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

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

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

· The declaration of transparency from the corresponding author.

· Clinical trial registration, if applicable.

· Institutional review board (IRB) approval or exemption.

· Informed consent.

· Any explanations related to reporting guidelines.

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

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

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

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

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

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

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A separate title page should list;

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

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

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

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

Precis

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

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

Abstract

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

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

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

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

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

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

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

Keywords

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

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

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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;

Introduction

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

Materials and Methods

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

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

Results

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

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

Discussion

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

Study Limitations

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

Conclusion

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

Conflict of Interest

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

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

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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.

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

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

Examples

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

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

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TURKISH JOURNAL OF OBSTETRICS AND GYNECOLOGY

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LETTER FROM THE PRESIDENT

Dear Colleagues,

2021 has been a tough and challenging year for everybody. As the new year approaches I would like to congratulate everyone's new year and state that we are looking forward to the new year full of hope. New steps are taken everyday to improve our conditions.

It was not possible to conduct our yearly National Congress of Obstetrics and Gynecology last year; however we were able to meet with our colleagues and have shared the latest breakthroughs face-to-face in the meeting at the start of December. I was heartwarming to be able to meet again and we are eternally grateful and thankful to anyone who joined us in the convention.

I would like to thank our Editor-in-Chief Eray Çalışkan Prof, MD, for his efforts and contributions for the Journal, and all colleagues who contributed who made this journal the number one scientific journal. We aim to make this journal even better and more successful in the future. As the Turkish Journal of Obstetrics and Gynecology, we are looking forward to your valuable submissions to publish in our journal and so we can contribute to medicine together.

Sincerely,

Ateş Karateke, Prof. MD
President of TJOD

Clinical Investigation / Araştırma

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Turk J Obstet Gynecol 2021;18:267-271

Increased sulfiredoxin-1 levels as compensatory mechanism against reactive oxygen species in women with gestational diabetes mellitus

Gestasyonel diabetes mellituslu kadınlarda reaktif oksijen türlerine karşı telafi edici bir mekanizma olarak sülfiredoksin-1 düzeyleri artmaktadır

Burak Tatar, Uğur Turhan

University of Health Sciences Turkey, Samsun Training and Research Hospital, Clinic of Obstetrics and Gynecology, Samsun, Turkey

Abstract

Objective: This study aimed to investigate the correlation between serum Sulfiredoxin-1 (Srx-1) levels and gestational diabetes mellitus (GDM).
Materials and Methods: A total of 40 patients diagnosed with GDM according to the American Diabetes Association Criteria and 40 age matched and gestational age-matched healthy pregnant women as a control group were included in this cross-sectional study. Serum Srx-1 levels and other demographic and laboratory variables were analyzed.
Results: Fasting plasma glucose, first and second-hour plasma glucose levels, fasting insulin levels, homeostasis model assessment of insulin resistance (HOMA-IR), and Srx-1 levels were significantly different in patients with GDM than control ($p<0.05$). Plasma Srx-1 levels significantly correlated with fasting plasma glucose, first and second-hour plasma glucose levels, fasting insulin levels, and HOMA-IR of patients with GDM ($p<0.05$), whereas no correlation in the control group.
Conclusion: This is the first study demonstrating an association between serum Srx-1 levels and GDM. Our results suggest increased serum Srx-1 levels may be a novel predictive marker for GDM. More randomized-controlled trials are needed to evaluate Srx-1 as a marker for adverse fetal results; closer monitoring is warranted with high Srx-1 levels.
Keywords: Sulfiredoxin-1, gestational diabetes mellitus, pregnancy, peroxiredoxin, reactive oxygen species

Öz

Amaç: Bu çalışma serum Sülfiredoksin-1 (Srx-1) düzeyleri ile gestasyonel diabetes mellitus (GDM) arasında bir korelasyon olup olmadığını araştırmayı amaçlamaktadır.
Gereç ve Yöntemler: Amerikan Diyabet Derneği Kriterleri'ne göre GDM tanısı alan toplam 40 hasta ve kontrol grubu olarak yaş ve gebelik haftası eşleşen 40 sağlıklı gebe bu kesitsel çalışmaya dahil edildi. Serum Srx-1 düzeyleri ile diğer demografik ve laboratuvar değişkenleri analiz edildi.
Bulgular: Açlık plazma glukoz, birinci ve ikinci saat plazma glikoz düzeyleri, açlık insülin düzeyleri, insülin direncinin homeostaz modeli değerlendirmesi (HOMA-IR) ve Srx-1 düzeyleri GDM hastalarında kontrol grubundan anlamlı olarak farklıydı ($p<0,05$). Plazma Srx-1 düzeyleri açlık plazma glikoz, birinci ve ikinci saat plazma glikoz düzeyleri, açlık insülin düzeyleri ve GDM hastalarının HOMA-IR ($p<0,05$) ile anlamlı olarak ilişkiliyken, kontrol grubu için bir korelasyon yoktu.
Sonuç: Bu çalışma, serum Srx-1 düzeyleri ile GDM arasındaki ilişkiyi gösteren ilk çalışmadır. Sonuçlarımız, serum Srx-1 seviyelerinin artmasının GDM için yeni bir belirteç olarak kullanılabileceğini göstermektedir. Srx-1'in advers fetal sonuçların bir göstergesi olup olmadığını değerlendirmek için daha fazla randomize kontrollü çalışmaya ihtiyaç vardır; böylece yüksek Srx-1 seviyeleri olan hastalara daha yakın izleme gereksinimi belirlenebilir.
Anahtar Kelimeler: Sülfiredoksin-1, gestasyonel diabetes mellitus, gebelik, peroksiredoksin, reaktif oksijen türleri

PRECIS: This is the first study to demonstrate an association between serum Srx-1 levels and gestational diabetes mellitus.

Address for Correspondence/Yazışma Adresi: Burak Tatar MD, University of Health Sciences Turkey, Samsun Training and Research Hospital, Clinic of Obstetrics and Gynecology, Samsun, Turkey
Phone: +90 533 347 95 71 **E-mail:** buraktatar@yahoo.com **ORCID ID:** orcid.org/0000-0002-6495-0174
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Introduction

Gestational diabetes mellitus (GDM) is defined as carbohydrate intolerance with an onset during pregnancy[1]. GDM is associated with a higher risk of complications, such as pre-eclampsia, increased cesarean delivery, macrosomia, birth trauma, and neonatal hypoglycemia[2,3]. The risk of type 2 DM increases and offspring of these women also have an increased risk of developing DM later in life[4].

Peroxiredoxins (Prxs) are a family of bifunctional proteins that are involved in chaperone and antioxidant activities controlling cytokine-induced peroxide levels, which are also involved in signal transduction[5,6]. Reduced Peroxiredoxin reduces hydrogen peroxide and alkyl hydroxiperoxides therefore becomes oxidized and reduced back by thioredoxin[7]. Murine knock-out models revealed that Prx1 inactivation reduces the lifespan by 15% and Prx 6 and Prx 3 knock-out models display no obvious pathology but a more sensitive phenotype to oxidative stress[8-10]. Prxs are also involved in the circadian rhythm of many species[11,12].

Sulfiredoxin (Srx) is a redox protein discovered in 1994[13]. The cysteine-sulfinic acid residues of certain Prxs are selectively reduced by Srx and play an important role in oxidative stress resistance, thereby affecting Prx in regulating downstream transcription factors and kinase pathways[14]. Glutathionylation is an important post-translational modification of proteins. Many diseases, such as Parkinson's disease and DM, correlated with increased glutathionylation of specific proteins[15,16]. Srx is also involved in the deglutathionylation of proteins and is a potential target of treatment[16].

Hyperglycemia induces reactive oxygen species (ROS) and is responsible for many DM complications[17]. Consequently, the organism produces antioxidants to compensate ROS, in the form of Prx and its function, are restored via Srx. Srx expression is significantly increased to protect Prxs in diabetic rats[18]. In addition, Srx-1 protects podocytes from high glucose-induced injury by activating Nrf2/ARE signaling[19]. As GDM similarly affects the fetus to type 2 DM, our hypothesis was that serum Srx-1 levels increased in hyperglycemic conditions such as GDM, which leads to ROS generation.

The literature revealed no previous report of human Srx-1 levels in women with GDM. This study aimed to investigate the correlation between SRx-1 levels and GDM.

Materials and Methods

A cross-sectional analysis of maternal plasma levels of Srx-1 between January 2019 and February 2020 was performed in 40 patients diagnosed with GDM according to the American Diabetes Association Criteria in their routine screening of 75 g oral glucose tolerance test (OGTT) between 24 and 28 gestational weeks. The control group was 40 gestational-age and age-matched healthy pregnant women with normal 75 g OGTT screening results. The inclusion criteria were singleton pregnancy and gestational age between 24 and 28 weeks. The exclusion criteria were patients with chronic diseases, DM history, hypertension history, multiple pregnancies, and maternal or fetal conditions that require preterm delivery, such as pre-eclampsia, preterm labor, or intrauterine growth retardation, and any fetal anomaly.

All patients were followed from the first trimester of pregnancy, and the crown-rump length at the first visit was the reference for the last menstrual period day. The study was approved by the local ethics committee in Health Sciences University Samsun Research and Training Hospital, the approval number SBUSEAH/KAEK 2019/2/12.

Serum Srx-1 concentration was measured using an enzyme immunoassay (catalog no: EH15392, Wuhan Fine Biological Technology Co., LTD., China) with a minimum detectable concentration of 0.313 ng/mL and intra- and inter-assay variation coefficients of <8% and 10%, respectively. Absorbance at 450 nm was measured using a Smart Microplate Reader 16.1 (USCN KIT INC.). The optical density was read on a standard automated plate at 450 nm (1420 Victor 3; Perkin Elmer, Waltham, MA). Insulin resistance is calculated using the homeostasis model assessment of insulin resistance (HOMA-IR). The HOMA-IR is calculated as follows: fasting glucose × (mmol) fasting insulin (lU/mL)/22.5.

Written informed consent for study participation and blood sample collection was obtained from all participants, according to the principles outlined by the Declaration of Helsinki (2013).

Statistical Analysis

The Statistical Package for the Social Sciences version 20 (SPSS Inc., Chicago, IL) was used for the statistical analyses. Data are presented as mean ± standard deviation. The categorical variables were expressed as percentages and the continuous variables were expressed as median (minimum and maximum). Correlations were assessed using Pearson and Spearman's correlation coefficient and a p-value <0.05 is considered statistically significant.

