-Mtx tedavisi başarılı olan grupta maternal serum M seviyesi düşük, K ve albümin seviyesi ise yüksek bulundu. TD-Mtx tedavisi öncesi kan elektrolit değerlerinin değerlendirilmesi ve var olan düzensizliğin düzeltilmesi, TD-Mtx tedavisinde başarıyı artırabilecek bir yaklaşım olabilir. Ayrıca düşük albümin değerlerinin de düzeltilmesi ektopik gebelik tedavisinde TD-Mtx tedavi başarısını artırabilir. Bu konu ile ilgili daha net kanıtlar elde edebilmek için daha geniş olgu sayıları ile daha fazla çalışmaya ihtiyaç vardır.

Anahtar Kelimeler: Ektopik gebelik, metotreksat, enflamasyon, β-hCG, serum elektrolitleri, albümin

Introduction

An ectopic pregnancy arises when a fertilized ovum implants itself outside the uterus, typically within the fallopian tubes, often in the ampulla[1]. Risk factors encompass a history of tubal surgery or ectopic pregnancy, pelvic inflammatory diseases, smoking, and specific assisted reproductive techniques[2]. The combined utilization of transvaginal ultrasonography and β-hCG levels aids in the prompt diagnosis[3], facilitating the selection of an appropriate treatment strategy to enhance outcomes and mitigate associated mortality and morbidity[4].

One prominent approach for managing unruptured ectopic pregnancies involves single-dose methotrexate (SD-Mtx) therapy[5]. This regimen involves administering a solitary intramuscular dose of 50 mg/m² SD-Mtx. In cases where β-hCG levels do not exhibit a decrease of less than 15% between the 4th and 7th days, a second dose is administered, a course of action necessitated in approximately 14-20% of cases[6]. Generally, the success rates of SD-Mtx treatment vary between 64-80%[7]. The achievement of success hinges on attaining β-hCG values below 5.000 IU/mL and the absence of fetal cardiac activity[8]. However, the risk of tubal rupture persists, underscoring the importance of identifying predictive factors for the success of medical treatment[2,8,9].

Methotrexate, acting as a folic acid antagonist, competitively inhibits the enzyme dihydrofolate reductase, thereby impeding cellular proliferation[8]. Much like other enzymes, specific co-enzymes or co-factors can influence this effect. A range of reductants and oxidants partake in these redox-type reactions. Electropositive alkali metals such as lithium, sodium, magnesium, iron, zinc, and aluminum function as potent reductive agents. Given their impact on numerous bodily enzymes, any fluctuations in electrolyte levels can reverberate across the system, maintaining overall bodily balance[10].

Substances such as drugs and hormones are primarily transported within the body through plasma proteins, with albumin being a prominent carrier[11,12]. When substances are bound to albumin, they remain inactive in the plasma. This suggests that the efficacy and duration of drug action could be influenced by fluctuations in plasma carrier proteins, notably albumin levels[13-15]. The presence of albumin's dual metal ion binding sites enables it to bind with a range of ions, including zinc, copper, cadmium, mercury, gold, silver, and nickel. Additionally, albumin interacts with calcium and magnesium, thereby exerting an impact on their concentrations within the blood[11,15]. Notably, these variations in electrolyte levels can also lead to alterations in enzyme activity, potentially resulting in individualized changes in methotrexate effectiveness.

The aim of our study is to conduct a comparative analysis of maternal serum electrolyte and albumin levels between patients who demonstrated successful response to TD-Mtx therapy and those who experienced treatment failure, requiring surgical intervention. Through this investigation, we aim to ascertain whether these serum levels have a discernible influence on the success of TD-Mtx treatment.

Materials and Methods

The study was conducted within the Department of Obstetrics and Gynecology at the Van Regional Training and Research Hospital, a tertiary center, spanning from 2012 to 2023. Approval for this non-interventional study was obtained from the local ethics committee (Van Training and Research Hospital Clinical Research Ethics Committee), under the reference number 2023/03-03. The study involved a total of 353 patients who presented at our clinic with ectopic pregnancy and subsequently underwent TD-Mtx treatment.

Ectopic pregnancy diagnosis was established through a combination of transvaginal ultrasound examination, clinical manifestations, and irregularly elevated β-hCG levels. Among the patients diagnosed with ectopic pregnancy, individuals with β-hCG levels surpassing 5.000, those with detectable fetal heartbeats, and those with a gestational sac measuring 2 cm or more were directed towards surgical intervention and were consequently excluded from our study. The participants were categorized into two groups: Group 1 comprised those who responded favorably to TD-Mtx treatment, while Group 2 consisted of individuals for whom TD-Mtx treatment proved ineffective, leading to subsequent surgical intervention.

Participants who underwent emergency surgery due to ectopic rupture, those who declined Mtx treatment, and individuals with pre-existing conditions such as diabetes, chronic kidney failure, and chronic heart disease were not included in the study. Furthermore, patients below 18 years of age or above 40 years were also excluded. The demographic information, clinical assessments, and laboratory results of the patients were acquired from the hospital's digital database and archival records. Throughout the study, the researchers diligently adhered to the guidelines stipulated by the Declaration of Helsinki pertaining to medical research involving human subjects.

Statistical Analysis

The statistical analysis was conducted utilizing the licensed SPSS 22.0 software. To compare two distinct independent groups with a normal distribution, the independent t-test was employed. The level of statistical significance was set at $p<0.05$.

Results

A total of 353 patients who underwent TD-Mtx treatment were enrolled in the study. Among these, 40 patients did not exhibit a favorable response to medical treatment and necessitated surgical intervention. The patients who demonstrated a positive response to TD-Mtx treatment were categorized as Group 1, while those who did not respond were labeled as Group 2. The mean age of the patients in Group 1 was 31.26 years, whereas Group 2 patients had a mean age of 30.58 years. No statistically significant difference in age was observed between the two groups of pregnant women.

The mean β-hCG level in Group 1 was recorded as 1996 IU/mL, while Group 2 exhibited a mean value of 2058 IU/mL. In terms of β-hCG levels, no statistically significant difference was identified between the two groups.

Table 1. Distribution of groups according to maternal characteristics

	Methotrexate (n=313)		Surgical (n=40)		
	Mean	SD	Mean	SD	p
Age	31.26	4.80	30.58	5.75	0.11
Gravida	2.83	1.45	2.85	1.69	0.24
Parity	1.16	1.16	1.23	1.23	0.22
Abortion	0.73	0.79	0.78	1.20	0.38
EG	0.074	0.32	0.06	0.32	0.36
Height (cm)	162.85	6.67	162.50	7.20	0.13
Weight (kg)	67.70	10.34	68.5	8.4	0.17
BMI (kg/m^2)	25.87	4.05	25.38	3.13	0.16
Gestational week	6.62	0.64	6.47	0.97	0.23

SD: Standard deviation, BMI: Body mass index. Values in bold represent statistically significant results

Table 1 presents the distribution of the groups by maternal characteristics. No significant distinctions were observed between the groups concerning factors such as age, parity, gravidity, abortion history, body mass index, gestational week, prior ectopic pregnancy occurrences, weight, and height.

The distribution of the groups by complete blood count and coagulation parameters is detailed in Table 2. No discernible differences were noted between the groups in terms of pre-treatment hemoglobin and hematocrit values. Similarly, there were no significant differences between the groups regarding platelet count (PLT), activated partial thromboplastin time (aPTT), prothrombin time, and fibrinogen values.

Table 3 outlines the distribution of groups based on hormone and electrolyte levels. There were no significant differences in thyroid hormone values between the groups. However, notable differences emerged in terms of albumin and creatinine levels. Group 1 patients exhibited higher albumin and creatinine values in comparison to those in Group 2.

The analysis of electrolyte values revealed no statistically significant difference between the two groups concerning sodium and calcium values, while a statistically significant difference surfaced in terms of potassium and magnesium values. More specifically, potassium values were elevated in Group 1 patients, whereas magnesium values were notably higher in Group 2 patients.

Discussion

Ectopic pregnancy represents a significant gynecological concern with implications for maternal mortality and morbidity[16,17]. Methotrexate (MTX) typically serves as the primary treatment option for stable cases of unruptured ectopic pregnancies. MTX can effectively address ectopic pregnancies

Table 2. Distribution of groups according to hemogram and coagulation parameters

	Methotrexate (n=313)		Surgical (n=40)		
	Mean	SD	Mean	SD	p
WBC (10^9/L)	8.27	2.06	9.10	3.20	**0.002**
HGB (g/dL)	12.92	1.31	12.87	1.7	0.092
HCT (%)	38.75	3.53	37.09	5.83	0.093
APTT (sec)	29.03	3.92	28.15	3.63	0.076
PT (sec)	14.10	1.25	13.55	1.16	0.086
PTZ%	1.00	0.00	1.00	0.00	cannot be computed
INR	94.25	12.26	95.18	13.07	0.092
Fibrinogen	326.48	72.63	332.82	74.64	0.42

SD: Standard deviation, WBC: White blood cell, Hb: Hemoglobin, HCT: Hematocrit, MON: Monocyte ratio, MCV: Mean corpuscular volume, PLT: Platelet count, MPV: Mean platelet volume, aPTT: Activated partial thromboplastin time, values in bold represent statistically significant results

Table 3. Distribution of groups according to hormone and electrolyte values

	Methotrexate (n=313)		Surgical (n=40)		
	Mean	SD	Mean	SD	p
TSH (U/L)	2.33	1.83	2.59	1.73	0.18
ft3 (pmol/L)	3.27	0.43	3.23	0.73	0.44
fT4 (pmol/L)	1.11	0.29	1.04	0.21	0.64
BUN (mg/dL)	10.81	3.11	10.37	2.26	0.16
CRE (mg/dL)	**0.68**	**0.42**	**0.56**	**0.51**	**0.001**
Albumin (g/dL)	**46.20**	**2.99**	**38.08**	**3.10**	**0.001**
AST (IU/L)	21.12	5.62	21.91	9.33	0.29
ALT (IU/L)	20.05	8.25	19.95	11.24	0.92
LDH (IU/L)	**195.55**	**40.97**	**209.09**	**40.40**	**0.02**
Na (mg/L)	139.20	2.27	139.18	1.78	0.93
Ca (mg/L)	9.51	0.66	9.45	0.58	0.36
K (mg/L)	**4.32**	**0.16**	**4.03**	**0.13**	**0.026**
Mg (mg/L)	**1.89**	**0.16**	**2.13**	**0.07**	**0.025**
Bhcg	**1996**	**287**	**2058**	**327**	**0.07**

SD: Standard deviation, TSH: Thyroid stimulating hormone, fT4: Free T4, fT3: Free T3, BUN: Blood urea nitrogen, CRE: Creatinine, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, values in bold represent statistically significant results

situated in various locations such as the cervix and cornual region, while preserving the patient's ovarian reserve and future fertility prospects[18]. Global studies have reported MTX success rates ranging from 65% to 95%[19]. In our investigation, the success rate of MTX treatment reached 88.7%.

Numerous studies in the existing literature have explored factors influencing the efficacy of systemic methotrexate (TD-Mtx) treatment, aiming to curtail maternal mortality and morbidity associated with ectopic pregnancies. Among these, β-hCG levels have emerged as pivotal prognostic indicators for predicting and assessing the effectiveness of Mtx treatment. While a definitive consensus on the ideal threshold value for predicting the success or failure of β-hCG is lacking, a substantial body of data employs 5.000 IU/mL as a threshold indicative of higher failure rates[18]. Within our present study, the mean β-hCG values for both patient groups remained below 5.000 IU/mL. Specifically, in the successfully treated TD-Mtx group, the mean β-hCG level was noted as 1.996 IU/mL, while the unsuccessful group exhibited a mean value of 2.058 IU/mL, with no significant difference between the two groups.

Certain studies in the literature have delved into the relationship between the success and failure of TD-Mtx treatment for ectopic pregnancy and various factors, including inflammation markers, white blood cell (WBC) count, and PLT, among others. Nonetheless, it is recognized that employing inflammation markers, WBC, and PLT values as predictors of the success of medical ectopic pregnancy treatment might prove challenging. Changes in these inflammation-related values are influenced by a multitude of factors and lack specificity for ectopic pregnancy. As a result, ongoing research seeks to identify alternative factors that can enhance the efficacy of Mtx treatment[20,21].

Another study in the literature explored the combination of methotrexate with mifepristone and found no discernible difference. Moreover, this study recommended avoiding the concurrent use of potassium chloride with methotrexate[22]. In our current study, Group 1 patients displayed lower serum magnesium values but higher potassium levels. Our results indicate that the success of TD-Mtx treatment was more prevalent among patients with elevated pre-treatment serum potassium and albumin levels, along with lower magnesium levels. This suggests that fine-tuning electrolyte and albumin levels in patients undergoing treatment for ectopic pregnancies could potentially boost treatment success rates.

Our study investigated whether changes in maternal electrolyte levels influenced the activity and inhibition of the dihydrofolate reductase enzyme, a key player in the mechanism of methotrexate action. We also explored whether the success of TD-Mtx treatment was impacted by these electrolyte levels and associated parameters.

In the existing literature, no studies have investigated the influence of electrolytes on methotrexate's effects. In this study, we aimed to explore the potential impact of maternal electrolyte levels on methotrexate's mechanism of action, with the goal of enhancing the success of medical treatment. Our results showed that within the context of the mechanism of methotrexate, magnesium levels negatively affected the success of TD-Mtx treatment, while potassium levels had a positive influence. Nonetheless, we did not identify any specific electrolyte directly affecting methotrexate's actions.

Another crucial aspect we examined in our study was albumin. As a primary plasma protein, albumin plays a pivotal role in facilitating the transport of various hormones, drugs, and substances within the bloodstream. Additionally, albumin can act as a reservoir for the drugs it carries, thereby influencing their pharmacokinetic profiles. This pertains to albumin's involvement in the distribution and metabolism of many drugs, including methotrexate. While methotrexate has a half-life of 2-3 hours, albumin's half-life extends to 18-19 days. Elevated albumin levels can potentially lead to an increased distribution volume of methotrexate, potentially augmenting its therapeutic response[14,15]. Consequently, monitoring albumin levels in methotrexate-treated patients could offer valuable insights into optimizing therapeutic outcomes in ectopic pregnancy management. This could involve minimizing adverse reactions, reducing free radicals (as in various diseases), and potentially enhancing the body's responsiveness. Moreover, heightened albumin levels might extend methotrexate's half-life in the body, thereby prolonging its therapeutic effects and potentially contributing to more favorable outcomes in ectopic pregnancies.

As such, we propose that maintaining albumin levels within the normal range could potentially amplify the efficacy of methotrexate and lead to improved outcomes in the treatment of ectopic pregnancies.

Study Limitations

Nevertheless, our study does have certain limitations, including its retrospective nature, a limited number of patients, and the utilization of patient records from the healthcare system. On the positive side, our study sheds light on the previously unexplored relationship between TD-Mtx and albumin. It represents one of the few studies revealing a relationship between TD-Mtx and electrolyte values.

Conclusion

We hold the perspective that maternal electrolyte levels, particularly magnesium, and potassium, along with the plasma protein albumin, could emerge as valuable indicators for predicting the success of medical treatment in the context of ectopic pregnancy management, complementing the role of β-hCG. Additionally, we posit that the optimization of blood electrolyte and albumin values, aligning them with established reference ranges, might significantly enhance the efficacy of TD-Mtx in the management of ectopic pregnancy.

The results of our study are anticipated to serve as a foundational resource and reference for subsequent research endeavors focused on this subject matter. We believe that more extensive and comprehensive studies are warranted to yield more nuanced and well-defined data, thus contributing to a deeper understanding of this relationship.