Results

The demographic, clinical, and laboratory characteristics of patients in GDM and control groups are demonstrated in Table 1. Fasting plasma glucose, first and second-hour plasma glucose levels, fasting insulin levels, HOMA-IR, and Srx-1 levels were significantly different in patients with GDM than that of the control group ($p<0.05$).

Plasma Srx-1 levels significantly correlated with fasting plasma glucose, first and second-hour plasma glucose levels, fasting insulin levels, and HOMA-IR in patients with GDM, whereas no correlation in the control group was observed (Table 2).

The median Srx-1 level in GDM group was higher than in the control group (474.6±109 pg/mL vs. 316.7±48 pg/mL, respectively, $p<0.01$) (Figure 1). The receiver operating characteristic curve for blood Srx-1 concentrations in the GDM group is demonstrated in Figure 2. The area under the curve

Table 1. Clinical characteristics of GDM and control groups

	GDM (n=40)	Control (n=40)	p
Value			
Age (years)	29.9±2.8	29.2±4.6	0.438
BMI (kg/m^2)	25.1±2.1	24.8±2.6	0.494
FPG (mg/dL)	87.3±12.4	72.7±4.8	0.001*
G1H (mg/dL)	164.4±14.5	125±13.2	0.001*
G2H (mg/dL)	133±12	107±10	0.001*
FI (lU/mL)	14.5±4.1	8.9±2.2	0.001*
HOMA-IR	2.3±0.7	0.8±0.7	0.001*
HbA1c	4.94±0.3	4.86±0.2	0.074
GA at drawing	25.8±1.4	25.5±1.3	0.310
GA at birth	38.4±0.9	39.4±1.0	0.001*
Birth weight (g)	3705±300	3265±174	0.001*
SRX-1 (pg/mL)	474.6±109	316.7±48	0.001*

Data are expressed as mean (± SD), GDM: Gestational diabetes mellitus, BMI: Body mass index, FPG: Fasting plasma glucose level, G1H: First-hour plasma glucose level, G2H: Second-hour plasma glucose level, FI: Fasting insulin level, HOMA-IR: Homeostasis model assessment of insulin resistance

was 0.938 (95% confidence interval: 0.89-0.986). The optimal cut-off value was 362 pg/mL and ratios above this value were 80.5% sensitivity, 82.5% specificity, 77.5% positive predictive value, and 82.5% negative predictive value (p=0.0001).

Discussion

The present study showed that serum Srx levels were significantly higher in patients with GDM than in healthy pregnant women. In addition, fasting plasma glucose, first and second-hour blood glucose levels, and HOMA-IR results correlated with Srx-1 levels.

ROS formation in diabetes by glucose oxidation, nonenzymatic glycation of proteins, and lipid peroxidation lead to cellular protein damage and higher insulin resistance due to oxidative stress[20]. Contrarily, ROS are produced, with a negative effect on insulin signaling, leading to insulin resistance, which is the pathophysiology for type 2 DM in response to insulin[21]. Antioxidant administrations improved insulin resistance, which also suggests the involvement of ROS in insulin resistance progression[22]. Increased glucocorticoids and insulin-like growth factor in the placenta in GDM increases ROS due to increased glucose utilization and mitochondrial activity, similar to DM[23].

Insulin resistance in pregnancy is a natural process that aims increased glucose transportation to the fetus. This natural process exaggerates if insulin secretion is not increased to compensate for insulin resistance, leading to GDM development, particularly with pancreatic beta-cell dysfunction[24,25].

Table 2. Correlation analyses between serum SRX-1 and clinical parameters

SRX-1 n (40)		
GDM	**r**	**p-value**
Age	-0.215	0.182
BMI	-0.164	0.313
FPG	0.503	**0.001**
GL1H	0.365	**0.001**
GL2H	0.483	**0.02**
FI	0.339	**0.032**
Hba1c	0.197	0.224
HOMA-IR	0.32	**0.044**
GA at sampling	-0.083	0.097
GA at birth	-0.256	0.111
Birth weight	0.1	0.54
SRX-1 n (40)		
(b) Control Group	r	p-value
Age	0.061	0.7
BMI	0.216	0.18
FPG	0.187	0.247
GL1H	0.3	0.06
GL2H	0.038	0.818
FI	0.123	0.448
Hba1c	-0.175	0.281
HOMA-IR	0.215	0.183
GA at sampling	0.009	0.957
GA at birth	-0.204	0.207
Birth weight	-0.019	0.908

p-values for Pearson's correlation, GDM: Gestational diabetes mellitus, BMI: Body mass index, FPG: Fasting plasma glucose level, G1H: First-hour plasma glucose level, G2H: Second-hour plasma glucose level, FI: Fasting insulin level, HOMA-IR: Homeostasis model assessment of insulin resistance

According to considerable evidence, Prxs act as antioxidants in eukaryotic cells and silence Srx, leading to Prxs hyperoxidation[18,26]. Congenital deficiency leads to neurodegenerative diseases and tumors, such as thyroid, breast, and lung cancer[15,16,27,28].

Srx, as an antioxidant enzyme, was not extensively studied *in-vivo*. Thus, Srx was not previously studied in GDM either. *In-vitro* studies demonstrated that Srx-1 alleviates podocyte injury caused by ROS generated in diabetic nephropathy and cardiopathy[18,19]. Before the study, hyperglycemic GDM condition, which generates ROS, was hypothesized to increase serum Srx-1 levels. The 2-hour OGTT revealed blood glucose

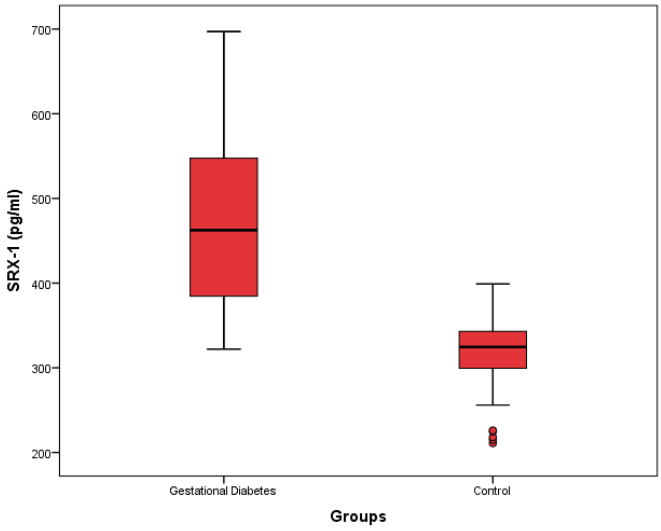

Figure 1. Srx-1 levels between GDM and control groups

GDM: Gestational diabetes mellitus, Srx-1: Sulfiredoxin-1

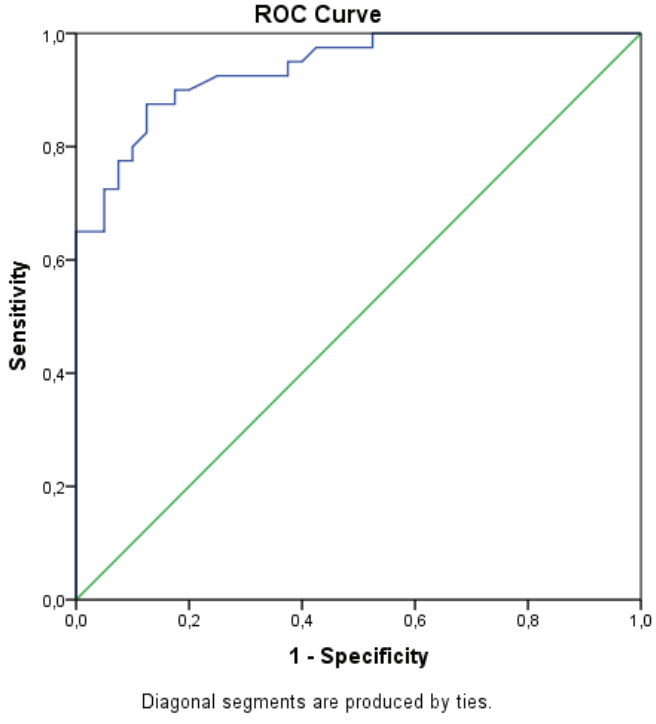

Figure 2. ROC curve for Srx-1 levels in GDM

GDM: Gestational diabetes mellitus, ROC: Receiver operating characteristic, Srx-1: Sulfiredoxin-1

level fluctuation that is significantly associated with beta-cell dysfunction, oxidative stress, and inflammation[29]. Our study revealed that the first-hour blood glucose levels in patients with GDM (164.4±14.5 in GDM vs. 125±13.2 in controls) significantly correlates with Srx-1 levels (474.6 in GDM±109 vs. 316.7±48 in controls). These results suggest that increased serum Srx-1 levels are used as a novel predictive marker for GDM.

Study Limitations

As the design of this study, obstetric outcomes were not evaluated between patients with high and low Srx levels, which is a limitation. In addition, the study had a small sample size and postpartum levels of Srx were not measured. Ergo, associations with obstetric outcomes and Srx-1 levels were not made. Nevertheless, elevated levels of Srx-1 are also associated with adverse gestational conditions, such as pre-eclampsia, intrauterine growth retardation, or placental implantation defects, with elevated ROS, which is beyond the scope of our preliminary study and requires further research[30].

Conclusion

This is the first study to demonstrate an association between serum Srx-1 levels and GDM. More randomized-controlled trials are needed to evaluate Srx-1 as a marker of adverse fetal results; then, closer monitoring is warranted with high Srx-1 levels.

Ethics

Ethics Committee Approval: The study was approved by the local ethics committee in Health Sciences University Samsun Research and Training Hospital, with approval number SBUSEAH/KAEK 2019/2/12.

Informed Consent: Written informed consent for study participation and blood sample collection was obtained from all participants, according to the principles outlined by the Declaration of Helsinki (2013).

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: U.T., Design: B.T., Data Collection or Processing: B.T., Analysis or Interpretation: U.T., Literature Search: U.T., Writing: B.T.

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

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The role of maternal serum catestatin in the evaluation of preeclampsia and fetal cardiac functions

Preeklampsi ve fetal kardiyak fonksiyonların değerlendirilmesinde maternal serum catestatinin rolü

Miraç Özalp[1], Hüseyin Yaman[2], Ömer Demir[3], Sümeyye Aytekin Garip[2], Turhan Aran[1], Mehmet Armağan Osmanağaoğlu[1]

[1]*Karadeniz Technical University Faculty of Medicine, Department of Perinatology, Trabzon, Turkey*

[2]*Karadeniz Technical University Faculty of Medicine, Department of Medical Biochemistry, Trabzon, Turkey*

[3]*Karadeniz Technical University Faculty of Medicine, Department of Gynecology and Obstetrics, Trabzon, Turkey*

Abstract

Objective: To compare the maternal serum catestatin (CST) levels in pregnant women with preeclampsia (PE) and with normal blood pressure and evaluate the relationship between the maternal serum CST levels and fetal cardiac functions

Materials and Methods: This cross-sectional study was conducted on 27 women with early-onset PE (EOPE), 28 women with late-onset PE (LOPE), and 28 healthy pregnant women. Maternal serum CST levels were measured using the enzyme-linked immunosorbent assay kits. Fetal cardiac functions were evaluated using the cardiac Doppler.

Results: Maternal serum CST levels were lower in the EOPE group; however, no statistically significant difference was found between the groups. Compared with the other two groups, a statistically significant difference was found in the fetal E/A ratio and myocardial performance index (MPI) values of the EOPE group (p=0.013, p=0.002, p=0.005, p<0.001, respectively). The fetal E/A ratio was positively correlated with the maternal serum CST levels in both the PE and control groups (p<0.001, p<0.001). The fetal isovolumetric relaxation time and MPI values were negatively correlated with maternal serum CST levels in both the PE and control groups (p<0.001, p=0.001, p<0.001, and p=0.002, respectively).

Conclusion: Lower CST levels are associated with fetal cardiovascular dysfunction, thus CST can be a critical biochemical marker in fetal cardiac function evaluation.

Keywords: Catestatin, cardiac function, Doppler, myocardial performance index, preeclampsia

Öz

Amaç: Preeklamptik (PE) ve normotansif gebelerin maternal serum catestatin (CST) düzeylerini karşılaştırmak ve maternal serum CST düzeyleri ile fetal kardiyak fonksiyonlar arasındaki ilişkiyi değerlendirmek.

Gereç ve Yöntemler: Bu kesitsel çalışma, erken başlangıçlı preeklampsili (EOPE) 27 gebe, geç başlangıçlı preeklampsili (LOPE) 28 gebe ve sağlıklı 28 gebe üzerinde yürütülmüştür. Maternal serum CST seviyeleri, enzime bağlı immünosorbent assay testleri kullanılarak ölçüldü. Fetal kardiyak fonksiyonlar, kardiyak Doppler ile değerlendirildi.

Bulgular: EOPE grubunda maternal serum CST düzeyleri daha düşük olmasına rağmen gruplar arasında istatistiksel olarak anlamlı fark bulunmadı. EOPE grubu diğer iki grupla karşılaştırıldığında, fetal E/A oranı ve miyokard performans indeksi (MPİ) değerlerinde istatistiksel olarak anlamlı fark bulundu (sırasıyla, p=0,013, p=0,002, p=0,005, p<0,001). Fetal E/A oranı hem preeklampsi hem de kontrol grubunda maternal serum CST seviyeleri ile pozitif korelasyon gösterdi (p<0,001, p<0,001). Hem preeklampsi hem de kontrol grubunda fetal IRT (izovolümetrik relaksasyon zamanı) ve MPİ değerleri maternal serum CST düzeyleri ile negatif korelasyon gösterdi (sırasıyla, p<0,001, p=0,001, p<0,001, p=0,002).

Sonuç: Düşük CST seviyeleri, fetal kardiyovasküler disfonksiyon ile ilişkilidir ve CST, fetal kalp fonksiyonunun değerlendirilmesinde kritik bir biyokimyasal belirteç olabilir.

Anahtar Kelimeler: Catestatin, kardiyak fonksiyon, Doppler, miyokardiyal performans indeksi, preeklampsi

PRECIS: We aimed to compare maternal serum catestatin levels of preeclamptic and normotensive pregnant women and to evaluation the relationship between maternal serum catestatin levels and fetal cardiac functions.

Address for Correspondence/Yazışma Adresi: Miraç Özalp MD, Karadeniz Technical University Faculty of Medicine, Department of Perinatology, Trabzon, Turkey
Phone: +90 505 223 40 93 **E-mail:** ozalpmirac@gmail.com **ORCID ID:** orcid.org/0000-0002-2255-1642
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Introduction

Preeclampsia (PE) is a critical disease affecting multiple organ systems in a normotensive woman, which occurs after the 20th week of pregnancy with hypertension and proteinuria or progresses to hypertension and end-organ damage without proteinuria[1]. Complicating 2%-8% of all pregnancies, this situation is one of the leading causes of maternal and neonatal morbidity and mortality[1,2].

The underlying mechanism of PE remains unclear; however, placental incompatibility appears as the main problem due to insufficient trophoblastic invasion of spiral arterioles[3]. Placental hypoxia and ischemia that develop due to this situation cause fetal heart expansion and increased aortic wall thickness, whereas the increased placental vascular resistance may cause fetal cardiac function changes by increasing fetal cardiac afterload[4,5].

The effective way to evaluate the global cardiac function in fetal life is using the Doppler-derived myocardial performance index (MPI), independent heart rate, and ventricular geometry[6]. This index, first proposed by Tei et al.[7], evaluates the myocardial function as a whole by combining both systolic and diastolic cardiac performance.

Catestatin (CST) is a hydrophobic and cationic structured peptide of 21 amino acids stored and released together with catecholamines in the storage vesicles of adrenal chromaffin cells and adrenergic neurons. It is obtained by degrading chromogranin A (CgA) with proteolytic enzymes, such as serine protease plasmin and cysteine protease cathepsin L[8,9]. CST modulates the sympathoadrenal system by inhibiting catecholamine release via the neuronal nicotinic acetylcholine (Ach) receptors[8]. CST, which stimulates histamine release from mast cells via heterodimeric G proteins, causes vasodilation and decreased blood pressure[9]. Other vital functions of CST include regulating the inotropy, lusitropy, and coronary tonus by increasing nitric oxide synthase, limiting apoptosis, providing proangiogenesis, and ultimately having cardioprotective effects[10,11]. A study found low plasma CST levels not only in patients with hypertension but also in their normotensive children[12].

Firstly, this study aimed to compare the serum CST levels of pregnant women with PE and those normotensive. Another aim is to evaluate the cardiac function in fetuses of women with PE and those normotensive and reveal the relationship between the maternal serum CST levels and the fetal cardiac function.

Materials and Methods

This cross-sectional study was conducted between November 2020 and May 2021 at the Karadeniz Technical University Faculty of Medicine Perinatology Clinic. The study was approved by the Faculty of Medicine Ethics Committee of our University and was conducted following the Declaration of Helsinki (ethics committee no: 2020/283, date: 16.11.2020). Written informed consent was obtained from all-volunteer pregnant women who participated in the study.

Study Design

A total of 55 patients with PE who were admitted to our clinic during the study period were randomly and consecutively chosen, wherein 27 were in the early-onset PE group (EOPE) and 28 in the late-onset PE group (LOPE). The normotensive control group consisted of 28 consecutive cases admitted to our clinic at the same date range (November 2020-May 2021), and whose ages and gestational ages matched with the study group. PE was diagnosed with the presence of hypertension (systolic and/or diastolic blood pressure of 140 and/or 90 mmHg in two measurements made at least 4 h apart) and proteinuria (≥300 mg in a 24-h urinalysis or urine protein/creatinine ratio of ≥0.3) that occurred after the 20th week of pregnancy in a woman who was previously normotensive[1]. Cases with new-onset hypertension without proteinuria were included in the PE group if they had a headache that is unresponsive to medical treatment, visual impairment, pulmonary edema, platelet count of $<100\times10^9$/L, and signs and symptoms of end-organ damage, such as elevated blood concentrations of liver transaminases to twice the normal concentration, and serum creatinine concentration above 1.1 mg/dL or a doubling of the serum creatinine concentration in the absence of other renal diseases[1]. PE was divided into two groups according to the gestational age of patient diagnoses; <34 weeks as EOPE and ≥34 weeks as LOPE[13]. The pregnant women in the normotensive control group consisted of singleton and term pregnancies with infants showing an appropriate development according to the gestational age. The exclusion criteria include chronic hypertension, pregestational diabetes, premature rupture of membranes, chorioamnionitis, multiple pregnancies, fetal anomalies, autoimmune diseases, and maternal chronic liver and kidney diseases. No patients were excluded after the study completion since inclusion and exclusion criteria were met. Pregnancy outcomes and maternal demographic characteristics were obtained from the participants' medical records by a single clinician blinded to the fetal cardiac evaluation. The gestational age was confirmed by the last menstrual period and sonographic measurement of the crown-rump length in the early pregnancy period.

Maternal CST Serum Concentrations

After 12 hours of fasting, 5 mL of venous blood samples were obtained from all participants and placed in vacuum tubes without anticoagulants. Blood samples of pregnant women with PE were taken at the first visit after diagnosis. These samples were then centrifuged at 1800 g for 10 min. Serum samples were stored at -80 °C until measurements were made. Serum CST levels were measured using an enzyme-linked immunosorbent assay (ELISA) kit (Bioassay Technology Laboratory, Shanghai, China, catalog no. E4996Hu). The test sensitivity was 0.046 ng/

mL (0.1-40 ng/mL). Absorbance in specimens was measured at a 450 nm wavelength on a VERSA microplate reader (Molecular Devices in California, USA). Results are presented in ng/mL. The intra-assay (CV) reliability of this ELISA method was <8%, and that of the inter-assay distribution was <10%.