Ethics

Ethics Committee Approval: Approval for the non-interventional study was obtained from the Van Training and Research Hospital Clinical Research Ethics Committeewith approval number 2023/03-03, date: 01.02.2023.
Informed Consent: Retrospective study.
Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Y.B., K.U., T.K., İ.Ç., Z.A., Concept: Y.B., K.U., T.K., İ.Ç., Z.A., Design: Y.B., K.U., T.K., İ.Ç., Z.A., Data Collection or Processing: Y.B., K.U., T.K., İ.Ç., Z.A., Analysis or Interpretation: Y.B., K.U., T.K., İ.Ç., Z.A., Literature Search: Y.B., K.U., T.K., İ.Ç., Z.A., Writing: Y.B., K.U., T.K., İ.Ç., Z.A.
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Effects of metformin and ganirelix on subcutaneous endometriosis in a mouse model of autophagy-related cell death

Otofaji ile ilişkili hücrelerde subkütan endometriozis fare modelinde metformin ve genireliksin etkisi

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Abstract

Objective: This study aimed to investigate the efficacy of metformin and ganirelix on subcutaneous endometriotic tissues created in an experimental mouse model.

Materials and Methods: Five groups were formed with eight animals in each group. One of the groups was set as the control group. Endometriotic lesions were created by transplanting 40 mouse autologous endomyometrial tissues into the mouse subcutaneous tissue to a highly vascular surface. Gene expression analyzes of tissues were performed as *HIF-1a*, *ATG5*, *ATG12*, *Beclin2*, *Beclin1*, *LC3BII*, *CateninB*, *GSK3b*, *TCF*, *WNT2*, *WNT7a*, and *WNT10a* gene analyzes. Drug effects were examined by histological examination. HIF1a and WNT2 protein expressions were examined immunohistochemically. Gene expression coefficients of control, metformin day 1 (Met1g), metformin day 7 (Met7g), ganirelix day 1 (Gnx1g), and ganirelix day 7 (Gnx7g) groups are shown in tables. Data are presented as mean and standard error.

Results: *Beclin2* gene expression coefficients of metformin 1st day, metformin 7th day, ganirelix 1st day, and general 7th day groups were found to have significantly decreased compared with the control group coefficient. *Beclin1* gene expression coefficients of metformin 1st day, metformin 7th day, ganirelix 1st day, and genirelix 7th day groups were found to have significantly decreased compared with the control group coefficient. *LC3BII* gene expression coefficients of metformin 1st day and metformin 7th day groups were found to have significantly decreased compared with *LC3BII* gene expression coefficients of control, genirelix 1st day, and genirelix 7th day groups. These findings were supported by histological and immunohistochemical staining.

Conclusion: These genes are actively involved in the autophagy pathway, and we think that the use of metformin in endometriosis might create an autophagy-based suppression mechanism.

Keywords: Endometriosis, ganirelix, metformin, subcutaneous endometriosis, mouse model

Öz

Amaç: Bu çalışmanın amacı, deneysel bir fare modelinde oluşturulan deri altı endometriotik dokular üzerindeki metformin ve genireliksin etkinliğini araştırmaktır.

PRECIS: We aimed to to investigate the efficacy of metformin and ganirelix on subcutaneous endometriotic tissues created in an experimental mouse model might create an autophagy-based suppression mechanism.

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Gereç ve Yöntemler: Her grupta 8 hayvan olacak şekilde 5 grup oluşturuldu. Gruplardan biri kontrol grubu olarak belirlendi. Endometriotik lezyonlar, 40 adet farenin otolog endomyometrial dokusunun fare deri altı dokusuna vasküler bir yüzeye nakledilmesiyle oluşturuldu. Dokuların gen ekspresyon analizleri *HIF1a*, *ATG5*, *ATG12*, *Beclin2*, *Beclin1*, *LC3BII*, *CateninB*, *GSK3b*, *TCF*, *WNT2*, *WNT7a*, *WNT10a* gen analizleri olarak yapıldı. Histolojik inceleme ile ilaç etkileri incelendi. İmmünohistokimyasal olarak HIF1a and WNT2 protein ifadelenmeleri incelendi.

Bulgular: Metformin 1. gün, metformin 7. gün, genireliks 1. gün, genireliks 7. gün gruplarının *Beclin2* gen ifadelenme katsayıları kontrol grubu katsayısı ile karşılaştırıldığında anlamlı olarak azalmış bulundu. Metformin 1. gün, metformin 7. gün, genireliks 1. gün, genireliks 7. gün gruplarının *Beclin1* gen ifadelenme katsayıları kontrol grubu katsayısı ile karşılaştırıldığında anlamlı olarak azalmış bulundu. Metformin 1. gün, metformin 7. gün gruplarının *LC3BII* gen ifadelenme katsayıları kontrol, genireliks 1. gün ve genireliks 7. gün gruplarının *LC3BII* gen ifadelenme katsayıları ile karşılaştırıldığında anlamlı olarak azalmış bulundu. Histolojik ve immünohistokimyasal boyamalar ile bu bulgular desteklendi.

Sonuç: Araştırma kapsamına alınan genler aktif olarak otofaji yolunda yer almaktadır, araştırma bulguları endometrioziste etkin kabul edilen genireliks ile aynı araştırma düzeneğinde incelenen metforminin otofajiye dayalı bir baskılama mekanizması oluşturarak endometriotik odakları baskıladığını desteklemektedir.

Anahtar Kelimeler: Endometriozis, genirelix, metformin, subkütan endometriozis, fare modeli

Introduction

Endometriosis is a chronic inflammatory gynecological condition characterized by the implantation and estrogen-dependent growth of endometrial glands and stromal structures outside the uterine cavity[1]. It usually causes chronic pelvic pain, infertility, menstrual irregularity, and dyspareuni[1]. It is a common disease affecting 7-11% of women of reproductive age and 40% of women undergoing infertility treatment worldwide[2]. It brings a serious economic burden because of a delay in diagnosis, misdiagnosis, and not providing effective treatments despite its high prevalence.

Although the pathophysiology of the disease is not fully known, a wide variety of genetic and environmental factors are thought to be effective[3]. Possible mechanisms such as retrograde menstruation, altered immunity, celomic metaplasia, and metaplastic distribution are considered likely factors in the disease pathophysiology. Although endometriosis is a benign disease, it possesses migration and invasive features similar to cancer[4]. Many studies have reported many factors contributing to the development of this disease, including inflammation, angiogenesis, vascularization, oxidative stress, apoptosis resistance, and immunological dysregulation[5].

The human endometrium is a dynamic tissue undergoing monthly cyclic changes such as proliferation, differentiation, and degeneration. These regular changes, due to alterations in sex hormone concentrations, cause ischemic necrosis in the functional layer of the endometrium, usually because of the contraction of the spiral arteries[6]. Recent studies have reported that apoptosis, a form of programed cell death, is observed in endometrial epithelial cells in the late secretory and menstrual phases and is reduced during the proliferative phase or the beginning of the secretory phase[7]. During the late secretory and menstrual phases of the menstrual cycle, aging endometrial cells are detached from their functional layer by apoptosis[8]. However, non-apoptotic forms of programed cell death have also been observed in human endometrial tissues[8].

Apoptosis and autophagy are two tightly regulated biological processes that play an important role in maintaining tissue homeostasis and cell development. Studies have classified cell death into three forms; apoptosis, autophagic cell death, and necrosis. Autophagy is a double-edged sword with protective and harmful effects[9]. Autophagy is a conservative lysosome-dependent intracellular catabolic process that degrades aged or dysfunctional proteins to regenerate energy and intracellular structures to maintain cellular homeostasis under various stress conditions such as nutrient deprivation, energy depletion, and hypoxia[10]. Autophagy, characterized by the accumulation of autophagic vacuoles, is considered a type II programed cell death known as autophagic cell death[11]. However, uncontrolled autophagy may play a harmful role in cell survival by promoting autophagic cell death[11]. Autophagy plays a significant role in the normal development and maintenance of homeostasis in various tissues, including the female reproductive system[7]. Recent studies have shown the abnormal autophagic activity in endometriosis[11].

In a study investigating Beclin 1 (Becn1) expression in human adenomyosis and its relation to clinical features, it was revealed that Becn1 mRNA and protein expression were significantly decreased in the eutopic endometrium of women with adenomyosis. Besides, Beclin 1 was negatively correlated with serum CA125 and pelvic pain[7]. According to current findings, Becn1 contributes to the initiation and progression of endometriosis. Becn1 is the autophagy protein required for autophagy. Initially, Beclin 1 was identified as a tumor suppressor inducing autophagy and was the first natural link identified between autophagy and carcinogenesis[12]. Beclin 2 (Becn2) has been recently identified as a Becn1 homolog with both autophagy-dependent and independent functions targeting G protein-coupled receptors (GPCRs) for degradation by the endosomal-lysosomal pathway[12]. The LC3BII protein also plays an active role in the autophagy pathway[13].

Ganirelix is a third - generation GnRH antagonist and is released in a dose-dependent and sustained manner. It stops the release of FSH and LH from the pituitary by binding to GnRH receptors. It is used to treat pain due to endometriosis[24]. Recent studies have shown that metformin has several effects and is responsible for autophagic changes in this context[14]. Since the autophagic biomarkers Beclin1, Beclin2 and LC3BII are actively involved in the autophagy pathway, we think that the use of metformin in endometriosis may constitute an

autophagy-based suppression mechanism. Our study aimed to examine the changes in these biomarkers when metformin was used together with ganirelix, a GnRH inhibitor, in a mouse endometriosis model.

Materials and Methods

Study Design

Ethics committee approval of the study was obtained from Sivas Cumhuriyet University (SCU) Animal Experiments Local Ethics Committee (decision no: 65202830-050.04.04-306, date: 28.08.2019). In this study, 40 mice weighing between 20 and 30 g were used and all experiments were performed in the Laboratory of Experimental Animals of SCU Faculty of Medicine. During the experiment, the mice were kept in an environment with ad libitum access to food (standard rat feed) at a constant temperature of 22°C, with a 12-h day and 12-h night cycle. The animals to be used in the experiment were fasted 12 h before and were allowed to drink only water.

Five groups were formed, with 8 animals in each group. One of the groups was set as the control group. Endometriotic lesions were created by transplanting 40 mouse autologous endomyometrial tissues into the mouse subcutaneous tissue to a highly vascular surface. Metformin was started to be administered to one group by adding it to the drinking water of the animals 24 h after the operation and continued until the end of the study. In another group, Ganirelix application was continued until the end of the study at a dose of 10 mg/kg, starting 24 h after the operation. Metformin was added to drinking water 7 days after the operation in one group and continued until the end of the study. Ganirelix at a dose of 10 mg/kg was started 7 days after the operation in one group and continued until the end of the study. The second operation was performed 2 weeks after the first operation, and the endometriotic lesions were evaluated. The degree of shrinkage in endometriotic lesions was recorded. The lesion regression rates in the treatment groups were compared with the control group. The treatments were discontinued after the second operation, and a necropsy operation was performed to evaluate their condition. Biopsies were taken from endometrial lesions in all operations for histopathological score evaluation. For genetic analysis, tissue pieces were placed in pre-sterilized and labeled 1.5 mL Eppendorf tubes and 1 mL of Ribo Saver (Gene All, Seoul, Korea) solution was added to them. Tissue samples were stored at -80 °C until total RNA isolation.

Gene Expression Analyzes

The GeneAll® Hybrid-RTM Kit was used for total RNA isolation (GeneAll® Hybrid-RTM - Seoul, Korea. Cat. No: 305-101- Lot. No: 30519L09056) and procedures were performed according to the manufacturer's instructions. The concentration and purity control of the obtained samples were measured spectrophotometrically (Denovix DS nanodrop). Care was taken to keep the total RNA in the range of approximately 20-40 ng for each sample. WizScript™ cDNA Synthesis Kit (South Korea) was used to obtain cDNA. In the cDNA synthesis stage, the reaction content was created from 10X reaction buffer, 20X dNTP mix, random hexamer, WizScript™ RTase, RNase inhibitor, and RNase free water components, and the kit procedure was applied.

Applied Biosystem Step One Plus real-time PCR (USA) device was used for gene expression analysis. GeneAll Real Amp TM SYBR master mix (Seoul, Korea) was used in Real Time qPCR stage. SYBR Green and passive reference dye ROX were used for quantitative analysis of the synthesized cDNA samples. In the Real-Time qPCR stage, the reaction medium was prepared in 10 µL, including Master mix (2X), ROX (50X), forward and reverse primers (10pmol each), nuclease-free water, and cDNA. For RT-qPCR, reaction conditions of 40 cycles of 10 min of initial denaturation at 95 °C, 15 s of denaturation at 95°C, and 1-minute annealing/extension/melting at 60 °C were used. The primers used for the genes whose expressions were investigated in this study are given below (Table 1). *ACTB* gene was used as a housekeeping gene in the real time qPCR stage. The delta delta Ct Method (ΔΔCt) was used for relative quantification. ΔΔCt values were taken as 2-ΔΔCt, and the fold change of expression level was calculated.

Histopathological Evaluation

The endometriotic cysts were fixed in 10% buffered neutral formalin for 30-36 hours. After routine histological processing, the cyst walls were cut into 5-µm thick sections and stained with hematoxylin-eosin (H&E) for glandular tissue and stromal tissue evaluation in the cyst walls. Glandular and stromal tissues were histopathologically evaluated and scored as described previously[15]. Briefly, while determining the average percentage of stromal tissue-containing areas in each of the 10 high-power areas (HPF), glandular tissue scoring was performed by the average of the gland numbers in these 10 HPF. Then, the score was evaluated as follows: score 0, no stromal and glandular tissue; score 1, <25% stromal tissue and 1 glandular tissue; score 2, 25% to 50% stromal tissue and 2-3 glandular tissue; and score 3, >50% stromal tissue and ≥4 glandular tissue.

The implants were excised and fixed with 10% formalin solution for histological examination. After routine histological follow-ups with alcohol, xylene, and paraffin, the tissues were embedded in paraffin blocks. Serial sections of 5-micrometer thickness were taken from endometriotic foci. After routine deparaffinization, sections from each sample (four sections from each sample) were stained with hematoxylin and eosin, and routine histopathological evaluations were made under a light microscope (Olympus BX50). In control and experimental groups;

Endometrial surface epithelium in each cyst was evaluated as a- well preserved, b- moderately preserved, c- poorly preserved epithelial layer, and d- absence of epithelial cells.

Glandular tissue amount was evaluated as 0 if there is no gland, 1 if there is one gland, 2 if there are one to three glands, and 3 if

Table 1. Primer information used for expression analysis

Primer Name	Primer Sequence
LC3BII-F	TTATAGAGCGATACAAGGGGGAG
LC3BII-R	CGCCGTCTGATTATCTTGATGAG
Beclın1-F	ATGGAGGGGTCTAAGGCGTC
Beclın1-R	TCCTCTCCTGAGTTAGCCTCT
ATG5-F	AGCCAGGTGATGATTCACGG
ATG5-R	GGCTGGGGGACAATGCTAA
ATG12-F?	TCCCCGGAACGAGGAACTC
ATG12-R?	TTCGCTCCACAGCCCATTTC
Beclın2-F	TCAGCCGGAGACTCAAGGT
Beclın2-R	CACAGCGGGTGATCCACATC
HIF1a-F	ACCTTCATCGGAAACTCCAAAG
HIF1a-R	ACTGTTAGGCTCAGGTGAACT
CateninB-F	ATGGAGCCGGACAGAAAAGC
CateninB-R	CTTGCCACTCAGGGAAGGA
GSK3b-F	TGGCAGCAAGGTAACCACAG
GSK3b-R	CGGTTCTTAAATCGCTTGTCCTG
TCF-F	CGCACCAGCAGTACAGATGAG
TCF-R	CAGCTTGGTCTGCGCCTTA
WNT7a-F	TCAGTTTCAGTTCCGAAATGGC
WNT7a-R	CCCGACTCCCCACTTTGAG
WNT10a-F	GCTCAACGCCAACACAGTG
WNT10a-R	CGAAAACCTCGGCTGAAGATG
WNT2-F	CTCGGTGGAATCTGGCTCTG
WNT2-R	CACATTGTCACACATCACCCT
ACTB-F	GGCTGTATTCCCCTCCATCG
ACTB-R	CCAGTTGGTAACAATGCCATGT

there are four or more glands after examining the cyst wall with 20X and 40X magnification.

Stromal tissue amount; After examining the cyst wall with 20X and 40X magnifications, endometrial stroma intensity was evaluated semiquantitatively and scored from 0 to 3.