Doppler Velocimetry

Ultrasonographic evaluation of all participants was performed by the only specialist (M.O.) experienced in the fetal heart using the Voluson E10 (General Electrics Healthcare, Zipf, Austria) ultrasound system. MPI measurements were performed from the fetal left ventricle using the technique specified by Hernandez-Andrade et al.[14]. After obtaining the four-chamber of the heart in the transverse section of the fetal thorax, the probe was angled toward the left ventricular outflow tract. The Doppler sample was set to 3 mm and was positioned to contain both the lateral wall of the ascending aorta and the inner leaflet of the mitral valve (MV) so that the mitral inlet and the aortic outlet were simultaneously captured. During the MPI waveform measurement, attention was paid to the absence of fetal breathing or movement. The insonation angle was set to <15 degrees, the Doppler scan speed at 5 cm/s, and the wall motion filter at 300 Hz. Measurements of three-time intervals were used: İsovolumetric contraction time (ICT), the time between the MV closure and the aortic valve (AV) opening; isovolumetric relaxation time (IRT), the time between AV closure and MV opening; and ejection time, (ET), the time between the AV opening and closure. MPI value was calculated with the formula: (ICT + IRT)/ET[7]. The peak velocity of the E wave represents early diastole with the MV opening and the peak velocity of the A wave resulting from atrial contraction in late diastole was determined as positive flow, and then the E/A ratio was calculated (Figure 1). Uterine artery Doppler evaluation was made as specified in the International Society of Ultrasound in Obstetrics and Gynecology guideline[15].

The intraobserver reproducibility was assessed by calculating the intraclass correlation coefficients (ICC). ICC was 0.86 (95% confidence interval: 0.82-0.90) for interobserver agreement. Women in the reliability study were excluded from the control group of the study.

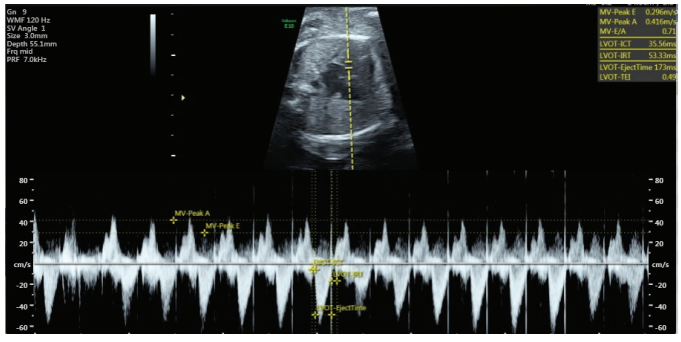

Figure 1. The myocardial performance index, E/A ratio, E and A waves peak velocity ratio at the mitral valve

Statistical Analysis

Statistical Package for the Social Sciences 21 program (IBM, NY) designed for Windows was used for the statistical analysis. All continuous variables were defined as mean and standard deviation, whereas categorical variables were defined as a percentage of the total group. A p-value of <0.05 was statistically significant. The three groups were first compared using the Kruskal-Wallis test, then paired groups were compared using the Mann-Whitney U test and chi-square or Fischer test. The Mann-Whitney U test was used to compare continuous variables in two groups. The chi-square or Fischer test was used to compare categorical variables. The relationship between the maternal serum CST levels and the fetal echocardiographic parameters was tested using the Pearson and Spearman correlation analyzes.

Results

Demographic and clinical characteristics and biochemical results are presented in Table 1. No significant difference was found between the groups in terms of age, gravida, parity, body mass index, aspartate aminotransferase, and platelet levels. When the control group was compared with the other two groups, a statistically significant difference was found between the groups in terms of systolic blood pressure, diastolic blood pressure, and uterine artery mean pulsatility index (p<0.001). The proteinuria value, blood sampling time, and ultrasound time in the EOPE group were statistically significantly different than the LOPE group (p<0.001, p=0.015, p=0.015, respectively). The maternal serum CST levels were low in the EOPE group; however, no statistically significant difference was found between the groups.

Perinatal results of cases are summarized in Table 2. The comparison of the EOPE group with the other two groups in terms of the gestational age at birth, fetal weight, 5th minute Apgar score, and neonatal intensive care unit admission was statistically significant (p<0.001). No significant difference was found in the cord pH values between the groups.

The echocardiography results, which evaluate the fetal cardiac functions, are shown in Table 3. The comparison of the EOPE group with the other two groups was statistically significant different in the fetal E/A ratio and MPI values (p=0.013, p=0.002, p=0.005, p<0.001, respectively). When the EOPE group and the control group were compared, a significant difference was found between the fetal E wave and IRT results (p=0.046, p=0.040, respectively).

The correlation of the maternal serum CST levels with the fetal echocardiographic parameters for both the PE and control groups is presented in Table 4. The fetal E/A ratio was positively correlated with maternal serum CST levels in both the PE and control groups (p<0.001, p<0.001, respectively). The fetal IRT and MPI values were negatively correlated with the maternal serum CST levels in both the PE and control groups (p<0.001, p=0.001, p<0.001, p=0.002, respectively).

Table 1. The comparison of the clinical and biochemical profiles of the study subjects

	EOPE	LOPE	Control	p-value		
	(n=27)	(n=28)	(n=28)	1-2	1-3	2-3
Age (years)	31.3±6.6	29.1±6	29.7±5.7	0.218	0.873	0.527
Gravidity	2 (1-5)	3 (1-6)	2 (1-5)	0.842	0.229	0.129
Parity	1 (0-3)	1 (0-4)	1 (0-4)	0.922	0.323	0.335
BMI (kg/m²)	31.4±5.3	30.8±4.7	30.5±2.6	0.655	0.755	0.935
SBP (mmHg)	160.5±18.7	145.1±7.8	117.8±6.9	**<0.001**	**<0.001**	**<0.001**
DBP (mmHg)	104.2±11.5	95.8±7.5	76.2±5.5	**0.004**	**<0.001**	**<0.001**
Proteinuria (g/day)	2900.8±3392.1	418±490.1	-	**<0.001**	-	-
AST (U/L)	30.3±29.6	24.9±10.5	21.3±6.8	0.980	0.311	0.313
ALT (U/L)	23.2±21.4	16.5±14.2	13.2±8.5	0.231	**0.032**	0.120
Platelet (10⁹/L)	198.8±72.1	204.9±70.6	226.9±61.9	0.686	0.084	0.201
Creatinine (mg/dL)	0.77±0.45	0.59±0.1	0.49±0.1	0.064	**<0.001**	**0.001**
Uric acid (mg/dL)	5.4±1.3	4.9±1.4	3.4±0.8	0.129	**<0.001**	**<0.001**
LDH (U/L)	300.8±128.4	227.1±77	193.1±49.2	0.051	**0.002**	0.131
UA mean PI	1.44±0.4	1.06±0.2	0.73±0.2	**<0.001**	**<0.001**	<0.001
GA at USG (weeks)	31.5±4.5	34.6±0.5	34±1	**0.015**	0.062	0.055
GA at blood sampling (weeks)	31.5±4.5	34.6±0.5	34±1	**0.015**	0.062	0.055
Catestatin (ng/mL)	3.77±2.83	5.20±4.6	5.23±4.43	0.242	0.162	0.851

EOPE: Early onset preeclampsia, LOPE: Late onset preeclampsia, BMI: Body mass index, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, LDH: Lactate dehydrogenase UA: Uterin artery, GA: Gestational age, USG: Ultrasonography

Table 2. Perinatal outcomes of EOPE, LOPE and control groups

	EOPE	LOPE	Control	p-value		
	(n=27)	(n=28)	(n=28)	1-2	1-3	2-3
GA at birth (weeks)	31.5±4.5	36.6±1	38.8±1	**<0.001**[a]	**<0.001**[a]	**<0.001**[a]
Cesarean section rate (%)	24/27 (88.9%)	25/28 (89.3%)	17/28 (60.7%)	**1**[c]	**0.016**[b]	**0.014**[b]
Birth weight (g)	1735±1061	2952±658	3334±409	**<0.001**[a]	**<0.001**[a]	**0.024**[a]
Cord pH	7.29±0.1	7.33±0.1	7.33±0.1	0.214[a]	0.124[a]	0.565[a]
Apgar score 5th min	5.85±2.8	8.3±1.1	8.3±1.2	**<0.001**[a]	**<0.001**[a]	1[a]
NICU admission (%)	25/27 (92.6%)	12/28 (42.9%)	10/28 (35.7%)	**<0.001**[b]	**<0.001**[b]	0.584[b]

[a]Mann-Whitney U test, [b]Chi-square test, [c]Fischer's exact test, GA: Gestational age, NICU: Neonatal intensive care unit, EOPE: Early onset preeclampsia, LOPE: Late onset preeclampsia

Discussion

This study evaluated the maternal serum CST levels and the fetal cardiac functions in pregnant women with PE and with normal blood pressure. Based on the known cardioprotective properties of the molecule, the relationship was examined between the maternal serum CST levels and the fetal cardiac functions. Only one recent study was reported in the literature evaluating the maternal serum CST levels in pregnant women with PE[16]. The study by Tüten et al.[16] revealed that the maternal serum CST levels were significantly higher in the PE group than that in the control group. Our study revealed low maternal serum CST levels in the EOPE group, but no statistically significant difference was observed between the groups. To the best of our knowledge, our study is the first study in the literature that evaluates the fetal cardiac functions in preeclamptic and control group pregnancies and examines their relationship with the maternal serum CST levels.

While the pathophysiology of PE is still controversial, partial or complete failure of placental implantation and trophoblastic

Table 3. Fetal echocardiography results of EOPE, LOPE and control groups

	EOPE	LOPE	Control		p-value	
	(n=27)	(n=28)	(n=28)	1-2	1-3	2-3
Mitral E (cm/s)	35.7±3.7	37.6±3.6	37.9±3.7	0.770	**0.046**	0.934
Mitral A (cm/s)	59.4±10.5	56.7±6.5	56.6±4.1	0.203	0.215	0.532
Mitral E/A ratio	0.61±0.06	0.66±0.08	0.67±0.07	**0.013**	**0.002**	0.812
ICT (ms)	35.1±5.3	33.8±5	34.2±4.9	0.413	0.715	0.709
IRT (ms)	48.9±4.7	45.7±6.5	44.9±7.3	0.068	**0.040**	0.576
ET (ms)	145±14.3	146.4±14.5	150.1±15.8	0.846	0.270	0.275
MPI	0.58±0.03	0.54±0.05	0.53±0.05	**0.005**	**<0.001**	0.197

ICT: Isovolumetric contraction time, IRT: Isovolumetric relaxation time, ET: Ejection time, MPI: Myocardial performance index, EOPE: Early onset preeclampsia, LOPE: Late onset preeclampsia

Table 4. Correlation of maternal serum catestatin levels with fetal echocardiographic parameters in preeclampsia and control groups

	Catestatin			
	Preeclampsia group (n=55)		Control group (n=28)	
	r*	p	r**	p
Mitral E wave	-0.103	0.454	0.625	**<0.001**
Mitral A wave	-0.580	**<0.001**	-0.395	0.370
Mitral E/A ratio	0.662	**<0.001**	0.935	**<0.001**
ICT	0.269	0.470	0.007	0.972
IRT	-0.838	**<0.001**	-0.603	**0.001**
ET	0.339	0.110	-.0.037	0.852
MPI	-.0.758	**<0.001**	-0.557	**0.002**

*Pearson correlation, **Spearman's rho ICT: Isovolumetric contraction time, IRT: Isovolumetric relaxation time, ET: Ejection time, MPI: Myocardial performance index

invasion are the main focus mechanisms[17,18]. Impaired Ach-mediated vasorelaxation due to developing endothelial dysfunction, decreased production of vasodilators such as nitric oxide and prostacyclin, increased production of vasoconstrictors such as endothelins and thromboxane, oxidative stress, and the changing rate of antiangiogenetic factors are other mechanisms that are thought to have a role in the disease. Another critical factor in the pathogenesis of PE is the increased sympathetic nervous system response[19]. Schobel et al.[20] observed an increased sympathetic nerve activity in the muscles of women with PE compared to those who are normotensive. While antihypertensive responses to nonselective adrenergic receptor blockage are high in women with PE, baroreflex sensitivity has decreased[19,21]. Increased sympathetic activity in the early period was associated with placental hypoxia/reperfusion. Pro-hypertensive placental factors that develop due to hypoxia play a role in the development of PE by joining the maternal circulation[22]. PE is divided into two groups according to the age of gestation upon diagnosis. While EOPE seems to be associated with poor placentation in the first trimester and represents a more severe clinical spectrum, the main problem in LOPE is the exaggerated maternal systemic inflammatory response[13].

CST regulates the autonomic cardiovascular control at the central systemic level through the baroreceptor afferent fibers of the nucleus tractus solitarius[10]. CST, effective in the peripheral system by stimulating histamine release, inhibiting catecholamine release, and lowering blood pressure[11]. Fung et al.[9] reported that decreased CST levels are associated with the risk of hypertension and the vasodilatory effect of local infusion of exogenous CST. Exogenous CST infusion provided normotension in CgA knock-out mice, representing a monogenic model of mouse hypertension[12]. *In vivo* CST also showed a supportive effect on angiogenesis/arteriogenesis and vasculogenesis in a unilateral mouse hindlimb ischemia model[23]. In our study, the maternal serum CST levels were lower in the EOPE group than that of the other two groups, but without a statistically significant difference. Tüten et al.[16] found higher maternal serum CST levels in pregnant women with severe PE than those of the control group and stated that their results were not consistent with other studies conducted on patients with hypertension in the literature. Differences

in the classification of PE (EOPE-LOPE/mild-severe) among studies caused changing results. In addition, Kiranmayi et al.[24] revealed that the Gly364Ser allele, a common naturally occurring genetic variation of CST, is associated with high blood pressure levels. Genetic variants of CST that can affect autonomic activity can also change the risk of hypertension.

Our study revealed that in fetuses in the EOPE group, MPI, which is a total indicator of cardiac function, significantly increased, whereas the E/A value, which was used to evaluate the diastolic cardiac function, significantly decreased. The high IRT value in the EOPE group is another parameter that indicates diastolic dysfunction in these fetuses. In a recently published study evaluating >2000 fetuses, the longitudinal reference ranges of fetal cardiac Doppler parameters were determined according to the weeks of gestation[25]. According to the study results, the mean values of fetal cardiac Doppler parameters and the 5th and 95th percentile values were similar in the mean ultrasound weeks of our EOPE and LOPE groups. Therefore, we think that the difference between the ultrasound times is not the main reason for the results in the EOPE and LOPE groups. We think that the mechanism that causes cardiac dysfunction in fetuses in the EOPE group is related to increased fetal cardiac afterload due to the increased placental vascular resistance. Compatible monitoring of fetal cardiac parameters in the control and LOPE groups may be associated with the milder placental involvement of the disease. Api et al.[26] evaluated the cardiac functions of the PE group and the control group fetuses and could not find any differences between the MPI and E/A values of the groups. The study by Balli et al.[27] evaluated the cases with mild PE and control groups and found that the IRT value was high in the PE group, but the MPI value was low, without any differences between the groups for the E/A value. In these studies, the case analyses in the PE group was not performed as early and late-onset subgroups, which may have a difference with the results of our study since the cause of cardiac dysfunction seems to be an increased cardiac afterload secondary to a more severe placental involvement in the EOPE group. Another recent study compared 60 fetuses with EOPE with 60 normotensive pregnant fetuses, which found a significantly lower E/A value in the EOPE group[28]. Considering the studies suggesting that babies of mothers with PE are more likely to develop heart diseases later in life, this may be related to changes in the cardiac function that begin in the in-utero period[17-19].

Evidence accumulated in the literature points to the cardioprotective effects of CST. The sympathetic nervous system may contribute to the atherosclerosis process and the development of coronary artery disease[29]. Adrenergic overactivity may cause mechanical damage to the vascular wall due to increased blood pressure and increased flow rate[29,30]. CST demonstrates a protective effect on cardiac hypertrophy by reducing the pressure signal, and cardiac afterload also inhibits adrenergic stimulation in the heart and reduces myocardial ischemia/reperfusion injury[24,30]. At the organ level, CST has a negative inotropic effect in the myocardium and provides coronary dilatation through the β2-adrenergic receptor-nitric oxide-cGMP signal and plays a role in the cardiovascular function regulation[10,29,30]. Our study evaluated the correlation of the maternal serum CST levels with the fetal cardiac function markers in separate groups and obtained moderate/strong correlation results, especially for MPI, E/A ratio, and IRT. Lower CST levels are associated with fetal cardiovascular dysfunction. We believe that the cardiac afterload reduction effect of CST against the increased fetal cardiac afterload, which results from the pathophysiology of PE, is one of the crucial factors of these results.

Study Limitations

The primary strength of our study is its prospective design. Showing the effect of PE on fetal cardiac functions using the cardiac Doppler is important. This is the first study in the literature showing CST as an effective marker in evaluating fetal cardiac functions. However, our current study has some limitations. The relatively low number of cases in this cross-sectional study may have prevented us from finding a significant difference for CST in cases with PE. In addition, the genetic variants of CST were not studied.

Conclusion

In summary, no significant difference was found between the maternal serum CST levels between the PE and the control groups in this study. EOPE can cause fetal cardiac function changes. Lower CST levels are associated with fetal cardiovascular dysfunction. CST may be a critical biochemical marker in the evaluation of fetal cardiac function.

Ethics

Ethics Committee Approval: The study was approved by the Faculty of Medicine Ethics Committee of our University and was conducted following the Declaration of Helsinki (ethics committee no: 2020/283, date: 16.11.2020).

Informed Consent: Written informed consent was obtained from all-volunteer pregnant women who participated in the study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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The association between increased maternal PARK7 (DJ-1) levels and the occurrence of preterm premature rupture of membranes - A randomized prospective study

Artmış maternal parkinson hastalığı proteini 7 düzeyinin preterm prematür membran rüptürü ile ilişkisi - Prospektif randomize bir çalışma

Uğur Turhan[1], Burak Tatar[2]

[1]*University of Health Sciences Turkey, Samsun Training and Research Hospital, Clinic of Obstetrics and Gynecology, Division of Perinatology, Samsun, Turkey*

[2]*University of Health Sciences Turkey, Samsun Training and Research Hospital, Clinic of Obstetrics and Gynecology, Division of Gynecological Oncology, Samsun, Turkey*

Abstract

Objective: Parkinson's disease protein 7 (PARK7/DJ-1) is an important antioxidant multiprotein against inflammation and oxidative stress. We therefore planned this study to demonstrate the association between maternal PARK7 levels and the occurrence of preterm premature rupture of membranes (PPROM).

Materials and Methods: We enrolled 80 pregnant women; 50 PPROM patients and 30 healthy controls, into this cross- sectional study between the 24th and 36th gestational weeks. Furthermore, we measured serum PARK 7 levels using enzyme-Linked immunosorbent assay method.

Results: Plasma PARK7 levels were higher in PPROM patients compared to those in the healthy controls ($p<0.001$). With a cutoff value of 2.57 pg/mL; PARK 7 had a 92% sensitivity, 86% specificity, 88% positive predictive value and 83% negative predictive value ($p<0.01$) for PPROM. PARK7 had positive correlation with blood leukocyte levels ($p<0.001$), C-reactive protein levels ($p<0.001$), and negatively correlated with birth weight and birth week.

Conclusion: PARK 7 is overexpressed in PPROM patients. Due to its anti-inflammatory and antioxidant properties, PARK7 may be a novel marker in better understanding the pathophysiology and prediction of the prognosis PPROM. Further large-scale studies are needed.

Keywords: Preterm premature rupture of membranes (PPROM), oxidative stress, inflammation, Parkinson's disease protein 7 (PARK7), DJ-1

Öz

Amaç: Parkinson hastalığı protein 7 (PARK7) enflamasyon ve oksidatif strese karşı önemli bir antioksidan multiproteindir. Bu çalışmayı preterm prematüre membran rüptürü (PPROM) hastalarında maternal PARK (DJ-1) düzeyini araştırmak için tasarladık.

Gereç ve Yöntemler: Kesitsel çalışmaya 24-36. gebelik haftaları arasında 50 PPROM ve 30 sağlıklı kontrol hastası olmak üzere toplamda 80 hasta dahil edildi. Enzim-Bağlı immünosorbent tahlili yöntemiyle maternal serum PARK 7 seviyeleri ölçüldü.

Bulgular: PPROM hastalarında sağlıklı kontrollere göre plazma PARK7 düzeyleri daha yüksekti ($p<0,001$). PARK7, kanda lökosit düzeyleri ($p<0,001$), CRP düzeyleri ($p<0,001$) ile pozitif, doğum ağırlığı ve doğum haftası ile negatif korelasyon gösterdi.

Sonuç: PARK 7, PPROM hastalarında aşırı eksprese edilmektedir. Anti-enflamatuvar ve antioksidan özelliklerinden dolayı PARK7, PPROM patofizyolojisini ve prognozunu öngörmede yeni bir belirteç olabilir. Daha geniş çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: Preterm prematür membran rüptürü (PPROM), oksidatif stres, enflamasyon, Parkinson hastalığı protein 7 (PARK 7), DJ-1

PRECIS: Maternal serum PARK7 levels are elevated in PPROM patients. PARK 7 levels can be combined with WBC, CRP, PCT in PPROM follow-up.