The presence and severity of inflammation were evaluated as absent, mild, moderate, and severe according to the intensity of inflammatory cells (macrophages, lymphocytes, plasma cells, and eosinophils) in the cyst wall and periphery.

The scoring system used in the evaluation of the examined parameters is summarized in Table 2.

Immunohistochemical Evaluation

Wnt2 and Hif1a expressions were immunohistochemically evaluated in cyst walls. For immunohistochemistry, after

Table 2. Histopathological scoring

Score	Epithelium	Gland Amount	Stroma Amount	Inflammation
0	Absent	No gland	Not observed	Absent
1	Poorly	Decreases: 1	Sparse	Mild
	Preserved	Gland		
2	Moderately	Moderate: 2-3	Moderate	Moderate
	Preserved	Glands		
3	Well	Abundant: ≥4	Abundant	Severe
	Preserved	Glands		

deparaffinization and rehydration, the slides were immersed in boiling 0,01M sodium citrate buffer (pH 6.0) for 10 min, and then incubated in 3% hydrogen peroxide to deactivate endogenous peroxidase. After incubation with ultra V block (Thermo Fisher Scientific), the slides were incubated with primary antibodies (Wnt2, 1:100, BTLAB and BT-AP00075 and Hif1a, 1:100, BTLAB and SKNDRANTK) overnight at 4 °C. The next day, after washing in PBS, sections were incubated with Primary Antibody Enhancer Solution for 20 min (TL-015-PB, Thermo Fisher Scientific, USA) followed by horseradish peroxidase (HRP) Polymer TL-015-PH, Thermo Fisher Scientific, USA) for 30 min at room temperature and subsequently treated with 3,3-diaminobenzidine (DAB) solution for color development. The sections were counterstain with Harris' hematoxylin and evaluated under a microscope (Olympus BX50). The slides were scored as described previously[16]. Wnt2 and HIF1a immunostaining were scored based on intensity and percentage of positive staining cells. The intensities were scored as follows: 0, negative; 1, weak; 2, intermediate; 3, strong. The percentage of positive staining cells was scored as follows: 0, no staining; 1% to 10%; 2, 1% to 50%; 3, >50%. The final immunoreactive score was calculated by multiplying the staining intensity and the percentage of positive staining cells and the final staining score ranged from 0 to 9.

Tissue sections taken from the blocks used in the histopathological examination on the adhesive (silanized) slides with a thickness of 5 micrometers were incubated at 37 °C for 12 h, then deparaffinized in xylol, dehydrated in alcohol, and lowered into the water. Before anti-Wnt2 and Hif1a antibody application, an "antigen retrieval" procedure was applied. Trisodium citrate buffer solution (antigen retrieval solution) prepared as 0.01M (pH 6.0) was used for this procedure. Sections were placed in citrate solution and boiled in a microwave oven at 750 W, 500 W, respectively, for 4.5 min at each stage. It was then treated with 0.3% H_2O_2 for 15 min at room temperature to suppress the peroxidase activity. Before primary antibody application, Ultra V Block solution (Thermo Fisher Scientific) was dripped onto the sections and blocked for 5 min, then primary antibodies Wnt2 (1:100,

BRAND and CATALOG number) and Hif1a (1:100, BRAND and CATALOG number) were applied to the sections without washing and incubated at +4 °C overnight. The next day, the sections were washed with PBS (phosphate buffered saline) and left for 20 min by dripping Lab Vision Primary Antibody Enhancer (Thermo Fisher Scientific) solution and washed with PBS again. Sections washed with PBS 30 min after the HRP Polymer (Thermo Fisher Scientific) solution of Lab Vision was dropped and kept in diaminobenzidine (DAB, Thermo Fisher Scientific) for 3 min as a chromogen to ensure the visibility of the reaction. Harris hematoxylin was used for background staining. Finally, the sections passed through alcohol, and xylol was closed with entellan.

Statistical Analysis

The analyzes were conducted using the Statistical Package for Social Sciences (SPSS) version 23 (IBM Corp., Armonk, NY, USA). The data are presented as mean and standard error. A comparison of gene expression coefficients was performed using the ANOVA test and then the Tukey test. A p-value of <0.05 was considered significant.

Results

The *Beclin2* gene expression coefficients of the metformin day 1, metformin day 7, ganirelix day 1, and ganirelix day 7 groups were found to be significantly decreased compared to the control group. (1.19±0.25 versus 0.42±0.12, 0.23±0.04, 0.50±0.08, and 0.39±0.04, respectively). The *Beclin1* gene expression coefficients of metformin day 1, metformin day 7, ganirelix day 1, and ganirelix day 7 groups were found to be significantly lower than the control group (2.39±0.51 versus 0.95±0.10; 1.03±0.20; 0.68±0.11 and 0.88±0.30, respectively) (Figure 1).

The *LC3BII* gene expression coefficients of the metformin day 1 and metformin day 7 groups were found to be significantly decreased compared to the control, ganirelix day 1, and ganirelix day 7 groups *LC3BII* gene expression coefficients (0.45±0.10 to 0.24±0.05 versus 1.29±0.38, 0.68±0.11 and 1.27±0.32, respectively; p<0.05).

The *CateninB* gene expression coefficients of metformin day 1, metformin day 7, ganirelix day 1, and ganirelix day 7 groups were significantly lower than the gene expression coefficient of the control group (2.53±1.24 vs. 0,17±0.03;0.14±0.06; 0.47±0.09 and 0.55±0.10, respectively).

The *TCF* gene expression coefficient of the ganirelix day 1 group was lower than the gene expression coefficients of the control, metformin day 1, metformin day 7, and ganirelix day 7 groups. (0.41±0.07 versus 1.30±0.31; 0.83±0.34; 0.49±0.10 and 0.59±0.22, respectively).

The *WNT2* gene expression coefficient of the metformin day 7 group was significantly higher than the gene expression coefficients of the control, metformin day 1, ganirelix day 1, and ganirelix day 7 groups (22.17±11.57 vs 1.27±0.27; 3. 32±1.21; 0.93±0.16 compared to 1.35±0.29).

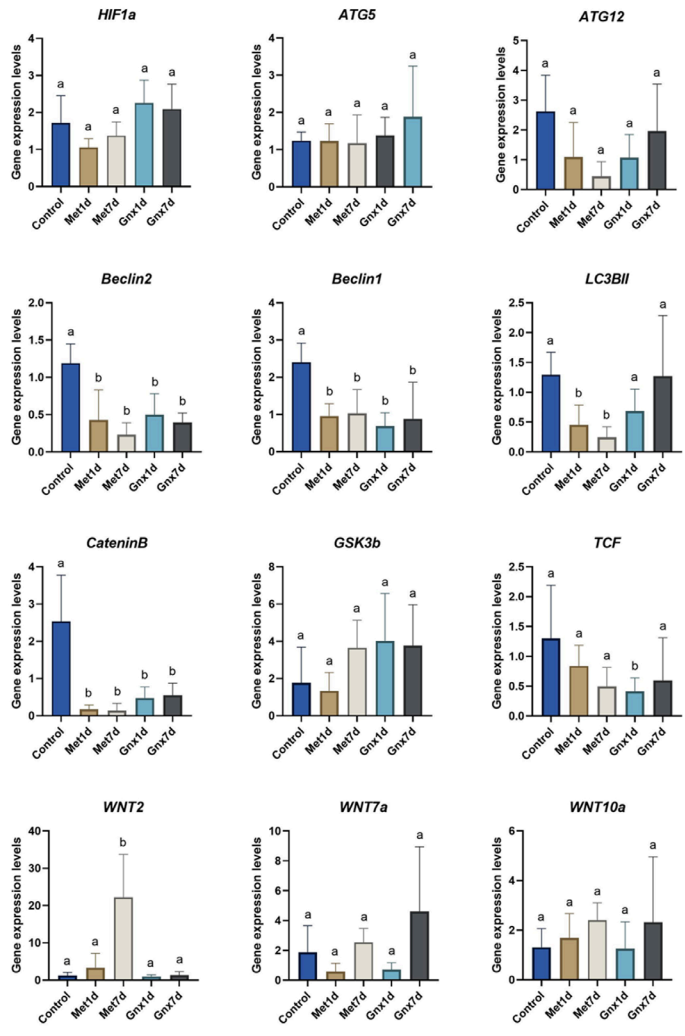

Figure 1. *HIF1a*, *ATG5*, *ATG12*, *Beclin2*, *Beclin1*, *LC3BII*, *CateninB*, *GSK3b*, *TCF*, *WNT2*, *WNT7a*, and *WNT10a* gene expressions. Data are presented as median with interquartile range

Considering endometriosis histomorphology data, the epithelium score of the ganirelix day 7 group was significantly lower than that of the control (denoted by a; p<0.05), and the amount of gland score of the ganirelix day 7 group was significantly lower than that of the control group (denoted by b; p<0.05). Metformin caused a partial decrease, but this difference did not reach significance (p>0.05). Considering immunohistochemical staining data regarding WNT2 and HIF1a (Figures 2 and 3), under the effects of study drugs, the expressions of the study provided some changes, but they did not reach statistical significance and are not conclusive (p>0.05).

Discussion

In this animal model of endometriosis, we tested the effects of ganirelix and metformin and the relationship of their effects with the expression of autophagy-related genes. Under the

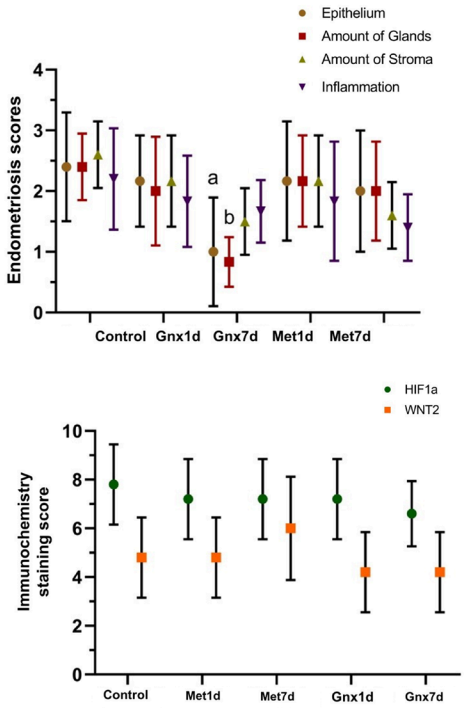

Figure 2. Endometriosis scores and immunochemistry staining scores for all control, gnx1d, gnx7d, met1d, and met7d groups. Data are presented as median with interquartile range. Considering endometriosis histomorphology data, the epithelium score of the gnx7d group was significantly lower than that of the control (denoted by a; $p<0.05$), and the amount of gland score of the gnx7d group was significantly lower than that of the control group (denoted by b; $p<0.05$). Considering immunohistochemical staining data regarding WNT2 and HIF1a

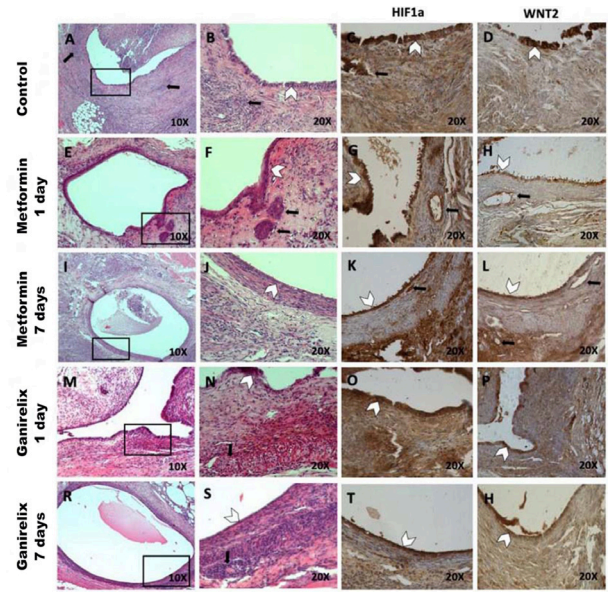

Figure 3. *HIF1a* and *WNT2* gene immunohistochemical evaluation of the control, metformin day 1, metformin day 7, ganirelix day 1, ganirelix day 7 groups. Hif1a immunoreactivity in the control group (C); intensely positive areas in the epithelium, glands, and stroma (immunohistochemistry score: 9, DAB). Wnt2 immunoreactivity in the control group (D); strong positive areas in the epithelium and glands, and medium positive areas in the stroma (immunohistochemistry score: 6, DAB). Metformin day 1 (E-F); endometrial glands in the endometriosis cyst wall with normal epithelium (Epithelial score; 3, Gland score: 3, Stroma score: 2). Hif1a immunoreactivity in the metformin day 1 group (G); strong positive areas in the epithelium and glands and medium positive areas in the stroma (immunohistochemistry score: 6, DAB). Wnt2 immunoreactivity in the metformin day 1 group (H); medium - positive areas in the epithelium and glands, and weak - positive areas in the stroma (immunohistochemistry score: 3, DAB). Metformin day 7 (I-J); endometrial glands in the endometriosis cyst wall with moderately preserved epithelium (Epithelial score; 2, Gland score:1, Stroma score:1). Hif1a immunoreactivity (K) in the metformin day 7 group; strong positive areas in the epithelium and glands and medium positive areas in the stroma (immunohistochemistry score: 6, DAB). Wnt2 immunoreactivity (L) in the metformin day 7 group; strong positive areas in the epithelium and glands and medium positive areas in the stroma (immunohistochemistry score: 6, DAB). Ganirelix day 1 (M-N); endometrial glands in the endometriosis cyst wall with a well-preserved epithelium (Epithelial score; 3, Gland score: 2, Stroma score: 2). Hif1a immunoreactivity (O) in the Ganirelix day 1 group; strong positive areas in the epithelium, glands, and stroma (immunohistochemistry score: 9, DAB). Wnt2 immunoreactivity (P) in the Ganirelix day 1 group; medium-positive areas in the epithelium, glands, and stroma (immunohistochemistry score: 6, DAB). Ganirelix day 7 group (R-S); endometrial glands in the endometriosis cyst wall with poorly preserved epithelium (Epithelial score; 1, Gland score:1, Stroma score:1). Hif1a immunoreactivity (T) in the Ganirelix day 7 group; medium positive areas in the epithelium, glands, and stroma (immunohistochemistry score: 6, DAB). Wnt2 immunoreactivity (U) in the Ganirelix day 7 group; weakly positive areas in the epithelium, glands, and stroma (immunohistochemistry score: 3, DAB). (Arrows: Endometrial glands, arrowheads: epithelium) (H&E, x200)

effects of study agents, *Beclin2* and *Beclin1*, and *CateninB* gene expressions presented a meaningful decrease; however, *LC3BII* gene expression presented a decrease with only metformin. *TCF* and *WNT2* gene expression revealed no strong decrease related to ganirelix and metformin, respectively. Overall, considering the immunohistochemical findings, these gene expression data support that these study drugs can inhibit the growth of endometriotic tissue and they can use autophagy-related pathways.

Although medical treatment methods for endometriosis have increased considerably in recent years, an agent with definite therapeutic efficacy has not yet been developed. The agents in use prevent the implantation, invasion, vascularization, and development of the endometriotic focus by both hormonal and anti-inflammatory mechanisms. The most important goal for treating endometriosis is to reduce or prevent disease-related pain[17]. Combined oral contraceptives (OCS) and progestins are the first-line therapy for treating deep endometriosis, which causes severe pain in women[17]. However, due to the side effects of the drugs in use, new drug searches have emerged.