Address for Correspondence/Yazışma Adresi: Uğur Turhan MD, University of Health Sciences Turkey, Samsun Training and Research Hospital, Clinic of Obstetrics and Gynecology, Division of Perinatology, Samsun, Turkey

Phone: +90 505 895 40 00 **E-mail:** drugurturhan@hotmail.com **ORCID ID:** orcid.org/0000-0002-0653-0630

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Introduction

As the cause of approximately one-third of preterm births, preterm premature rupture of membranes (PPROM), has an incidence of 3% of all pregnancies. PPROM is defined as the rupture of the amniotic membrane which leads to leakage of amniotic fluid before 37 weeks of gestation[1]. Although risk factors like history of PPROM in previous pregnancies, second or third trimester vaginal bleeding, vitamin deficiency, connective tissue diseases, smoking, alcohol use, low body mass index (BMI), diabetes mellitus are known, the etiopathology is not fully understood. Besides, PPROM cannot be predicted[2-4]. The complications associated with PPROM include chorioamnionitis (CA), oligohydramnios, prematurity, and intrauterine death[5,6]. Among the predisposing factors, the most potential risk factor is infection and inflammation. Infection leads to releasing of proinflammatory cytokines from amniotic membrane and decidua[7]. Infection is seen with a frequency of 15%-25% in the antepartum period and 15%-20% in the postpartum period in PPROM patients[8]. The aim of the follow-up period is to monitor closely the clinical signs of infection and then deciding the time of active labor with close monitoring of signs of CA until reaching the 34th week of gestation, if possible.

Currently, there are no accepted diagnostic markers predicting the risk of CA. White blood cell count (WBC), C-reactive protein (CRP), Procalcitonin (PCT), interleukin-6 (IL-6), fasting blood glucose and heat-shock proteins are the most studied markers[9-11]. WBC increases physiologically during gestation and is increased further with exogenous antenatal steroids administered. Thus, its value in CA clinical diagnosis is limited. CRP, another marker increased physiologically during pregnancy, has limited value in the clinical practice of CA because it increases in the late period of the infection. PCT is an acute phase reactant that shows monocyte activity and does not increase in normal pregnancy. Many studies are inconsistent with its predictive power for CA[12-14].

PARK 7, also known as Parkinson's disease protein 7 (PARK7/DJ-1), has the gene on the short arm of chromosome 1 (1p36.12- 1p36.33), and was first identified with Parkinson's disease[15,16]. It has been identified in more than 20 tissues including fetal membranes and placenta, and its main role is to provide cellular protection against oxidative stress[17]. In neural tissue, it is responsible for astrocyte activation during oxidative stress, regulating genes such as glutamate cysteine ligase, extracellular superoxide dismutase (SOD3) or manganese superoxide dismutase[18].

PARK-7 expression increases especially in sepsis, in concordance with its role in impairing bacterial clearance with decreasing ROS production[19]. The increased PARK-7 binds to p47phox, a critical component of NADPH, thereby disrupting the NADPH oxidation complex. PARK-7 performs this function by facilitating *Nox2* (gp91phox) ubiquitination[19]. Macrophage and concurrent cytokine stimulation from the bone marrow due to increased endotoxin levels secondary to infection increases DJ-1 mRNA and protein expression[20].

To this respect, we hypothesized that PARK-7 expression and its maternal serum levels in PPROM patients should be higher. In this pilot study, we investigated the efficacy of measuring PARK7 levels in maternal serum in subclinical infections such as PPROM without CA.

Materials and Methods

This cross-sectional study was carried out in the Samsun Training and Research Hospital between January 1, 2019 and January 1, 2020. The study was approved by the Local Ethics Committee with the approval number of CAEK 2019/2/13 in accordance with the 2003 Helsinki Declaration. We enrolled 80 pregnant women between 24 and 34 weeks gestation: 50 women with a history of PPROM were included in the study group while 30 women with healthy pregnancy course were accepted as healthy controls.

The gestational age (GA) of patients were confirmed with the last menstrual period and Crown-rump length. PPROM diagnosis was made based on the patient's history, vaginal speculum assessment and sonographic evaluation of the amniotic fluid and for indecisive cases, sampling from vaginal fluid was made with Amnisure (Qiagen, Germany).

Any chronic systemic disease (pregestational or gestational diabetes, hypertension, preeclampsia, hypo-hyperparathyroidism, hepatitis, asthma, cardiac pathologies and renal disease), any cervical dilatation in administration, presence of placental abnormalities (placenta previa, morbidly adherent placenta), cerclage presence in cervix, any acute infection symptoms, emergent labor indication such as non-stress test. Abnormality or abruption of placenta, and presence of fetal anomalies were excluded from the study.

After admission to inpatient clinics, urine cultures and recto-vaginal Beta Streptococcus cultures were obtained from all patients. Serum Park-7 levels measured at the time of admission to the hospital, no more than 12 hours after the PPROM diagnosis. Besides, Park-7 level is measured before administration of antibiotics, steroids or magnesium sulfate. Routine prophylactic antibiotics were started (for 48 hours IV ampicillin and oral azithromycin for 6 days, and then oral ampicillin until labor) All PPROM patients received two doses of betamethasone treatment 24 hours apart for antenatal lung maturation. For fetal neuroprotection, intra-venous 4 g loading dose in 15 minutes continued by 1 g/hour magnesium sulfate was given for patients under 32 weeks of gestation for 24 hours. Patients were monitored closely with daily WBC, PCT and CRP for CA risk.

CA was diagnosed in the presence of at least three of the following symptoms: fever (≥38 °C), vaginal discharge, maternal tachycardia (>100 beats/min), fetal tachycardia (>160 beats/min), abdominal pain, uterine tenderness, and leukocytosis[21]. Pregnancy termination was performed in patients diagnosed

with CA and placenta was sent to pathology for histopathological confirmation of diagnosis.

Patients without inevitable labor, without a sign of CA and amniotic fluid index ≥5 were followed-up until 34 week of gestation and then delivered.

Biochemical Analysis

Serum procalcitonin, CRP and WBC count were measured in the first day of admission to inpatient clinics. WBC level was determined with the Sysmex XP-300(®) kit. Hematology Analyzer (Sysmex, America, Inc.) CRP was measured using the immunoturbidimetric assay. Pars Azmin, Tehran, Iran) Procalcitonin value was measured with BRAHMS PCT kit (Roche Diagnostic, Mannheim, Germany). A serum level equal to or greater than 2 ng / mL was considered positive for CA[7].

We collected 5 cm^3 of maternal serum after 12 hours of fasting on the first day of hospitalization in a biochemistry tube. Then, we centrifuged it at 1000 rpm and 2 °C-8 °C temperature for 15 minutes. The supernatant of the serum was placed in 1.5 mL Eppendorf tubes and stored at -80 °C. Maternal serum plasma PARK 7 level was measured using the ELISA kit, following the manufacturer's instructions (Recombinant Human PARK7/DJ-1 Protein (His Tag); catalog no: PKSH030826, Cusabio Biotech Co. Ltd. China).

Statistical Analysis

Data was analyzed using the Statistical Package for the Social Sciences version 20 (SPSS Inc). The distributions of all the continuous variables were tested using the Kolmogorov-Smirnov test. The variables with normal distribution were compared between two groups using an independent samples t-test, and were compared among more than two groups using ANOVA.

The results were expressed as the mean ± standard deviation. The Mann-Whitney U test was used to analyze the nonnormally distributed variables involving two groups, while the Kruskal-Wallis test was used to analyze variables involving more than two groups, and the results were expressed as median and interquartile range. If the values were significantly different in the Kruskal-Wallis test and ANOVA, then, the Bonferroni-adjusted Mann-Whitney U and Tukey-Kramer Post-hoc tests were performed, respectively.

The chi-squared test was used to compare the categorical variables, and data obtained were presented as proportions. The correlations were assessed using Spearman's correlation coefficient, along with their related p-values. A two-tailed p-value of less than 0.05 was considered statistically significant.

Results

The demographics and laboratory results of the patients are demonstrated in Table 1. There was no difference in maternal age, BMI, and GA between the PPROM and control groups. WBC accounts, serum CRP levels, and serum PARK 7 levels were significantly higher in the study group ($p<0.01$) (Figure 1).

GA at birth, birth weight and Apgar scores were significantly lower in the PPROM group (Table 2).

With a cutoff value of 2.57 pg/mL; PARK 7 had 92% sensitivity. 86% specificity, 88% positive predictive value and 83% negative predictive value ($p<0.01$) for PPROM. Receiver operating characteristic curve of PARK 7 for prediction of PPROM is demonstrated in Figure 2.

PARK 7 negatively correlated with GA at birth, birth weight, 10^{th} minute Apgar scores ($p<0.01$), and positively correlated with WBC and serum CRP levels ($p>0.05$) (Table 3).

Discussion

PARK 7 is overexpressed in PPROM patients and a cutoff value of 2.57 pg/mL seemed to have a high sensitivity and specificity for predicting PPROM. Due to its anti-inflammatory and antioxidant properties, PARK7 could be considered a novel marker in better understanding the pathophysiology and prediction of the prognosis of PPROM.