Subcutaneous endometriosis cases have been frequently recently, affecting the social lives of patients. Increased cesarean

section operations, surgeries related to infertility in recent years, surgeons performing laparoscopic surgeries more comfortably due to the increase in technology, and endometriosis surgeries performed more easily are among the reasons for its emergence. With the increase in intra-abdominal surgeries, endometriotic foci can be moved to different parts of the body and cause disease[18]. Subcutaneous endometriosis cases are therefore more common. Patients may present with complaints of pain, tenderness, and a palpable mass at the incision site after surgery[18]. This situation disrupts the social comfort of people, and the feeling of constant pain can even cause workforce loss. Metformin is an inexpensive and effective treatment option used in many diseases such as polycystic ovary syndrome. Our study aims to evaluate the results of metformin and ganirelix (frequently used, inexpensive, quickly available, and used in the control and treatment of different diseases) in cases of subcutaneous endometriosis, which are common recently. Although rats are seen as more suitable animals for creating an endometriosis model in the literature, endometriosis animal models can be created with mice when suitable conditions are provided. Autologous endometrial pieces were implanted into their peritoneal cavities by autotransplantation, and endometriosis foci similar to those in humans were formed. Thus, in these models, information about the pathophysiology of endometriosis can be obtained and the effectiveness of new treatment agents can be evaluated.

The endometriosis animal model created by Oner et al.[19] has shown that while there was no regression on endometriotic implants in the control group, a statistically significant regression of endometriotic foci in the groups receiving metformin and letrozole. They found the effect of metformin on endometriotic tissues to be at least as significant as letrozole[19]. Yilmaz et al.[20] created a model of endometriosis in rats and randomly divided the animals into groups. Oral metformin 25 mg/kg/day was given to one group, oral metformin 50 mg/kg/day to the other group, and saline to another group. Endometriotic foci were more reduced in groups receiving metformin than in those not taking it. Takemura et al.[21] suggested that metformin, which increases insulin sensitivity and is widely used for diabetes, is effective for treating endometriosis because it has both anti-inflammatory properties and a modulating effect on ovarian steroid production. To determine the efficacy of metformin for treating endometriosis, they evaluated the effects of this agent on the inflammatory response, estradiol production, and proliferation of endometriotic stromal cells (ESCs). ESCs derived from ovarian endometriomas were cultured with varying concentrations of metformin. They measured IL-8 production, mRNA expression and aromatase activity, and the incorporation of 5-bromo-2'-deoxyuridine into ESCs. Metformin dose-dependently suppressed IL-1beta-induced IL-8 production, cAMP-induced mRNA expression and aromatase activity, and the incorporation of 5-bromo-2'-deoxyuridine into ESCs. Thus with these results, they argued that further research is needed on metformin's unique therapeutic potential as an anti-endometriotic drug. Zhou et al.[22] reported that metformin was able to inhibit PGE2-induced CYP19A1 mRNA expression and aromatase activity in human ESCs through AMPK activation and inhibition of CREB to CYP19A1 PII, and they argued that metformin may have unique therapeutic potential as an anti-endometriotic drug in the future. Zhang et al.[23] investigated the effect of metformin on the crosstalk of stromal-epithelial cells in endometriosis. Metformin regulates stromal-epithelial cell communication in endometriosis via Wnt2/β-catenin signaling[24].

Conclusion

In this animal model of endometriosis, ganirelix, and metformin affected the expression of autophagy-related genes and provided an anti-endometriotic effect. In the search for new drugs for the treatment of endometriosis, it seems useful to include genirelix and metformin, which have been shown in our study to cause changes in gene expressions involved in important molecular mechanisms such as autophagy, in the scope of further research.

Ethics

Ethics Committee Approval: Ethics committee approval of the study was obtained from Sivas Cumhuriyet University (SCU) Animal Experiments Local Ethics Committee (decision no: 65202830-050.04.04-306, date: 28.08.2019).
Informed Consent: Not necessary.
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Authorship Contributions

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Protective and/or therapeutic effects of berberine in a model of premature ovarian failure induced by cyclophosphamide in rats

Sıçanlarda siklofosfomid ile indüklenen prematüre ovaryan yetmezlik modelinde berberinin koruyucu ve/veya tedavi edici etkileri

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Abstract

Objective: We conducted a biochemical and histological evaluation of whether berberine has a protective and/or therapeutic effect in a cyclophosphamide-induced premature ovarian failure (POF) model.

Materials and Methods: We divided 28 Wistar albino female rats into 4 groups [control group, POF group, cyclophosphamide (CP)+berberine (Bb) group, and POF+Bb group]. The POF model was established by intraperitoneal administration of 50 mg/kg CP on day 1 followed by 8 mg/kg/day CP dissolved in saline for the following 14 days. The CP+Bb group received Bb concurrently for two weeks with CP. The POF+Bb group received berberine for two weeks following the completion of CP administration. Left ovaries were used for histopathological evaluation and right ovaries were used for biochemical analysis [tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1, IL-6 levels in tissue].

Results: Ovarian damage scoring was significantly higher in the POF group than in the other groups ($p<0.005$). In the POF group, primordial and primary follicle counts were the lowest, while secondary and corpus luteum counts were the highest ($p<0.005$). There was no significant difference between the other groups. The POF group had significantly elevated levels of TNF-α, IL-1, and IL-6 in the biochemistry results ($p<0.005$).

Conclusion: We demonstrated that berberine could be effective in the protection and treatment of POF by reducing proinflammatory cytokines. We believe that our study can make a considerable contribution to the literature in terms of POF protection and/or treatment.

Keywords: POF, ovarian damage, TNF-α, follicle count, histopathologic evaluation

Öz

Amaç: Çalışmamızda siklofosfamid ile oluşturulan prematüre ovaryan yetmezlik (POY) modelinde berberinin koruyucu ve/veya tedavi edici etkisi olup olmadığı biyokimyasal ve histolojik olarak değerlendirdik.

Gereç ve Yöntemler: Çalışmada toplam 28 adet Wistar albino cinsi 180-220 g ağırlıklarında dişi ratlar 4 gruba [kontrol grubu, POY grubu, siklofosfomid (CP)+berberin (Bb) grubu ve POY+Bb grubu)] ayrıldı. POY modeli, 1. gün 50 mg/kg CP ve sonraki 14 gün 8 mg/kg/gün CP tuzlu su ile çözülerek intraperitoneal uygulanarak oluşturuldu. CP+Bb grubuna CP ile eşzamanlı 2 hafta Bb uygulandı. POY+Bb grubuna ise CP uygulamaları bittikten sonraki 2 hafta berberin uygulandı. Denekler sakrifiye edilirken, sağ ve sol overler eksize edildi. Sol ovaryumlar histopatolojik değerlenmede ve sağ ovaryumlar ise biyokimyasal analizde (dokuda tümör nekroz faktörü-alfa [TNF-α, interlökin (IL)-1, IL-6 düzeyleri] kullanıldı.

Bulgular: Overlerin hasar skorlaması, POY grubunda diğerler gruplara göre istatistiksel anlamlı yüksek bulundu ($p<0,005$). POY grubunda, primordial ve primer folikül sayımı en düşük, sekonder folikül ve korpus luteum sayımı en yüksek bulundu ($p<0,005$). Diğer gruplar arasında

PRECIS: We showed that Berberine had anti-inflammatory effects by decreasing IL-1, IL-6, TNF-alpha levels and thus prevented ovarian damage.

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istatistiksel farklılık gözlenmedi. Biyokimya sonuçlarında, POY grubunda TNF-α, IL-1, IL-6 düzey yüksekliği istatistiksel olarak anlamlı bulundu (p<0,005).

Sonuç: Berberinin proenflamatuvar sitokinleri azaltarak POY profilaksisinde ve tedavisinde etkili olabileceğini gösterdik. Çalışmamızın POY profilaksisi ve/veya tedavisi açısından literatüre önemli katkısı olacağını düşünmekteyiz.

Anahtar Kelimeler: POY, ovaryan hasar, TNF-α, folikül sayımı, histopatolojik değerlendirme

Introduction

Premature ovarian failure (POF), also known as primary ovarian failure, was coined by Fuller Albright in 1942 to describe hypergonadotropic hypogonadism in women younger than 40 years of age. In women under 40 years of age, amenorrhea is defined as the presence of amenorrhea lasting for at least four months and follicle-stimulating hormone levels greater than 40 mIU/L at two different intervals. Primary ovarian insufficiency occurs because of decreased follicular reserve and accelerated follicular atresia.

Currently, there is no clear understanding of the exact mechanism of POF. However, programed cell death, also known as apoptosis, has been closely associated with ovarian granulosa cell quality and condition. Ovarian granulosa cells play a promoting and estrogen-mediated regulatory role in the maturation and development of oocytes, maintaining hormonal balance in the ovarian niche to promote oocyte maturation through autocrine and paracrine mechanisms. It has been well documented that the sensitivity of ovarian granulosa cells and the decline in reserves and reduced function that occurs as a result of apoptosis, stimulation of ovarian atresia and early insufficiency in ovarian function as well as a significant reduction in the follicle pool result in POF. Thus, high-quality ovarian granulosa cells are needed to maintain ovarian function, oocyte viability, and normal ovulation[1,2].

Alkylating chemotherapeutics such as cyclophosphamide (CP) may cause POF by affecting nondividing oocytes and primordial follicles. It is common for women with POF to experience infertility, and a small proportion may still have the ability to conceive. Therefore, there is increasing interest in the preservation and/or treatment of fertility in cancer patients. In the literature, research on the etiology and/or treatment of POF involves creating a POF model with CP in rats or mice[3,4].

Berberine (Bb) is a nonbasic herbal quaternary benzylisoquinoline alkaloids with proven medicinal history in Ayurveda and Chinese medicine. It is present in the roots, rhizomes, and bark of a number of medicinally important plants, including hydrastis canadensis, coptis chinensis, berberis aquifolium, berberis vulgaris, and berberis aristata (tree turmeric, family berberidaceae). It is a shrub that can reach heights of up to 3 meters, growing at altitudes of 2000 to 3000 meters, with a wide distribution in the Himalayan region and the Nilgiri hills in southern India[5]. Its active components are Bb, Berbamine and Palmatine. Bb is currently also produced by chemical synthesis. The chloride or sulfate salt of Bb is often used for clinical purposes.

It is an odorless, dense yellow powder with characteristic alkaloidal bitter taste. The use of Bb in Ayurvedic and Chinese medicine dates back at least 3000 years due to its potent antimicrobial, antiprotozoal and antidiarrheal properties. Furthermore, various clinical trials over the years have revealed that Bb has a broad range of pharmacological effects. Many studies have suggested that it has significant antihypertensive, antiarrhythmic, antihyperglycemic, anticancer, antidepressant, anxiolytic, neuroprotective, antioxidant, anti-inflammatory, analgesic and hypolipidemic activities[6,7]. Furthermore, various studies have shown that Bb possesses nephroprotective[8], hepatoprotective[9], cardioprotective[10] and cerebroprotective[7] properties.

The potent antioxidant, anti-inflammatory and antiapoptotic effects of Bb may suppress ovarian damage and prevent or ameliorate POF by impeding the reduction in ovarian reserve. Hence, in our study, we aimed to investigate the protective and therapeutic effects of Bb in a CP-induced POF model by biochemically evaluating interleukin (IL)-1β, IL-6, and tumor necrosis factor-alpha (TNF-α) levels in tissue and histologically by ovarian damage scoring and follicle counting.

Materials and Methods

The experimental procedure was approved by the Dokuz Eylül University Local Ethics Committee (protocol no: 09/2021, date: 10.03.2021). This study was conducted at the Dokuz Eylül University Experimental Animal Laboratory in January 2022.

The study employed 28 Wistar albino female rats weighing 180-220 g. Throughout the experimental period, the rats were followed under normal environmental conditions and dietary habits (21±2 °C, water and food were provided ad libitum). To equalize the effects of sex hormones on female rats, vaginal smears were performed at the beginning of the study and estrus rats were included in the study.

The rats (n=28) were randomly assigned to four groups. Group I (n=7): 1 mL saline (SF) was given by oral gavage to the control (sham) group. Group II (n=7): The POF group was administered 50 mg/kg CP (Endoxan, EIP Eczacibasi, Istanbul, Turkey) intraperitoneally on the first day, followed by 8 mg/kg/day CP SF for the subsequent 14 days, and the POF model was created[3,4]. Group III (n=7): The CP+Bb group was administered CP intraperitoneally by dissolving 50 mg/kg CP on the first day, followed by 8 mg/kg/day CP SF on the following 14 days. A single dose of 200 mg/kg/day Bb (Bb chloride, Sigma-Aldrich, cas. no: 633-65-8) prepared in 1 mL SF was administered by oral gavage for two weeks accompanied by CP administration[11,12].

In this group, the protective (prophylactic) effects of Bb against CP damage were studied[13,14]. Group IV (n=7): The POF+Bb group was administered 50 mg/kg CP intraperitoneally on the first day, followed by 8 mg/kg/day CP SF on the subsequent 14 days, and a POF model was created. For a duration of two weeks following the administration of the POF model, a single-dose oral gavage of 200 mg/kg/day Bb prepared in 1 mL SF was administered. In this group, the therapeutic effects of Bb on CP injury were studied.

At the end of the experiment, the subjects were sacrificed and the right and left ovarian tissues were collected. The left ovaries were fixed in formalin for histological evaluation, and the right ovaries were collected for biochemical analysis.

Histopathologic Evaluation

The left ovaries were fixed with formalin, dehydrated with different concentrations of alcohol, and embedded in paraffin. The maximum number of sections (4 µm thick) was collected from the ovaries and stained with hematoxylin and eosin. Follicle count and damage scoring were conducted in the histopathological evaluation of ovarian tissue[15]. Follicle counting included counting and averaging the primordial follicle, primary follicle, secondary follicle, tertiary follicle, and corpus luteum[16]. Damage scoring, on the other hand, assessed follicular cell degeneration, vascular congestion, hemorrhage, and inflammation parameters. Minimum five microscopic domains were examined to assign semiquantitative scores to the samples. Each sample was scored for each parameter using a scale ranging from 0 to 3 (0=absent; 1=mild; 2=moderate; 3=severe)[17,18]. Periodic acid-Schiff (PAS) staining was used to examine the zona pellucida structure[19].

Biochemical Evaluation

The biochemical evaluation included measurement of IL-1β, IL-6, and TNF-α levels in tissue. IL-1β, IL-6, and TNF-α (BTLAB, catalog numbers E0119Ra, E0135Ra, and E0764Ra) levels were analyzed by ELISA in accordance with the manufacturer's instructions.

Statistical Analysis

Statistical analysis of the study data was conducted using SPSS (Statistical Package for Social Sciences) 26.0 software. For analysis, the mean and standard deviation of the data were calculated. The difference between the groups was analyzed by the Kruskal-Wallis test, while the Mann-Whitney U test was utilized to determine which group caused the difference.

Results

There were no pathologic changes in the control group (Figure 1). The follicular degeneration, vascular congestion, edema, hemorrhage, and inflammation scores of the POF group were significantly higher than those of the other groups (control, CP+Bb, POF+Bb) ($p<0.005$). However, there was no significant difference between the control group, CP+Bb group, and

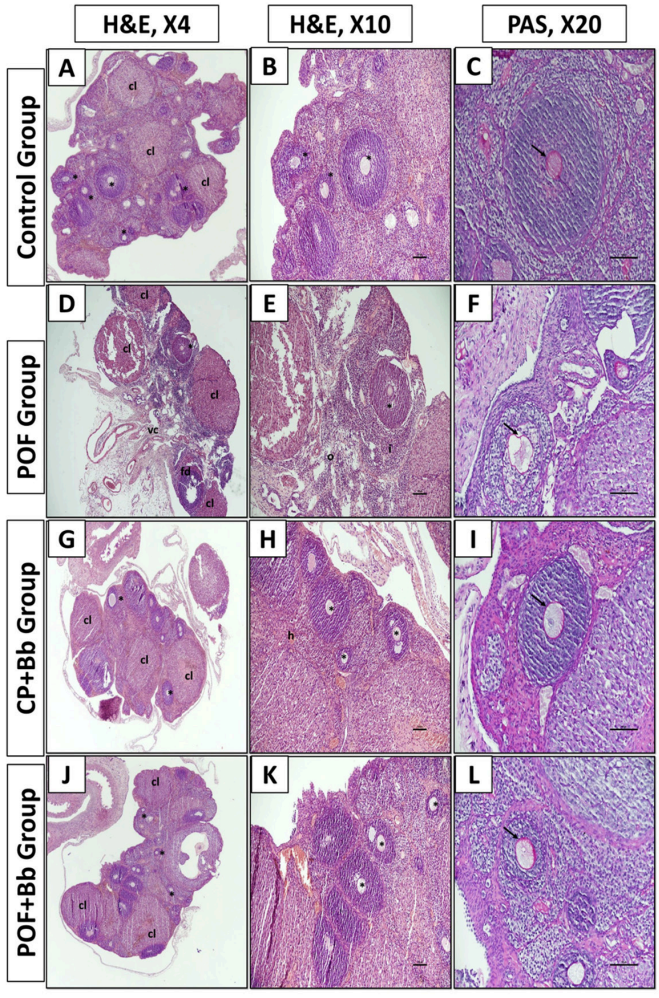


Figure 1. Photomicrographs of the ovarium tissue. *Follicles at different stages of development, corpus luteum (cl), vascular congestion (vc), hemorrhaging (h), edema (o), follicular degeneration (fd), inflammation (i). Black arrows show the zona pellucida. A, B, C: Control group, D, E, F: POF group, G, H, I: CP+Bb group, J, K, L: POY+Bb group, Scale bar: 100 µm

H&E: Hematoxylin and eosin staining, PAS: Periodic acid-Schiff staining, POF: Premature ovarian failure

POF+Bb group. Additionally, there was no significant difference between the CP+Bb group and POF+Bb group. Figure 2 shows the histopathologic damage scoring. In PAS staining, thinning of the zona pellucida was observed in the POF group.