PARK 7, denominated after the discovery of familial Parkinson's disease, proves to be an important antioxidant stress regulator especially in hypoxia-induced cellular response[22]. It protects endothelial cells, macrophages, fibroblast cells, neurons, and pancreatic islet cells against oxidative stress in high

Table 1. The basic characteristics and laboratory parameters

Parameters	PPROM n=50	Control n=30	p-values
Age (years)	31.9±3.2	31.5±4.2	0.84
BMI, (kg/m^2)	27.9±1.9	27.6±2.1	0.89
GA, sampling, (w)	28.9±1.9	29±1.7	0.75
WBC, On admission (count/mL)	12.4±2.6	9.5±1.9	**<0.001**
CRP, On admission (mg/dL)	13.4±4.1	6.3±2.1	**<0.001**
PCT, On admission (ng/mL)	0.52±0.41	0.50±0.35	0.43
PARK 7 values (pg/mL)	4.83±1.78	2.05±0.44	**<0.001**

PPROM: Premature rupture of membrane, BMI: Body mass index, GA: Gestational age, W: Week, WBC: White blood cells, CRP: C-reactive protein, PCT: Procalcitonin, SD: Standard deviation Data are expressed as median (interquartile) or mean (± SD). p-value <0.05 is significant

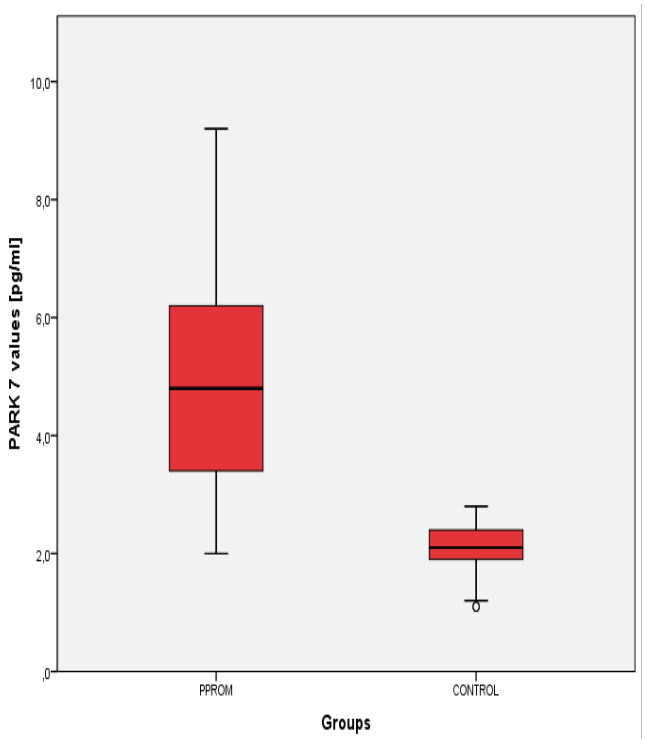

Figure 1. PARK 7 levels according to the groups

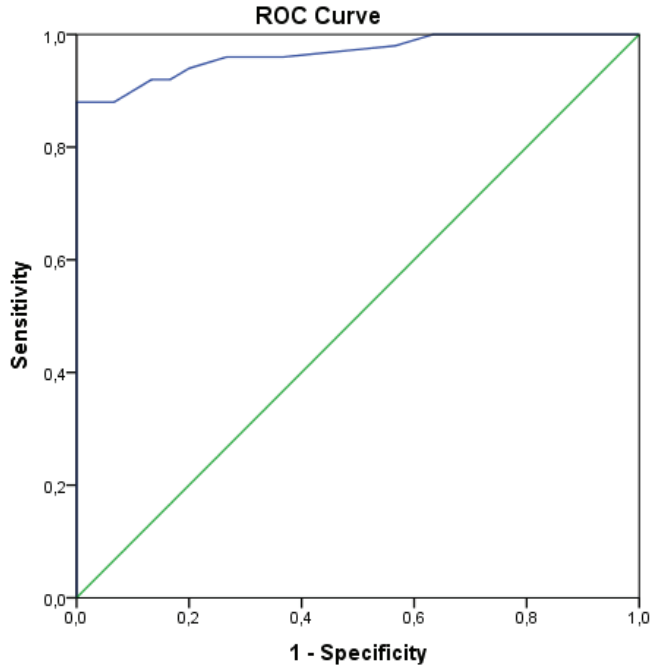

Figure 2. Receiver operating characteristic (ROC) curve of PARK 7 for prediction of PPROM

Table 2. Perinatal outcomes

Outcomes	PPROM n=50	Control n=30	p-values
GA at birth (w)	30.3±2.3	39.3±1.1	**<0.001**
Birth weight (g)	1598±543	3272±198	**0.009**
1-min Apgar score	5.8 (4-8)	8 (6-10)	**<0.001**
5-min Apgar score	8.5 (6-10)	9.2 (8-10)	**<0.001**

PPROM: Premature rupture of membrane, GA: Gestational age

Table 3. Correlation analyses between maternal serum PARK 7 and clinical parameters

PARK 7 n (50) PPROM	r	p
Age	-0.04	0.74
BMI	-0.19	0.18
GA at sampling	-0.07	0.59
GA at birth	-0.5	**0.001**
Birth weight	-0.49	**0.001**
Apgar 5	-0.07	0.58
Apgar 10	-0.45	**0.001**
WBC	0.29	**0.039**
CRP	0.57	**0.001**
PCT	0.17	0.23

r: Spearman's coefficient. p values <0.05 is considered statistically significant

oxidative stress conditions such as Hydrogen peroxide (H_2O_2), 6-hydroxydopamine and high glucose[23].

The mean range of serum PARK-7 levels in healthy individuals differs from study to study. In a study for chronic pancreatitis and pancreatic cancer, healthy controls had a mean level of 0.6236 (0.4221-2.0) ng/mL[24]. In two studies conducted for Parkinson's disease (PD), serum concentrations of PARK-7 were found at 18.1(±12.8) ng/mL and 2.49 (±0.60) ng/mL, and in both studies, no significant difference was observed in serum levels between PD and controls[25,26].

PARK-7 is stained immunohistochemically in the syncytiotrophoblast, cytotrophoblasts, vascular endothelial cells, and stroma, and the immunoreactivity is found to be higher in the syncytiotrophoblast of preeclamptic placentas compared to healthy placentas[27].

According to the aforementioned studies, PARK-7 seems to increase in diseases associated with inflammation and hypoxia induced cells such as pancreatitis pancreatic cancer, and preeclampsia, but serum level does not differ in PD, where expression is increased in neural tissue[26].

The main events attributed to the physiopathology of PPROM: inflammation, infection, oxidative stress and collagenolytic enzyme activation resulting impaired apoptosis of fetal membranes[28].

Increased mitochondrial activity and ROS production against oxidative stress induces PARK 7, which has increased expression in severe preeclampsia in patients with defective trophoblastic invasion in the placenta[29,30].

On the other hand, there must be a balanced matrix metallo-proteinase (MMP)/(tissue inhibitor of metallo-proteinase) TIMP system is involved in the production of collagen, which is necessary for membrane health[31]. PARK 7 down-regulation decreases the expression of MMP 2 and MMP 9[32]. To this respect, PARK-7 overexpression is expected in PPROM in which the MMP system is over-expressive. In this study, with a cutoff value of 2.57 pg/mL; PARK 7 had 92% sensitivity. 86% specificity, 88% positive predictive value and 83% negative predictive value ($p<0.01$) for PPROM.

Limited studies on PARK 7 were conducted in pregnancy and most of them focused on migration, cellular adhesion, and invasion in trophoblasts[30,32]. Especially, it is more expressed from syncytiotrophoblast and cytotrophoblast cells in the 1st trimester than the 3rd trimester[32]. In humans, fetal membranes play a role both during normal birth and in PPROM. Studies have not yet clearly defined how this balance is disturbed in PPROM, but proteases can activate MMPs with infection or inflammation of membranes thereby causing collagen catabolism and membrane rupture. PARK-7 levels were found to be positively correlated with WBC and serum CRP levels, consistent with the aforementioned hypothesis. After rupture of membranes, subclinical infection and inflammation rate increases with time, and this may increase serum PARK-7 levels[19]. In our study, we measured PARK-7 levels not more than 12 hours after diagnosing PPROM. Therefore, the effect of possible infection and inflammation on PARK-7 levels is minimized.

Study Limitations

Limitations of this study included small sample size and lack of follow-up of serum WBC, CRP and PARK-7 levels that we cannot conclude the rise of PARK-7 is limited to antenatal period.

Conclusion

To date, the relationship between plasma PARK-7 levels in healthy pregnancy and PPROM was unknown. To the best of our knowledge, this is the first study evaluating maternal serum PARK7 levels in PPROM patients. PARK 7 levels can be combined with WBC, CRP, PCT in the follow-up of PPROM. Further studies with large series are warranted to confirm these findings.

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Ethics

Ethics Committee Approval: The study was approved by the Local Ethics Committee with the approval number of CAEK 2019/2/13 in accordance with the 2003 Helsinki Declaration.

Informed Consent: Inform consent was obtained.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: B.T., Design: B.T., Data Collection or Processing: U.T., Analysis or Interpretation: U.T., Literature Search: U.T., Writing: B.T.

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Effects of growth hormone co-treatment on in vitro fertilization outcomes in women with expected normal ovarian response

Büyüme hormonu ko-tedavisinin normal ovaryan cevap beklenen infertil kadınların IVF sonuçlarına etkisi

Fırat Tülek[1], Alper Kahraman[2]

[1]*Memorial Ataşehir Hospital, Clinic of Obstetrics and Gynecology, İstanbul, Turkey*

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

Abstract

Objective: This study aimed to evaluate the effects of adjuvant growth hormone (GH) therapy on in vitro fertilization outcomes in women with infertility with expected normal ovarian response who underwent gonadotropin-releasing hormone (GnRH) antagonist protocol with dual triggering.

Materials and Methods: Records of women who underwent GnRH antagonist cycles with dual triggering in a single tertiary center between 2017 and 2020 were retrospectively analyzed. A total of 1054 women with expected normal ovarian response were evaluated, of which 131 were found to receive GH co-treatment (study group). Moreover, 950 women did not receive any adjuvant therapy (control group). Their cycle outcomes were compared.

Results: The number of retrieved oocytes, oocyte maturation rates, quality of embryos, miscarriage rates, and multiple pregnancy rates were comparable among women who underwent GnRH antagonist cycles with and without GH co-treatment. The number of obtained 2PN embryos (5.68±2.46 vs 5.06±2.5; p=0.003), fertilization rates (0.84±0.16 vs 0.76±0.18; p<0.001), implantation rates (0.34 vs 0.25; p=0.006), clinical pregnancy rates (50.4% vs 38%; p=0.008), and live birth delivery rates (41.8% vs 32.2%; p=0.007) were significantly higher in women who received GH co-treatment.

Conclusion: GH co-treatment significantly increased the clinical pregnancy rates and live birth delivery rates in women with infertility and expected normal ovarian response who underwent GnRH antagonist protocol with dual triggering for oocyte maturation, which was possibly due to the increasing endometrial receptivity or improving oocyte quality.

Keywords: Growth hormone, infertility, dual trigger, live birth, in vitro fertilization

Öz

Amaç: Adjuvan büyüme hormonu kullanımının GnRH antagonist sikluslarında dual trigger uygulanan normal ovaryan cevap beklenen infertil kadınlarda siklus sonuçları üzerine etkisini araştırmaktadır.