Figure 2 summarizes the follicle numbers and damage scoring of the groups. The POF group had the lowest mean number of primordial follicles, whereas the number of primordial follicles in the other groups was significantly higher than that in the POF group ($p<0.005$). However, there was no difference between the control, CP+Bb and POF+Bb groups. The mean number of primary follicles was similar to the mean number of primordial follicles. Conversely, the highest numbers of secondary and tertiary follicles and corpus luteum were observed in the POF group. Secondary and tertiary follicles and the corpus luteum

counts of the other groups were significantly lower than those in the POF group (p<0.005). However, the number of secondary and tertiary follicles and corpus luteum did not differ between the other groups.

Figure 3 presents biochemical results of TNF-α, IL-1, and IL-6 levels in tissue. TNF-α, IL-1, and IL-6 levels in the tissue of the POF group were significantly higher than those in the other groups (control, CP+Bb and POF+Bb) (p<0.005). However, there was no significant difference between the control group, CP+Bb group, and POF+Bb group.3

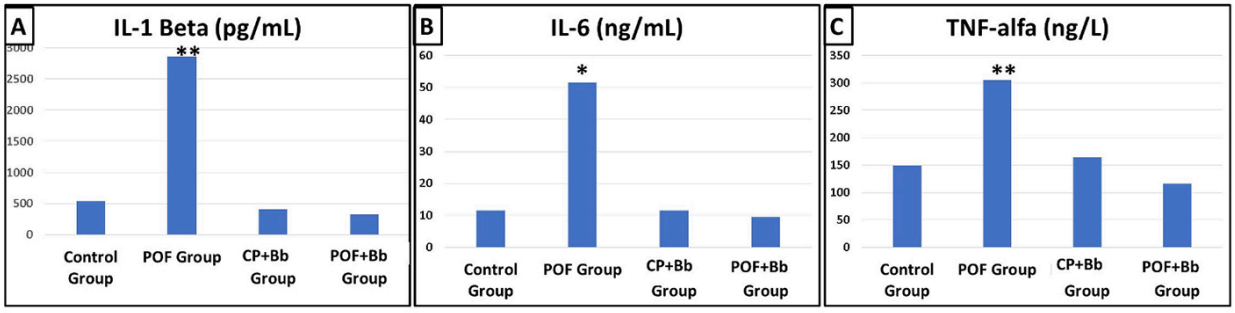
Discussion

Infertility is one of the most significant factors affecting the health of women today. POF, one of the leading causes of infertility, particularly in women under 40 years of age, is a multifactorial disease with an unclear etiology that results in ovarian dysfunction and early ovarian reserve depletion. Ongoing studies elucidate the etiology of infertility caused by POF and to develop protective and/or treatment protocols. To achieve this, appropriate experimental models are utilized, which can reproduce the clinical symptoms of the disease and

	Control Group	POF Group	CP+Bb Group	POF+Bb Group
Follicular Degeneration	0,2±0,4	2,5±0,5**[a]	1,2±0,5**[b]	1,0±0,5**[c]
Vascular Congestion	0,6±0,5	2,4±0,5**[a]	2,1±0,9	0,8±0,7**[c]
Haemorrhage	0,5±0,5	2,5±0,5**[a]	1,4±0,5*[b]	1,2±0,7*[c]
Inflammatory Cell	0,4±0,5	2,5±0,5**[a]	1,2±0,5**[b]	1,1±0,7**[c]
Primordial Follicles	6,0±1,1	1,2±0,5**[a]	4,1±1,0**[b]	6,0±0,8**[c]
Primary Follicles	4,8±0,7	1,7±0,5**[a]	4,0±0,8**[b]	4,4±1,2**[c]
Secondary Follicles	2,4±0,9	3,1±0,9**[a]	1,7±0,7**[b]	1,4±0,5**[c]
Tertiary Follicles	1,5±0,5	3,0±0,8*[a]	1,5±0,5*[b]	1,4±0,5**[c]
Corpus Luteum	1,4±0,5	4,8±2,1**[a]	2,1±0,9**[b]	1,2±0,4**[c]

Figure 2. Histopathological scores of the ovaries between groups, follicular cell degeneration, vascular congestion, hemorrhage, and inflammation parameters (0=none, 1=mild, 2=moderate, 3=severe). Primordial, primary, secondary, tertiary follicles and the corpus luteum were counted, and their averages were calculated. Data are expressed as the mean ± standard error of the mean. *p<0.05 **p<0.005. [a]Group POF vs. other groups, [b]group POF vs group CP+Bb, [c]group POF vs group POF+Bb. POF: Premature ovarian failure (n=7 for each group)

POF: Premature ovarian failure, CP: Cyclophosphamide, Bb: Berberine



D	IL-1 Beta (pg/mL)	IL-6 (ng/mL)	TNF-alfa (ng/L)
Control Group	541,3±413,8	11,5±2,9	149,1±41,4
POF Group	2856,5±1391,2**	51,64±63,9*	305,5±136,4**
CP+Bb Group	411,3±279,7	11,5±3,5	164,0±17,4
POF+Bb Group	330,0±313,3	9,5±1,9	116,6±19,5

Figure 3. The results of biochemical evaluation, A: IL-1, B: IL-6, C: TNF- alfa (TNF-α) levels in tissue. D: Comparison of IL-1, IL-6, and TNF-alpha (TNF-α) levels. *p<0.05, **p<0.005, Group POF vs. other groups

POF: Premature ovarian failure, IL: Interleukin, TNF-α: Tumor necrosis factor-alpha

induce ovarian damage, allowing histopathological evaluations to be performed. In our study, we created a POF model in rats with CP and formed four groups to investigate the role of Bb in both treatment and protection of POF. We administered Bb simultaneously with CP to investigate the prophylactic effect[13,14]. To evaluate the treatment process, we scrutinized the effects of Bb following CP administration (after the POF pattern was induced).

Known for its cytotoxic effects on ovaries, CP is an agent widely used in cancer treatment. The development of POF occurs as a secondary disease, particularly in women undergoing cancer treatment. Follicular atresia is a process in which multiple cell death processes are involved in ovarian granulosa cells. It is well known that apoptosis and autophagy play a primary role in this process[20]. Song et al.[21] administered 200 mg/kg CP on day 1 followed by 8 mg/kg/day for the subsequent 15 days in POF modeling. We did not prefer 200 mg/kg dose because of its high and strong toxic effect. Kilic et al.[22] preferred a dose of 200 mg/kg in their study, but they aimed to investigate the ovarian damage caused by ovarian cancers and medications used in cancer treatments. However, in our study, we administered a single dose of 50 mg/kg CP followed by 8 mg/kg/day CP for the following 14 days.

The process of declining the number and quality of oocytes and the failure of oocytes to regenerate is also called female reproductive aging. The term ovarian reserve refers to the quantity and quality of oocytes capable of forming dominant follicles during the late follicular phase of the menstrual cycle. Most women with decreased ovarian reserve have regular menstruation and a reduced number of ovarian follicles. Due to this condition, a limited response and decreased fecundity have been revealed in response to ovarian stimulation in the treatment of infertility[23]. Studies on POF have examined follicle numbers. In their POF model study, Özel et al.[19] demonstrated a decrease in the number of primordial and primary follicles, while indicating an increase in the number of corpus luteum and secondary follicles. Similarly, we observed a decrease in the number of primordial and primary follicles and an increase in the number of corpus luteum and secondary follicles in the POF group. The data we have obtained clearly indicate that we have successfully created a POF model fit for purpose.

The processes of inflammation, apoptosis, oxidative stress, and aging are considered interconnected. For instance, an increase in pro-inflammatory cytokines has been observed in aging vessels[24]. Another study noted an increase in inflammatory cytokines in ovarian failure, also called ovarian aging. The expression levels of pro-inflammatory cytokines such as TNF-α, TGF-β, IL-8, IL-6, IL-1β, and increased interferon-gamma increased by PCR in mice in the ovarian failure model induced by CP administration, and pro-inflammatory cytokines decreased after the administration of skin-derived mesenchymal stem cells (SMSCs). Thus, SMCs may play a role in restoring the function of the damaged ovary[25]. Park et al.[26] observed increased apoptosis and inflammation in mice treated with CP. With the increase in TNF-α and IL-6, it was hypothesized that oogenesis and ovarian function could be restored with an anti-inflammatory effect. Similarly, in our study where we created a POF model, we observed elevated pro-inflammatory cytokines in rat ovaries. These data suggest that proinflammatory cytokines may play a role in the decrease in the number and quality of oocytes.

Bb has a very wide range of pharmacological action profiles, ranging from antioxidant action to affect neurotransmitters, enzymes, molecular targets, and immunomodulation. Various clinical studies have best demonstrated the antioxidant and anti-apoptotic effects of Bb in a variety of diseases ranging from diabetes to hypercholesterolemia, Alzheimer's disease and cerebral ischemia[27,28]. Song et al.[11] investigated the potential therapeutic effect of Bb on diabetes-induced testicular damage in rats. They administered 200 mg/kg/day Bb for 28 days. The results showed that the testicular tissue improved histomorphological terms and the TUNEL assay revealed a decrease in apoptosis[11]. Zhang et al.[12] examined the effect of Bb in the PCOS model induced in rats. Twenty-eight days of 200 mg/kg and 400 mg/kg Bb administration improved the histomorphologic damage scoring of the ovary and increased follicle reserve. Another study examined the effect of 150 mg/kg Bb in the PCOS model created in rats, but no improvement findings were observed[29]. This might be due to the inadequate dosage of Bb. Zhu et al.[30] showed an anti-inflammatory effect by decreasing IL-1, IL-6 and TNF-α levels by administering 50 mg/kg Bb for 4 weeks in an ulcerative colitis model. Huang et al.[31] demonstrated the anti-inflammatory and antioxidant effects of Bb in head trauma. In our study, we observed the anti-inflammatory effect of Bb and its healing effects on ovarian damage.

A similar study by Xue et al.[32] examined the effects of Bb at three different dosages for the treatment of POF and found that it was effective. In our study, we investigated and compared the preventive and therapeutic effects of Bb. We also showed that it has protective and therapeutic effects. Our study has contributed to the literature by demonstrating that it can be used in POF prophylaxis.

Study Limitations

Our study has some limitations. First, we did not assess oxidative stress markers and apoptosis parameters in serum or tissue samples. Second, the subjects were rats and clinical data are needed to confirm its efficacy and dose estimation in humans. These limitations should be considered in future studies.

Conclusion

In conclusion, we have revealed that Bb use may have POF protective and therapeutic effects. We suggest that the anti-inflammatory effect of Bb (by decreasing proinflammatory cytokines) and its effects on follicle reserve may preserve

fertility. We believe that our study will make a significant contribution to the literature regarding the prophylactic and/or therapeutic use of POFs.

Ethics

Ethics Committee Approval: The experimental procedure was approved by the Dokuz Eylül University Local Ethics Committee (protocol no: 09/2021, date: 10.03.2021). This study was conducted at the Dokuz Eylül University Experimental Animal Laboratory in January 2022.
Informed Consent: Not necessary.
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Authorship Contributions

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Clinical and genetic aspects of termination of pregnancy; tertiary center experience

Gebelik terminasyonlarında klinik ve genetik yönler; tersiyer merkez deneyimi

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Abstract

Objective: The aim of the study was to retrospectively analyze the indications Techniques and complications of pregnancy termination performed in a tertiary center.

Materials and Methods: All cases between 10 and 33 weeks of gestation between January 2021 and June 2023 were retrospectively analyzed. The patients were divided into two groups as group 1 with 11+0 to 21+6 gestational weeks and group 2 for those at 22+0 and 33+0 gestational weeks.

Results: A total of 568 pregnancy terminations were included in the study. Among all terminations the most common fetal indications were central nervous system anomalies (148 cases, 26%) and trisomy 21 (53 cases, 9%) and the most common maternal/obstetrical Indication was previable premature rupture of the membranes (179 cases, 31.5%). Abnormal genetic results were found in 50 of 173 cases (28.9%) with a termination indication of Structural malformation who accepted invaziv genetic testing. The number of terminations with

fetal indications performed after 22 weeks were 148 (41%) and 11 (7.4%) cases of these late terminations of pregnancy were anomalies expected to be diagnosed in the first trimester. Complication rates (12.4%) and abdominal termination rates (3.5%) were significantly higher in group 2 than in group 1 ($p<0.05$).

Conclusion: Improvements in prenatal genetic screening and diagnostic techniques will undoubtedly decrease the gestational ages in terminations of pregnancies. However, there will always be cases that can neither be diagnosed earlier nor can be treated due to the nature of the anomaly. In the management of such cases, terminations will always occupy an important place in prenatal care.

Keywords: Fetal ultrasonography, congenital anomaly, pregnancy complications, prenatal diagnosis

Öz

Amaç: Bu çalışmanın amacı üçüncü basamak bir merkezde gerçekleştirilen gebelik terminasyonu endikasyonlarını, tekniklerini ve komplikasyonlarını retrospektif olarak değerlendirmektir.

Gereç ve Yöntemler: Ocak 2019 ile Ağustos 2022 arasında 10 ila 33. gebelik haftaları arasındaki tüm olgular değerlendirildi. Hastalar; 11+0 ile 21+6 gebelik haftaları arasındakiler grup 1 ve 22+0 ile 33+0 gebelik haftaları arasındakiler grup 2 olmak üzere iki gruba ayrıldı.

Bulgular: Çalışmaya toplam 568 gebelik terminasyonu dahil edildi. Tüm terminasyonlar arasında en sık görülen fetal endikasyonlar merkezi sinir sistemi anomalileri (148 olgu, %26) ve trizomi 21 (53 olgu, %9) iken en sık görülen maternal/obstetrik endikasyon ise previable preterm erken membran rüptürüydü (179 olgu, %31,5). Genetik testi kabul eden terminasyon endikasyonu yapısal malformasyon olan ve invaziv genetik tanı testi yaptırmayı kabul eden 173 olgunun 50'sinde (%28,9) anormal genetik sonuç saptandı. Yirmi iki haftadan sonra fetal endikasyonla yapılan gebelik terminasyonu sayısı 148

PRECIS: We evaluated terminations of pregnancy after 10th gestational week, performed in a 2.5 year period, in terms of indications, timing, process and complications.

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(%41) olup, bu geç terminasyonların 11'i (%7,4) ilk trimesterde saptanması beklenen anomalilerdi. Grup 2'de komplikasyon oranları (%12,4) ve abdominal terminasyon oranları (%3,5) grup 1'e göre anlamlı olarak yüksekti (p<0,05).

Sonuç: Prenatal genetik tarama ve tanı tekniklerindeki gelişmeler, gebelik terminasyonlarının daha erken gebelik haftalarında yapılmasını sağlayacaktır. Ancak doğası gereği erken teşhis edilemeyen veya tedavi edilemeyen olgular her zaman olacaktır. Bu tür olguların yönetiminde gebelik terminasyonları, doğum öncesi bakımda her zaman önemli bir yer tutacaktır.

Anahtar Kelimeler: Doğumsal anomaliler, fetal ultrasonografi, gebelik komplikasyonları, prenatal tanı

Introduction

Termination of pregnancy (TOP) is a medical procedure performed to terminate undesired pregnancies, pregnancies with maternal life-threatening morbidity or fetal incurable anomalies potentially leading to serious sequelae. Advances in prenatal genetic diagnosis and fetal imaging technologies as well as the widespread use of routine prenatal screening programs have made it possible to diagnose many fetal abnormalities during the antenatal period. Despite advances in fetal medicine and fetal therapies as well as postnatal therapies, TOP still occupies an important place in prenatal care due to limited treatment options for many congenital anomalies.

In Turkey, until the 10th gestational week, the pregnancy can be terminated at the request of the parents. After the 10th week, the pregnancy can be legally terminated due to a life-threatening maternal condition or a fetal abnormality that will lead to a serious outcome (Family planning law no: 2827, May 27, 1982). Therefore, TOP is frequently performed medically regardless of the gestational week, especially in tertiary centers. In this report, we retrospectively evaluated the indications, procedures, and complications of terminations between 11 and 33 weeks of gestation.

Materials and Methods

This study was conducted retrospectively at University of Health Sciences Turkey, Zeynep Kamil Women and Children Diseases Training and Research Hospital. All terminations of pregnancy performed due to fetal, maternal or obstetric indications beyond the 10th gestational week from January 2021 to June 2023 were evaluated. Patient demographics and clinical characteristics were derived from patient files and electronic archives. The study was approved by the local ethics committee (no: 76-2022, date: 22.06.2022 - University of Health Sciences Turkey, Zeynep Kamil Women and Children Diseases Training and Research Hospital Clinical Research Ethics Committee) and it was carried out in accordance with the principles of the Declaration of Helsinki. Malformations that affect more than one organ system with unknown genetic results were considered complex malformations. Chorionic villus sampling or amniocentesis were offered and performed for genetic diagnoses in necessary cases. Congenital malformations with abnormal genetic results were grouped according to genetics results that constitute the indication for the TOP. Genetic work-up was also performed for structural anomalies incompatible with life or causing severe sequelae. These cases were considered as structural anomalies as termination indications. Genetic work-ups were not waited to conclude the TOP. Previable preterm premature the rupture of membranes cases were included in the study only if the pregnancy was actively terminated by parents request. Terminations were grouped as before and after 22 weeks, which constitutes the life expectancy limit (group 1, before 22 weeks and group 2 after 22 weeks)[1]. Each pregnancy termination request was discussed and concluded by a committee consisting of consultants from relevant departments. If a live born neonate is expected, predominantly in pregnancies after 22 weeks of gestation, the procedure was initiated by performing a feticide with intracardiac or umbilical venous potassium chloride injection[2]. Three methods were used for termination: pharmacological, mechanical, and combined. Misoprostol (PGE1) was administered according to FIGO recommended regimens[3]. Extraamniotic cervical balloon dilatation was used as the mechanical method. In some cases, mechanical dilation was combined with pharmacological agents (misoprostol/oxytocin). Hysterotomy was decided in case of complication or failure of the other termination methods depending on the patient's gestation week and uterine surgery history. The need for surgery or transfusion as a result of massive bleeding after termination was considered bleeding complications. Endometritis was diagnosed with clinical and laboratory findings. The indication for curettage was evaluated by sonographic examination after the abortion.

Statistical Analysis

The study data were analyzed using IBM SPSS statistics version 22.0 (IBM Corporation, Armonk, New York, United States. Descriptive statistical parameters such as the mean ± standard deviation, median along with 25-75% interquartile range, numbers and percentages for categorical variables were used for statistical evaluation. Kolmogorov-Smirnov test was used to test the distribution of continuous data. Independent samples t-test compared two independent gestational week-based groups where numerical variables are normally distributed. The Mann-Whitney U test is used to compare differences between two independent groups when the dependent variable is either ordinal or continuous, but not normally distributed. The Pearson chi-square test or Fisher exact test was used to compare the differences between categorical variables were appropriate. In all statistical analyzes, the significance level (p-value) was determined at 0.05.

Results

A total of 568 pregnancy terminations were analyzed. The mean age of the patients undergoing pregnancy termination was 30.2±6.2, and the gestational week at which the termination was performed was 19.8±4.3. The current study revealed that 47.2% of pregnancy terminations were performed due to fetal structural malformations, 16.4% to genetic abnormalities and 36.4% to maternal/obstetrical indications. Table 1 shows the indications for the 568 pregnancy terminations performed. In 3 of the 93 patients who underwent TOP for a chromosomal/genetic abnormality, genetic research was carried out due to the history of a child with a genetic disease (metachromatic leukodystrophy, spinal muscular atrophy, Pompe disease). Of the remaining 90 patients, 66 (73.3%) were diagnosed with abnormal ultrasound findings and 24 (26.7%) were diagnosed with further investigations due to high risk in the first trimester screening test. The mean TOP week of the patients diagnosed through abnormal ultrasound finding was 22±3.6 whereas the mean TOP week of the patients diagnosed with a genetic

Table 1. Indications for termination of pregnancy

A. Fetal Structural Malformations (± genetic abnormalities)			**268 (47.2%)**
Central Nervous System (n=148)			
-Spina Bifida	50	-Agenezis of the corpus callosum	3
-Acrania/Exencephaly/Anencephaly	49	-Cortical developmental malformations	3
-Cephalocele	11	-Vermian agenezis/ hypoplasia	4
-Hydrocephalus	8	-Ischemic/ hemorrhagic lesions	6
-Craniorachischisis/Iniencephaly	6	-Intracranial tumor	1
-Holoprosencephaly	6	-Rhombencephalosynapsys	1
Complex Malformations (n=36)			
Cardiovascular System (n=5)			
-Right isomerism	2	Hypoplastic Left Heart (HLHS)	2
-Left isomerism	1		
Genitourinary System (n=29)			
-Bilateral Renal Agenezis	9	-Bilateral Multicystic Kidney	6
-Lower urinary tract obstructions (LUTO)	10	-Renal dysplasia	4
Abdominal and Thoracic Malformations (n=7)			
-Diaphragmatic Hernia	4		
-Abdominal wall defect§	3		
Lethal Skeletal Dysplasia (n=13)			
Fetal Akinesia Deformation Sequence (FADS) (n=6)			
Non-immun Hydrops Fetalis (n=15)			
Limb Body Wall Complex (n=9)			
B. Fetal Chromosomal/Genetic Abnormalities (± sonographic findings)			**93 (16.4%)**
Trisomy 21	53	Xq22 deletion	1
Trisomy 18	15	Trisomy 9	1
Trisomy 13	3	4p deletion (Wolf Hirschhorn)	1
Meckel Gruber	3	Metachromatic Leukodystrophy	1
Turner Syndorme (45,X0)	5	Spinal Muscular Atrophy	1
22q11 deletion (Di-George)	2	Prader Willi	1
7q11 deletion (Williams Beuren)	2	RASophathy	1
Xp22 deletion (Ichthyosis, X-linked)	2	Pompe Disease	1
C. Maternal/Obstetrical Indications			**207 (36.4%)**
Previable PPROM + Anhydramnios	179	Abnormal Placentation	8
Previable Severe FGR†	11	Hypertension	1
Embryo Reduction	7	Sexual Abuse	1
Total			**568 (100.0%)**

§: Includes exomphalos and gastroschisis, †: With pathologic fetal Doppler findings and fetal chronic hypoxia signs on ultrasound. PPROM: Premature preterm rupture of the membranes, FGR: Fetal growth restriction

investigation following high risk the first trimester screening was 19±3.3. The difference was significant (p=0.002). Genetic research was conducted in 173 of 268 structural malformations that indicate TOP. 50 (28.9%) abnormal genetic test results were detected in the structural malformations that constitute the indication for the TOP. Genetics analyses and abnormal results in structural malformations are shown in Table 2.

Indications for TOP according to gestational age are summarized in Table 3. In group 2, TOP rates due to fetal structural malformations (59.0% vs. 41.8%, p<0.001) and chromosomal/genetic abnormalities (24.2% vs. 12.8%, p=0.001) were higher than group 1. Central nervous system malformations and numerical chromosomal anomalies comprised the leading anomalies in group 2. Maternal/obstetric indication rates were higher in group 1 than in group 2 (45.4% vs. 16.8%, p<0.001). When TOP processes were compared between groups, time for family to decide on termination, duration of induction and hospitalization time was longer in group 2 than group 1 (p<0.05). There was no difference in the history of uterine surgery between groups (p=0.503). Need for hysterotomy (abdominal TOP) (3.5% vs. 0.5; p=0.001), need for combined method for vaginal termination (31.4% vs. 17.2%; p<0.001) and complication rates (12.4 vs 5.6%; p=0.005) were higher in group 2 than group 1. Ten of 11 patients who underwent

Table 2. Genetic work-ups and results of the structural malformations shown in Table 1, section A

Affected System (Patients with genetic work-up/ Total cases)	Chromosomal abnormalities/ Performed Karyotyping (n)	Microarray abornomalities/ Performed CMA (n)	Single gene disorders/ Performed Molecular Genetic Analyzes (n)	Abnormal Genetic Results / Patients with genetic work-up(%)
Central Nervous System (83/148)	**8/83** -Trisomy 18: (4) -Trisomy 13: (3) -Triploidy: (1)	**7/56** -22q11 del: (1) -Xp22 del: (1) -8p21 del : (1) -4p14 del: (1) -7q 31 del : (1) -Xq28 dup: (1) -19q13 del: (1)	**8/10** -Joubert: (1) -Caudal Regression: (1) -Coffin-Siris: (1) -Walker Warburg: (2) -Incontinentia Pigmenti: (2) -Jarcho Levin: (1)	**23/83 (27.7)**
Cardiovascular System (5/5)	**0/5**	**0/4**	**0/1**	**0/5 (0.0)**
Genitourinary System (14/29)	**2/14** -Trisomy 21: (1) -Trisomy 18: (1)	**1/7** -17q12 del: (1)	**2/3** -Nephrolithiasis/osteoporosis hypophosphatemic-1: (1) -AR PKD: (1)	**5/14 (35.7)**
Abdomen (6/7)	**0/6**	**1/5** -12p dup: (1)	**0/1**	**1/6 (16.6)**
Skeletal (12/13)	**1/12** -45X0: (1)	**0/9**	**4/6** -Achondrogenesis: (2) -Osteogenesis Imp. type 3: (1) -FGFR3 gene mutatation: (1)	**5/12 (41.6)**
Hydrops (12/15)	**2/12** -Trisomy 21: (1) -Mosaic 45X0 + trisomy 18: (1)	**0/8**	**2/3** -Achondrogenezis: (1) -PTPN-11 mut: (1)	**4/12 (33.3)**
FADS (6/6)	**0/6**	**0/4**	**2/3** -Neu-Laxova: (1) -FADS-3: (1)	**2/6 (33.3)**
Limb body wall (5/9)	**0/5**	**0/3**	**0/0**	**0/5 (0.0)**
Complex Malformations (30/36)	**5/30** -Trisomy18: (3) -Triploidy: (1) -der(5)t(5;7): (1)	**3/23** -22q11 del: (1) -16p12.2: (1) -1p36 del: (1)	2/4 -Schinzel-Giedion: (1) -Acrofacial Dysostosis: (1)	**10/30 (27.7)**
Total (173/268)	**18/173**	**12/119**	**20/31**	**50/173 (28.9)**

CMA: Chromosomal microarray, del: Deletion, dup: Duplication, der: Derivation, FADS: Fetal akinesia deformation sequence, AR: Autosomal recessive, PKD: Polycystic kidney disease, Imp: Imperfecta

Table 3. TOP indications according to gestational weeks

		Group 1 n (%)	Group 2 n (%)	p-value
Structural	CNS CVS GUS Abdominal/Thoracic Skeletal System Hydrops FADS Limb Body Wall Complex Malformations	93 3 18 4 6 11 1 9 18	55 2 11 3 7 4 5 0 18	
	Total	**163 (41.8)**	**105 (59.0)**	**<0.001**
Chromosomal/Genetic	Numerical Structural İnherited	43 3 4	34 5 4	
	Total	**50 (12.8)**	**43 (24.2)**	**0.001**
Maternal/Obstetrical		177 (45.4)	30 (16.8)	<0.001
Total		**390 (100.0)**	**178 (100.0)**	

Values given as number or numbers (percentage). CNS: Central nervous system, CVS: Cardiovascular system, GUS: Genitourinary system, FADS: Fetal akinesia deformation sequence, TOP: Termination of pregnancy

abdominal TOP and had a history of uterine surgery. Hysterectomy was performed in only two patients, one due to cesarean scar pregnancy and the other due to massive bleeding during the termination procedure. Medical method utilization for vaginal termination (79.6% vs. 59.0%; p p<0.001) and need for curettage after abortion (79.9% vs. 36.0%; p<0.001) rates were higher in group 1 than group 2 (Table 4).

Table 4. Clinical characteristics and complications of the gestational age groups†

	Group 1 (n=378)	Group 2 (n=178)	p-value
Decision time for TOP (days)	0 [0-2]	2 [0-5]	<0.001
History of uterine surgery	97 (25.7)	41 (23.0)	0.503
Termination Route Abdominal Vaginal	 2 (0.5) 376 (99.5)	 9 (3.5) 169 (94.9)	 0.001
Vaginal Termination Method Medical Mechanical Combined	 301 (79.6) 10 (2.7) 65 (17.2)	 105 (59.0) 8 (4.5) 56 (31.4)	 <0.001 0.210 <0.001
Duration of induction in vaginal terminations (days)	1.5±0.9	1.9±1.0	<0.001
Complication Bleeding Uterine Rupture Endometritis Hysterectomy	21 (5.6) 12 (3.2) 1 (0.3) 7 (1.8) 1 (0.3)	22 (12.4) 11 (6.2) 2 (1.1) 8 (4.5) 1 (0.6)	0.005
Need for Curettage	302 (79.9)	64 (36.0)	<0.001
Hospitalization time (days)	2.7±1.2	3.1±1.4	<0.001

†: 12 cases (7 embryo reductions and 5 selective fetocides due to fetal anomaly) were excluded from the table. Values given as median (Q1-Q3), number (%), mean ± standard deviation, TOP: Termination of pregnancy

Discussion

The aim of prenatal care is prediction and prevention of the maternal and fetal risks, to detect the fetal anomalies, and to provide the necessary prevention or treatment modalities to the patients. However, most fetal malformations or genetic disorders lead to serious disabilities that are currently uncurable or are known to have treatment methods with unfavourable results. Therefore, TOP may be an option for such cases. Although there is no legal upper limit of gestational weeks for TOP due to maternal or fetal indication in Turkey, according to the 2011 Ankara declaration, pregnancy termination after the 24th week is not recommended[4,5]. Therefore, it is important for reference centers to publish their termination series.

In this study, it was shown that the most common indication of TOPs was fetal structural malformations in line with the literature[6,7]. Anomalies of the central nervous system system, complex malformations and anomalies of the genitourinary system were the most frequently observed structural malformations. Chromosomal and genetic causes have also been shown as the main indications for pregnancy terminations in the reported publications[8,9]. In the current study, chromosomal/genetic abnormalities were found to be 16.4% consistent with the literature. The rate of abnormal genetic results (28.3%) was found to be quite high in genetic analyses of TOPs performed due to structural anomalies. Comprehensive genetic studies have utmost importance in fetal structural malformations to

find out the genetic etiology leading to the malformation -if exists- even if the malformation alone constitutes the indication for TOP independent of genetic results. This may reveal some of these the hereditary genetic abnormalities and provide alternatives like preimplantation genetics diagnosis or invasive prenatal diagnosis chance at earlier weeks to the couples in subsequent pregnancies, particularly in nations like ours where consanguineous marriages are widespread.

The rate of cardiovascular system anomalies in TOP indications was quite low compared to recent studies[7,8,10,11]. Since the surgical intervention for congenital heart diseases can be performed effectively in our country, the termination demand may be less for fetuses with congenital heart disease. In addition to the indications for pregnancy terminations, the timing of termination is another issue that needs to be discussed. The timing of termination is closely dependent on the gestational weeks at which the ultrasonographic or genetic diagnosis is made. Syngelaki et al.[12] reported that all cases of acrania, holoprosencephaly, encephalocele, exomphalos, gastroschisis and body stalk anomalies can be detected in the first trimester by anatomical scanning. In our series 43 of 78 cases including these anomalies were terminated after 14 weeks and 40 of them could not be diagnosed in the first trimester for several reasons. Mean diagnosis and termination weeks of these 78 cases were 15.7±3.8 and 16.4±4.2 respectively. As we realize that most of the above-mentioned cases were diagnosed in their first appeal to our center, we can advocate that sonographic basic fetal screening is lacking in primary obstetrical care centers. In addition, the mean TOP weeks in chromosomal abnormalities detected with abnormal ultrasound findings was higher than those detected by high risk in the aneuploidy screening test. Despite the earlier TOP weeks in the abnormal aneuploidy screening test group, the majority of the genetic abnormalities indicated terminations were owing to ultrasound signs. This reflects the paucity of the screening tests in our country especially considering that the majority of the cases were trisomy 21 which could have been detected by 11-14 weeks combined screening tests with 90% sensitivity. All these findings in line with the literature, major fetal abnormalities can be diagnosed earlier with a first trimester aneuploidy screening including early fetal anatomical scan[13,14].

In the literature, terminations made after the 22nd-24th week of pregnancies have been considered as late pregnancy terminations and have been reported with a frequency of 25%[6,7]. In this study, the rate of late TOPs was 31.3% (178 cases) and slightly above the reported publications. As in many studies, most of the indications in late TOPs were fetal structural anomalies that could be detected on ultrasound at earlier gestational weeks (neural tube defects, complex malformations, lower urinary tract obstructions…)[6,9,15]. For only fetal indications, the rate of late TOPs was 41% (n=148), consistent with the literature[16]. Eleven cases (7.4%) were anomalies expected to be diagnosed in the first trimester. In a study by Aslan et al.[17], the late termination rate was reported as 46.2%. Current study conducted 15 years later in the same region of Turkey found that the late the termination rate appeared to have slightly decreased. The widespread use of screening programs in Turkey may have been responsible for this decline. However, in our country, where aneuploidy screening programs are common and access to healthcare is easier, the significant portion of the above-mentioned detectable anomalies in the first trimester is still being terminated after the 22nd week. This raises questions about the sufficiency of the 11-14-week of examination. The absence of a legal upper gestational age limit for TOP in the countries where all these publications were reported including ours might be mitigating the obligation of obstetricians to make these diagnoses in earlier weeks. The upper gestational the age limit may contribute to focus the obstetricians' attention on first trimester anatomic scans which enhances the experience in the first trimester ultrasound. On the other hand, many patients were referred to our clinic on suspicion of a fetal anomaly in this study. Therefore, inadequate fetal anatomical scanning may not be the only reason for higher rates of late TOPs. High rates of pregnancies with no antenatal follow-up in particular regions, latency in application to the referral center, the time necessary to complete the prenatal genetic studies and prolongation of decision time of the families might be the other reasons for the failure in terminating these pregnancies on time. In a different region of Turkey, Can and Kaleli[18] find that in pregnancies after 22 weeks, the rate of TOP for fetal indication was 11.4%, and there were no cases in which TOP was performed after viability owing to chromosomal/genetic abnormalities. One can explain the inconsistency between these rates with socio-cultural factors differences between the regions and differences in healthcare providers' attitudes to approve late terminations in different regions.

We also observed that complications of the procedure were more frequent and the duration of hospital stay was longer in late TOPs, as the recent study reported[19]. Garofalo et al.[20] declared that the history of previous uterine surgery and advanced gestational week were the main risk factors for performing pregnancy terminations with hysterotomy. In our study, the fact that the rate of hysterotomy was higher in group 2 and that most of the patients were patients who underwent hysterotomy had a history of uterine surgery (n=10, 90.9%) supports the literature.

Maternal health in pregnancy terminations is influenced by psychological factors as well as medical procedures and complications. Posttraumatic stress disorder and depression symptoms in pregnancy terminations were reported in 44% and 28% of patients, respectively, and advanced gestational week was significantly associated with psychiatric complications[21]. Although it is recommended that psychiatrists participate in the process especially in late pregnancy terminations, what their role should be is a matter of debate[22]. In the current study, the time it took for the parents to make a TOP decision was longer

in group 2 than group 1. This situation can be interpreted as TOPs performed in the advanced gestational weeks have more psychological aspects.

Despite the abovementioned disadvantages of late TOPs, there is a group of abnormalities that can not be diagnosed in the first or early second trimesters such as cortical developmental defects, intracranial ischemic/hemorrhagic lesions, tumors, or severe FGR with fetal chronic hypoxia signs and severe maternal morbidities. It is obvious that such cases may also have severe consequences and require expensive palliative care. In addition to the moral burden that a baby with severe sequelae will bring to the family, it is inevitable that it will lead to financial victimization in countries where social assistance facilities are limited.

Study Limitations

The major limitation of the study is that genetic testing was not standardized in all patients. Genetic research could not be performed on patients who did not accept the invasive diagnostic procedure. Furthermore, since our center is a public hospital, genetic studies were carried out step by step. Therefore, the applied genetic tests differed between patients according to the indications. Results could have been different if whole genome sequencing or whole exome sequencing could be studied further. The second limitation is the fact that our center serves as a tertiary referral center may have led to patient selection bias.

Conclusion

In the near future, the hopes are in favor of outstanding improvements in fetal therapies and prevention of serious maternal morbidities leading to obligation of terminating the pregnancies. However, for the time being, one of the main goals of obstetricians should be to detect these anomalies as early as possible so that the frequency of related complications can be reduced. It seems that there is a need for termination in advanced gestational age weeks can be significantly reduced with early and adequate screening. Improvements in prenatal genetic screening and diagnostic techniques will undoubtedly decrease the costs of these facilities make them more accessible. This can result in a shift in rates of TOP indications from 'fetal structural malformations to "genetic disorders" and from late TOPs to first trimester TOPs. However, there will always be cases that can neither be diagnosed earlier or can be treated due to the nature of the anomaly. In the management of such cases, it is important that the laws allow pregnancy termination in the advanced weeks of gestation.

Ethics

Ethics Committee Approval: The study was approved by the local ethics committee (no: 76-2022, date: 22.06.2022 - University of Health Sciences Turkey, Zeynep Kamil Women and Children Diseases Training and Research Hospital Clinical Research Ethics Committee).

Informed Consent: Retrospective study.

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Authorship Contributions

Concept: Ö.G.E., L.U., O.D., Design: Ö.G.E., L.U., O.D., Data Collection or Processing: Ç.Ö., Ü.T., Analysis or Interpretation: A.Ö., Literature Search: A.Ö., Writing: Ö.G.E., M.E.Ö.

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Threatened miscarriage and recurrent miscarriage: Expert opinions on progesterone therapy and treatment challenges

Düşük tehdidi ve tekrarlayan düşük: Progesteron tedavisine ilişkin uzman görüşleri ve tedavi zorlukları

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Abstract

It is crucial to gain a better understanding of threatened and recurrent miscarriages, including the existing knowledge and unknowns, as well as to discuss medical approaches and assess the situation. These issues are outstanding problems, causing significant physical and emotional burdens on women and their families, not only in Turkey but also worldwide. This article aims to explore the topic of miscarriages, including the implications, challenges, and potential therapeutic approaches in Turkey. Nineteen statements were presented to 6 Turkish perinatologists and obstetricians and gynecologists to evaluate the management of threatened and recurrent miscarriage and to compare the results with literature recommendations in an expert opinion meeting. Turkish perinatologists and obstetricians & gynecologists provided their responses using a 5-point Likert scale and discussed every statement. Progesterone use, particularly oral dydrogesterone, was supported by most of the experts. Opinions varied on the preference for dydrogesterone in recurrent miscarriage treatment. Experts unanimously agreed on the efficacy and safety of dydrogesterone and its recommendation in guidelines for threatened and recurrent miscarriage. Regarding progesterone therapy, vaginal progesterone was not associated with an increased rate of live births. Side effects of vaginal and oral micronized progesterone were acknowledged, and compliance with oral progesterone treatment was generally agreed upon. Dydrogesterone activity and effectiveness in threatened miscarriage received positive responses, while opinions on its effectiveness in recurrent miscarriage were divided. The loading dose of dydrogesterone and the practice of checking blood progesterone levels had different opinions among experts.This manuscript provides valuable insights in the management of threatened and recurrent miscarriages, highlighting the role of progesterone therapy, specifically dydrogesterone, and the need for adherence to relevant guidelines. Further research and a national Turkish guideline are warranted to address areas of uncertainty and optimize the management of these conditions.

Keywords: Threatened miscarriage, recurrent miscarriage, progesterone, treatment, diagnosis

Öz

Düşük tehdidi ve tekrarlayan düşüklerin daha iyi anlaşılması, mevcut bilgi ve bilinmezliklerin incelenmesi, tıbbi yaklaşımların tartışılması ve durumun değerlendirilmesi son derece önemlidir. Bu durum, sadece Türkiye'de değil, aynı zamanda tüm dünyadaki kadınlar ve aileleri üzerinde önemli fiziksel ve duygusal yükler yaratan önemli sorunlardır. Bu makale, Türkiye'de düşük konusunu, sonuçlarını, zorluklarını ve potansiyel terapötik yaklaşımları araştırmayı amaçlamaktadır. Uzman görüşleri ile düşük tehdidi ve tekrarlayan düşük yönetimi ve literatür önerilerini değerlendirmek amacıyla 6 Türk perinatolog ve kadın hastalıkları & doğum uzmanına 19 önerme sunulmuş ve uzman görüş toplantısında sonuçlar 5 puanlık Likert ölçeği kullanılarak değerlendirilmiştir. Türk perinatolog ve kadın hastalıkları & doğum uzmanları, her önermeyi ayrı ayrı değerlendirmiş ve tartışmışlardır. Uzmanların cevapları daha sonra analiz edilmiştir. Özellikle oral didrogesteron dahil progesteron kullanımı, uzmanların çoğunluğu tarafından desteklenmiştir. Ancak,

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didrogesteronun tekrarlayan düşük tedavisindeki yeri konusunda görüşler farklılık göstermiştir. Uzmanlar, didrogesteronun etkinliği ve güvenliliği ile düşük tehdidi ve tekrarlayan düşük tedavisinde kılavuzlardaki önerilerle aynı fikirde olmuşlardır. Vajinal progesteronun canlı doğum oranında artışla ilişkilendirilmediği görülmüştür. Vajinal ve oral mikronize progesteronun yan etkileri ve oral progesteron tedavisine daha iyi uyum sağlandığı genel olarak kabul görmüştür. Didrogesteronun düşük tehdidindeki etkinliği olumlu değerlendirilmiş, ancak tekrarlayan düşükteki etkinliği konusunda farklı görüşler belirtilmiştir. Didrogesteronun yükleme dozu şeklinde kullanımı ve kan progesteron seviyelerinin kontrolü konusundaki uygulamalar arasında farklı görüşler vardır. Bu yazı, düşük tehdidi ve tekrarlayan düşük yönetimi konusunda, progesteron tedavisinin, özellikle didrogesteronun rolünü ve ilgili kılavuzlara uyumunu vurgulayan değerli görüşler sunmaktadır. Belirsizlik alanlarını ele almak ve bu durumların yönetimini optimize etmek için daha fazla araştırma ve ulusal bir kılavuz gerekmektedir.

Anahtar Kelimeler: Düşük tehdidi, tekrarlayan düşük, progesteron, tedavi, tanı

Introduction

Miscarriage, defined as the loss of a spontaneous pregnancy before 20 weeks of gestation, can occur due to various reasons such as chromosomal anomalies, implantation dysfunction, and clinical complications[1,2]. Despite advancements in the prevention and management of clinical miscarriages, it remains a significant concern for healthcare professionals.

The most common complication of early pregnancy is threatened miscarriage, characterized by vaginal bleeding with or without abdominal cramps. It is estimated to occur in approximately 20% of pregnancies before the 20th week of gestation[3]. Recurrent miscarriage is typically defined as the occurrence of three or more consecutive documented pregnancy failures before 20 weeks of development[4]. However, according to the European Society of Human Reproduction and Embryology (ESHRE), recurrent miscarriage is defined as two or more consecutive pregnancy losses confirmed by ultrasound or histopathology. Nevertheless, only about 5% of women who conceive experience two consecutive miscarriages, and only 1% undergo three or more miscarriages[5].

Progesterone, known as the "pregnancy hormone," plays a crucial role in early pregnancy by preparing the endometrium for implantation and maintaining the gestational sac in the uterus[6]. Low levels of serum progesterone have been associated with threatened miscarriage. Progesterone is commonly used worldwide as the standard treatment for threatened miscarriage. Progesterone promotes muscle protein synthesis in the uterus, increases sensitivity to prostaglandin and estrogen, and prevents premature contractions of the myometrium[7].

Progesterone can be administered orally, intramuscularly, or as a vaginal suppository. Oral administration ensures maximum compliance, but the effectiveness of oral progesterone varies[8]. A Cochrane review in 2018 found that oral progesterone "probably reduces miscarriage rates" compared with no treatment, but the evidence from the included trials was of moderate quality[4]. In a study conducted by Abrar et al.[9], the effectiveness of oral versus vaginal progesterone was compared in terms of the absence of vaginal bleeding and the continuation of pregnancy beyond the 20th week. They found that the oral progesterone group had an efficacy of 90%, compared to 71% in the vaginal progesterone group.

Threatened miscarriage and recurrent miscarriage impose substantial physical and emotional burdens on women and their families. The aim of this manuscript is to explore the topic of threatened and recurrent miscarriages and discuss the implications, challenges, and potential therapeutic approaches in Turkey. Expert opinions from medical specialists in the field have been consulted to provide valuable insights into this subject.

Materials and Methods

This manuscript is an expert opinion approach in the evaluation of the management of threatened miscarriage and recurrent miscarriage by Turkish perinatologists and obstetricians and gynecologists and comparing the results with the recommendations of the literature. Six opinion leaders with studies/publications on miscarriage were invited to the expert opinion meeting. Relevant guidelines and literature approaches were provided to the experts prior to the meeting. During the expert opinion meeting, a total of 19 statements were presented to 6 Turkish perinatologists and obstetricians and gynecologists, who provided their responses using a 5-point Likert scale. There were 19 statements (Table 1), and the experts responded to the statements with "Strongly Agree", "Agree", "Undecided", "Disagree" and "Strongly Disagree" options. Experts answered and discussed all the statements with an independent moderator, and all the discussions were turned into a final report.

Results and Key Findings

The experts discussed 19 statements. The key findings of this expert opinion meeting are summarized in Table 2.

Discussion

This manuscript offers valuable insights into the real-world management of threatened and recurrent miscarriages in Turkey, providing information on the diagnostic and treatment approaches by Turkish perinatologists and obstetricians and gynecologists. The results demonstrate a high level of awareness and adherence to international guidelines and recommendations, reflecting the commitment of Turkish perinatologists and obstetricians and gynecologists to deliver optimal care for women with threatened miscarriage and recurrent miscarriage.

Experts highlight the influence of ethnicity on pregnancy outcomes, emphasizing that different ethnic groups in

Table 1. Statements

The prevalence of threatened miscarriage in Turkey is similar to the global prevalence.
The prevalence of recurrent miscarriage in Turkey is similar to the global prevalence.
Traditional methods, such as bed rest, are still effective in the treatment of threatened miscarriage and recurrent miscarriage.
Progestogen use is recommended in pregnant women experiencing threatened miscarriage.
It is recommended to investigate the cause of recurrent miscarriage in pregnant women.
In pregnant women experiencing idiopathic recurrent miscarriage, progestogen use is recommended to support the luteal phase.
In pregnant women experiencing idiopathic recurrent miscarriage, progesterone use is recommended in the first trimester.
In pregnant women experiencing idiopathic recurrent miscarriage, natural micronized progesterone treatment is not recommended in the first trimester.
Vaginal progesterone does not increase the rate of live births in recurrent miscarriage.
In the vaginal use of progesterone for threatened miscarriages, there are potential side effects such as discharge, irritation, itching etc.
Due to its sedative and hypnotic effects, oral micronized progesterone has a higher incidence of dizziness and drowsiness.
The oral use of progestogens enhances the compliance of the pregnant woman with the treatment.
While dydrogesterone exhibits progestogenic activity through strong progesterone receptors, its effects on androgen, glucocorticoid, and mineralocorticoid receptors are negligible/insignificant.
Dydrogesterone is effective in the treatment of threatened miscarriage.
Dydrogesterone is effective in the treatment of recurrent miscarriage.
Dydrogesterone treatment should be started with an initial loading dose in threatened miscarriage
Based on the studies, dydrogesterone is a safe treatment option.
According to ESHRE, the European Progestogen Club, Russia, and China guidelines, dydrogesterone is recommended for the treatment of threatened miscarriage and recurrent miscarriage.
It is recommended to check the blood progesterone level in cases of threatened miscarriage.

Turkey may experience variations in preterm birth rates[10]. While the overall preterm birth rate in Turkey is reported to be around 1-2%, it can vary significantly and reach up to 5% based on different studies. Additionally, the prevalence rate of miscarriage in Turkey is estimated to be 13.5%, with approximately 168,000 reported cases based on data from the Turkish Statistical Institute[11].

Recurrent miscarriage, defined as the loss of three or more consecutive pregnancies, affects approximately 1% to 3% of women, although some definitions consider two or more failed pregnancies[12,13].The condition is more common in sexually active couples and poses a higher risk for women over 35 years of age[14]. Experts acknowledge the challenges in diagnosing recurrent miscarriages, particularly in distinguishing between actual miscarriages and pregnancies with fetal heartbeats seen on ultrasound or those with initially high β-HCG levels that later decline. Different criteria are used to define recurrent miscarriage, with the World Health Organization[15] requiring three miscarriages confirmed histopathologically/ultrasonographically and ESHRE[5] considering two consecutive miscarriages with positive β-HCG results. Experts emphasize the importance of considering additional factors beyond β-HCG levels when diagnosing recurrent miscarriages. Experts also mention that gestational weeks are also an important factor, and some doctors diagnose miscarriage as early as 5 weeks of gestation, while others consider a loss at 19 weeks as a miscarriage.

Regarding the treatment of threatened and recurrent miscarriages, traditional methods such as bed rest are considered ineffective by most of experts[16]. However, 83.34% of experts advocate the use of progestogens, citing the role of progesterone in preparing the endometrium and preventing endometrial defects that may contribute to miscarriage. Progesterone therapy, specifically oral dysrogesterone, is supported by most experts due to its minimal side effects and effectiveness in preventing miscarriages. Investigating the causes of miscarriage in cases of recurrent miscarriage is unanimously agreed upon by experts experts[17,18].

Experts emphasize the importance of administering progesterone and providing appropriate bed rest and treatment in cases of threatened miscarriage. They strongly recommend the use of progesterone therapy due to the absence of observed side effects or adverse outcomes, indicating its safety and efficacy.

Regarding recurrent miscarriage, experts unanimously agree on the significance of investigating the underlying causes. In cases of idiopathic recurrent miscarriage, progestogen's use agents to support the luteal phase is recommended. However, there are differing opinions among experts regarding the preference for dydrogesterone and the overall importance of progesterone therapy for recurrent miscarriages. Some experts argue that dydrogesterone has minimal side effects and higher effectiveness compared with other options. However, one expert expresses skepticism about the proven benefits of progesterone therapy in such cases. It is noted that progesterone therapy is administered to exclude uterine septum or anomalies and to mitigate the impact of immunological factors. Experts report variations in the diagnostic rate of idiopathic recurrent miscarriages among competent physicians, highlighting the need for a differentiated evaluation of these cases. The importance of progesterone support in in vitro fertilization is also mentioned. Genetic factors,

Table 2. Key Findings

The prevalence of threatened miscarriage in Turkey similar to global prevelance: 33.33% of the experts responded with "Strongly Agree" and 66.67% responded with "Agree".
The prevalence of recurrent miscarriage in Turkey similar to global prevelance: 6.67% of the experts responded with "Strongly Agree", 50% responded with "Agree", and 33.33% responded with "Undecided".
Traditional methods effectiveness: 33.33% of the experts responded with "Agree", 16.67% responded with "Disagree", and 50% responded with "Strongly Disagree".
Progestogen use in threatened miscarriage: 66.67% of the experts responded with "Strongly Agree", 16.67% responded with "Agree", and 16.67% responded with "Disagree".
The cause of recurrent miscarriage investigation: 66.67% of the experts responded with "Strongly Agree", and 33.33% responded with "Agree".
Progesterone use in the first trimester in idiopathic recurrent miscarriage: 66.67% of the experts responded with "Strongly Agree", and 33.33% responded with "Agree".
Progesterone use in the first trimester in idiopathic recurrent miscarriage:16.67% of the experts responded with "Strongly Agree", 50% responded with "Agree", 16.67% responded with "Undecided", and 16.67% responded with "Disagree".
Natural micronized progesterone treatment is not recommended in idiopathic recurrent miscarriage: 33.33% of the experts responded with "Agree", 50% responded with "Disagree", and 16.67% responded with "Strongly Disagree".
Vaginal progesterone: 100% of the experts agree that the use of vaginal progesterone does not increase the rate of live births.
Vaginal progesterone side effects: 33.33% of the experts responded with "Strongly Agree", 50% responded with "Agree", and 16.67% responded with "Disagree".
Oral micronized progesterone side effects: The experts, with 50% stating "Strongly Agree" and 50% stating "Agree", expressed their consensus.
The compliance treatment with oral progesterone: 33.33% of the experts stated "Strongly Agree" while 66.67% stated "Agree", indicating their consensus. The statements refers to the evaluation of vaginal, intramuscular, subcutaneous, and oral use.
Dydrogesterone strong activity on progesterone receptors: 33.33% of the experts responded with "Strongly Agree", while 66.67% responded with "Agree".
Effectivity of dydrogesterone in threatened miscarriage: 33.33% of the experts responded "Strongly Agree", 50% responded "Agree", and 16.67% responded "Undecided".
Effectivity of dydrogesterone in recurrent miscarriage: 83.33% of the experts responded "Agree", and 16.67% responded "Disagree".
Loading dose of dydrogesterone use in threatened miscarriage: 16.67% of the experts responded "Agree", 66.67% responded "Undecided", and 16.67% responded "Strongly Disagree".
Dydrogesterone safety: The experts reported that dydrogesterone is a safe treatment option, with 50% responding "Strongly Agree" and 50% responding "Agree".
The recommendation of dydrogesterone in guidelines: The experts confirmed that in guidelines, dydrogesterone is recommended for threatened miscarriage and recurrent miscarriage treatment, with 16.67% responding "Strongly Agree" and 83.33% responding "Agree".
Checking the blood progesterone level in threatened miscarriage: 33.33% of the experts responded "Agree", 50% responded "Disagree", and 16.67% responded "Strongly Disagree".

structural anomalies, thrombophilia, and other endocrine causes are evaluated in cases of recurrent miscarriages, whereas idiopathic cases are reported to be less common.

Negative publications concerning medroxyprogesterone acetate (MPA) suggest that it should not be considered in the same category as dydrogesterone[19]. Overall, the experts' opinion highlighted the importance of progesterone therapy in threatened miscarriage and recurrent miscarriage, with considerations for individual cases, potential causes, and treatment options.

According to experts, guidelines indicate that micronized progesterone is considered ineffective for pregnant women with recurrent miscarriage, and recent studies have shown no significant difference in its effectiveness[20]. MPA has been excluded, and it is stated that any form of progesterone is effective. Experts comment that the desired effect of progesterone is primarily local, and although micronized progesterone lacks a specific vaginal indication, its ability to be used vaginally provides a psychological indication and is perceived as superior by some individuals.

Regarding the treatment of recurrent miscarriages, experts state that vaginal progesterone has lower efficacy compared with oral dysrogesterone, suggesting the need to consider a systemic effect. They reference a study by Lee et al.[21] in 2017

titled "The Influence of Oral Dydrogesterone and Vaginal Progesterone on Threatened Abortion: A Systematic Review and Meta-Analysis," which highlights the effectiveness of progesterone therapy, especially oral dydrogesterone, in preventing miscarriages in women with threatened abortion. They underlined that the vaginal form of progesterone is less effective. Furthermore, the experts unanimously agree that the statement "Vaginal progesterone does not increase the live birth rate in recurrent miscarriages" is supported by all experts with a 100% agreement rate. They state that vaginal administration of progesterone can cause discomfort in patients and should not be recommended if there is vaginal bleeding. The presence of vaginal bleeding increases the risk of infection and may serve as a contraindication; thus, oral treatment can be considered. In cases of vaginal bleeding, experts emphasize to completely discontinue vaginal progesterone application, as it will have no effect.

It is noted that oral micronized progesterone, due to its sedative and hypnotic effects, has a higher incidence of dizziness and drowsiness[22].

The experts' opinion highlights the varying efficacy of different forms of progesterone, with oral dydrogesterone considered more effective for recurrent miscarriages compared to vaginal progesterone. They emphasize the importance of individual considerations, potential contraindications, and the need for further research in this field. Progesterone can be administered orally, intramuscularly, or as a vaginal suppository[23]. An oral administration of progesterone ensures maximum compliance although its efficacy has shown varying results. A Cochrane review conducted in 2018 found that oral progesterone "probably reduces the rates of miscarriage" (relative risk 0.57, 95% confidence interval 0.38-0.85) compared with no treatment, although all included studies were noted to have moderate quality evidence[4]. In another study by Abrar et al.[9], the effectiveness of oral and vaginal progesterone was observed in terms of absence of vaginal bleeding and the ability of the pregnancy to progress beyond the 20th week. They noted that the oral progesterone group had an efficacy rate of 90% compared to 71% in the vaginal progesterone group. The experts clarified that the statement evaluated the effectiveness of different administration routes of protestogens compared with oral use. All experts unanimously agreed that an oral administration of protestogens improves compliance in pregnant women with the treatment.

Regarding the efficacy of dysrogesterone in the treatment of threatened miscarriage and recurrent miscarriage, 83.33% of the experts agree that the molecule is effective, while 16.67% express uncertainty due to the lack of conclusive evidence in the literature[24]. Some studies in the literature support the claim that dydrogesterone has beneficial effects on maintaining pregnancy in women with threatened miscarriage and recurrent miscarriage, as suggested by the majority of experts[25-27].

In the statement suggesting the use of an initial loading dose of dydrogesterone for treating threatened miscarriage, 66.67% of the experts expressed indecision. They provided various explanations for this uncertainty. One expert emphasized the variability of progesterone dosages and the need for a standardized definition of a loading dose, highlighting the lack of sufficient evidence in this regard. Another expert shared their clinical practice of using a loading dose with dydrogesterone, but acknowledged the absence of a standardized definition for such a dose. The third expert confirmed the absence of a loading dose in their practice and mentioned the existence of oral loading studies, but noted the lack of clear information regarding specific products and dosages[28].

Regarding the need to check blood progesterone levels in cases of threatened miscarriage, 66.67% of the experts stated that it is unnecessary. The experts held different opinions on this matter. They generally agreed that while measuring progesterone levels is feasible as a research topic, it is not meaningful or practical in the clinical setting. They highlighted the risks associated with delaying treatment or referring patients to other doctors while waiting for test results, especially in cases of bleeding. One expert suggested that measuring progesterone levels could be considered if it provides prognostic information, but emphasized that this test is not practical or cost-effective. Given the limited alternatives for miscarriage management, initiating treatment without measuring progesterone levels was deemed necessary. Experts noted that progesterone levels could potentially provide insights into prognosis and indicate the effectiveness of medication in target-oriented treatment. However, the mechanisms underlying changes in interoplacental shift and progesterone secretion capacity remain poorly understood. All experts unanimously reported that dydrogesterone is a safe treatment option. They referred to the guidelines of reputable organizations such as ESHRE, the European Progestogen Club, Russia, and China, which recommend dydrogesterone for the treatment of threatened and recurrent miscarriages.

In summary, the experts' opinion reflects their indecision regarding the use of an initial loading dose of dydrogesterone for threatened miscarriage and the need to check blood progesterone levels in such cases. They highlight the lack of standardized definitions and sufficient evidence in these areas in Turkey. While they recognize the potential benefits of measuring progesterone levels as a prognostic indicator, they emphasize the practical challenges and risks associated with delayed treatment. Overall, experts unanimously agree on the safety of dydrogesterone and its recommendation in the treatment of threatened and recurrent miscarriages according to relevant guidelines.

Conclusion

This manuscript reflects a high level of awareness among Turkish perinatologists and obstetricians and gynecologists regarding international guidelines and their commitment to

providing optimal care for women with these conditions. Experts acknowledge the influence of ethnicity on pregnancy outcomes and highlight the variability of preterm birth rates and the frequency of miscarriages in Turkey. They emphasize the challenges in diagnosing recurrent miscarriages. Experts consider the use of progestogens, especially oral dydrogesterone, in the treatment of threatened and recurrent miscarriages. However, there are differing opinions on the preference for dydrogesterone and the overall importance of progesterone therapy for recurrent miscarriages. The experts propose the need to investigate the underlying causes of recurrent miscarriage and highlight the importance of individual evaluations and potential contraindications in selecting appropriate treatment options. They underline the differences in efficacy among different forms of progesterone and suggest that oral dydrogesterone is a more effective option for recurrent miscarriages compared with vaginal progesterone. Overall, experts unanimously agree on the safety of dydrogesterone and its recommendation in the treatment of threatened and recurrent miscarriages according to relevant guidelines. Experts concluded that population - based research regarding miscarriage should be conducted because of the lack of data in Turkiye, and a national guideline is needed to address areas of uncertainty and optimize the management of threatened and recurrent miscarriage in clinical practice.

Key findings

• The results demonstrate a high level of awareness and adherence to international guidelines and recommendations, reflecting the commitment of Turkish perinatologists and obstetricians and gynecologists to deliver optimal care for women with threatened miscarriage and recurrent miscarriage.
• Oral dysrogesterone is a more effective option for recurrent miscarriage than vaginal progesterone.
• Experts unanimously agree on the safety of dydrogesterone and its recommendation in the treatment of threatened and recurrent miscarriage according to relevant guidelines.
• The experts agreed that there is a lack of data in the Turkish literature. A population-based study in Turkiye regarding miscarriage should be conducted.
• It has been highlighted that a Turkish guideline regarding threatened miscarriage and recurrent miscarriage should be prepared.

These key findings highlight the benefits and effectiveness of different forms of progesterone in miscarriage. These expert opinions emphasize the importance of progesterone administration in threatened miscarriage and recurrent threatened miscarriage, the significance of progesterone levels in predicting risk and guiding treatment. Further research is needed to enhance our understanding of these relationships and optimize progesterone therapy in clinical practice.

Ethics

Peer-review: Internally peer-reviewed.

Authorship Contributions

Concept: S.C.D., A.G., H.T., C.Ç., H.G.P., Ç.G., Design: S.C.D., A.G., H.T., C.Ç., H.G.P., Ç.G., Data Collection or Processing: S.C.D., A.G., H.T., C.Ç., H.G.P., Ç.G., Analysis or Interpretation: S.C.D., A.G., H.T., C.Ç., H.G.P., Ç.G., Literature Search: S.C.D., A.G., H.T., C.Ç., H.G.P., Ç.G., Writing: S.C.D., A.G., H.T., C.Ç., H.G.P., Ç.G.

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