Gereç ve Yöntemler: Üçüncü basamak bir infertilite merkezinde 2017 ile 2020 yılları arasında dual trigger ile GnRH antagonist siklusu uygulanan kadınların verileri retrospektif olarak taranmıştır. Toplamda 1054 normal ovaryan cevap beklenen infertil kadın çalışmaya dahil edilmiştir. Yüz otuz bir hasta adjuvan büyüme hormonu almış ve çalışma grubuna dahil edilmiştir. Dokuz yüz elli üç hasta kontrol grubu olarak belirlenmiş ve siklus sonuçları karşılaştırılmıştır.

Bulgular: Elde edilen oosit sayısı, oosit matürasyon oranları, embriyo kalitesi, düşük oranları, çoğul gebelik oranları açısından çalışma ve kontrol grupları arasında fark bulunmamıştır. Elde edilen 2PN embriyo sayısı (5,68±2,46 vs 5,06±2,5; p=0,003), fertilizasyon oranları (0,84±0,16 vs 0,76±0,18; p<0,001), implantasyon oranları (0,34 vs 0,25; p=0,006), klinik gebelik oranları (%50,4 vs %38; p=0,008) ve canlı doğum hızları (%41,8 vs %32,2; p=0,007) adjuvan büyüme hormonu verilen kadınlarda anlamlı olarak yüksek saptanmıştır.

Sonuç: Adjuvan büyüme hormonu tedavisi normal ovaryan cevap beklenen infertil kadınlarda klinik gebelik oranları ve canlı doğum oranlarını anlamlı şekilde artırmaktadır.

Anahtar Kelimeler: Büyüme hormonu, infertilite, dual trigger, canlı doğum, in vitro fertilizasyon

PRECIS: We retrospectively evaluated the effects of adjuvant GH administration on IVF outcomes of infertile women with normal ovarian response that were underwent GnRH antagonist cycles with dual trigger.

Address for Correspondence/Yazışma Adresi: Fırat Tülek MD, Memorial Ataşehir Hospital, Clinic of Obstetrics and Gynecology, İstanbul, Turkey
Phone: +90 530 608 48 74 **E-mail:** firattulek@yahoo.com **ORCID ID:** orcid.org/0000-0003-1668-8746
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Introduction

Infertility is defined as the inability to achieve a pregnancy despite 1 year of unprotected intercourse. Worldwide, it affects approximately 10% of couples[1]. Infertility treatments are demanding in terms of financial, psychological, and physical aspects. Hence, infertility treatment, as well as subfertility, leads to frustration, brings anxiety, and creates distress in couples. The ultimate goal of assisted reproduction is pregnancy. Live birth rates in in vitro fertilization (IVF) cycles vary between 8% and 34% worldwide[2]. Therefore, despite the substantial recent advances in IVF, most of the couples still could not reach this eventual goal in their first IVF attempts. Research about better techniques and convenient adjuvants to improve IVF outcomes are ubiquitously ongoing. Two of the under-studied therapies are the use of growth hormone (GH) as an adjuvant in IVF cycles and the triggering of oocyte maturation with a combination of gonadotropin-releasing hormone (GnRH) agonist and human chorionic gonadotropin (dual trigger).

GH is a peptide mainly secreted from somatotropic cells of anterior hypophysis that stimulate cellular growth and regeneration. Human granulosa cells, oocytes, and endometrial cells are known to express GH receptors[3]. GH was found to potentiate the effects of follicle-stimulating hormone (FSH) and to induce oocyte maturation[3]. Given these effects, the use of GH as an adjuvant in IVF cycles to increase success rates appears as a notable idea since the first study by Homburg et al.[4] three decades ago. Although some data indicated that GH supplementation could improve reproductive outcomes and increase live birth delivery rates in poor responders, a few studies have evaluated the effects of GH supplementation on normoresponders and women with expected normal ovarian response with controversial results[5-18]. Therefore, literature data regarding this topic are still scarce.

In this study, we evaluated the effects of GH supplementation on IVF outcomes in women with normal prognosis who underwent GnRH antagonist cycle with dual triggering.

Materials and Methods

This study was conducted as retrospective analysis of patient records in a single tertiary center (Üsküdar University Faculty of Medicine) in Istanbul. Records of women who underwent GnRH antagonist cycles with dual triggering, either used adjuvant GH as physician's preference or no adjuvant at all between 2017 and 2020, were screened, and patients were contacted by phone when required.Ethical approval was obtained from the Ethical Committee of Üsküdar University Faculty of Medicine (approval no: 61351342/April 2021-81). The need for informed consent was waived by the ethical committee due to the retrospective design.

Patients with high (>30 kg/m^2) or low (<18 kg/m^2) body mass index; patients with endocrine disorders such as congenital adrenal hyperplasia, hyperprolactinemia, Addison disease, diabetes mellitus, Cushing syndrome, and thyroid dysfunction; patients with corrected or present uterine anomalies; and patients with severe male factor infertility were excluded from the study.

According to the POSEIDON classification, the low prognosis group of patients consisted of women with diminished ovarian reserves or women with suboptimal ovarian response in previous IVF attempts[19]. Patients considered as having a low prognosis according to the POSEIDON classification were also excluded from the analysis. Women with anti-Müllerian hormone levels ≥1.2 ng/mL and antral follicle count ≥5 prior to the commencement of cycles who either have no other previous IVF attempts or at least 10 oocytes were retrieved in all previous IVF attempts were included in the study, and they were referred to as women with normal prognosis or women with expected normal ovarian response. Only outcomes of fresh embryo transfers were evaluated.

Controlled ovarian stimulation was initiated within the first 5 days of the menstrual cycle. Recombinant follicle-stimulating hormone (rFSH, Gonal-F, Merck Serono S.p.A, Italy), human menopausal gonadotropin (Merional, IBSA Institut Biochimique S.A, Menopur®, Ferring Pharmaceuticals, Switzerland), or a combination of recombinant luteinizing hormone and rFSH (Pergoveris, Merck Serono SA, Switzerland) were used for ovarian stimulation on practitioner's choice. Patients were monitored during stimulation for the follicular growth with serial transvaginal ultrasonography and serum hormone levels. Adjustments in gonadotropin doses were made based on each patient's follicular growth. Once the leading follicle was observed to reach a diameter of 12-14 mm, GnRH antagonist (Cetrotide 0.25 mg, Pierre Fabre Medicament Production, France) injections commenced to suppress premature LH peak and continued to the day of oocyte maturation triggering. Daily injections of 4 IU (1.2 mg) of GH [Genotropin 36 IU (12 MG) GoQuick, Pfizer Inc., Australia] were implemented starting from the day of cycle commencement to the day of oocyte triggering in women to whom adjuvant therapy was administered. The dual-triggering method was used to induce oocyte maturation with a GnRH agonist of 0.2 mg triptorelin acetate (Gonapeptyl, Ferring Pharmaceuticals) and 250 mcg recombinant human chorionic gonadotropin (Ovitrelle, Merck Serono) when at least one follicle reached a diameter of 18 mm. Oocytes were retrieved under transvaginal ultrasound guidance 35-36 h after oocyte maturation triggering. Fertilization was conducted by intracytoplasmic sperm injection. Acquired embryos were graded according to Istanbul Consensus Workshop[20]. Day 3 or day 5 embryos were transferred by using an embryo transfer catheter under abdominal ultrasonography guidance depending on the condition. A maximum of two embryos were transferred at once following legal regulations. Luteal phase support was initiated in every patient with 200 mg intravaginal progesterone (Lutinus, Ferring Pharmaceuticals) twice a day when endometrial thickness was observed to reach 7-8 mm and continued until 8th-10th gestational weeks.

Patients with expected normal ovarian response who underwent GnRH antagonist cycle with dual triggering to whom adjuvant GH administered were assigned to the study group. The control group consisted of patients with normal prognosis who underwent GnRH antagonist cycle with dual triggering without adjuvant therapy within the selected period.The cycle outcomes of the study and control groups were compared. The primary outcome was live birth delivery rate. The secondary outcomes were the number of retrieved oocytes, number of metaphase 2 (M2) oocytes, oocyte maturation rates (M2 oocytes/retrieved oocytes), fertilization rates (2pronuclear embryos/M2 oocytes), implantation rates (gestational sacs observed/transferred embryos), number of obtained embryos, and grades of obtained embryos. Outcome parameters were described according to the International Glossary on Infertility and Fertility Care (2017) [21].

Statistical Analysis

Statistical analyses were performed using IBM SPSS v23 (IBM Corp, Armonk, NY, USA). Descriptive statistics were expressed as mean ± standard deviations for normally distributed data. Categorical variables were expressed as numbers and percentages (%). Significance of differences in means and medians among groups were assessed by Student's t-test and Mann-Whitney U test. Categorical variables were evaluated with Pearson's chi-squared test or Fisher's exact test. P-values <0.05 are considered significant.

Results

Following the exclusion of patients with confounding factors, a total of 131 women with expected normal ovarian response at the beginning of treatment were found to undergo GnRH antagonist cycles with dual triggering and received daily GH injections within the selected period. These women constituted the study group. GnRH antagonist cycles with dual triggering without adjuvant therapy were applied in 923 women with expected normal ovarian response, and these patients were assigned to the control group. The mean ages of the study and control groups were 35.17±3.82 and 34.31±4.89 years, respectively. The mean ages of groups were significantly comparable (p=0.077). The mean body mass index of the study and control groups were 25.15±2.71 and 24.95±2.54, respectively. No significant difference was found between the body mass index values of the groups (p=0.431). The causes of infertility within the study population were mild male factor, anovulation, tubal factor, endometriosis, and combined and unexplained factors. The prevalence of these etiologic factors was comparable between the groups (p=0.992). The distribution and comparison of the etiologic factors of infertility are presented in Table 1.

The total doses of gonadotropins required, stimulation length, and progesterone levels at the day of triggering were significantly higher in the control group than in the study group (p=0.013, p=0.036, p=0.004, respectively). Estradiol levels at the day of triggering, number of 2PN embryos obtained, fertilization rate, implantation rate, clinical pregnancy rate, and live birth delivery rates were significantly higher in cycles with GH injection (p<0.001, p=0.003, p<0.001, p=0.006, p=0.008, p=0.007, respectively). In women with GH co-treatment, the number of retrieved oocytes (9.79±3.46 vs 9.40±3.70; p=0.128), number of M2 oocytes (6.83±2.83 vs 6.66±2.89; p=0.426), oocyte maturation rates (0.70±0.16 vs 0.71±0.16; p=0.275), biochemical pregnancy rates (13% vs 9.5%; p=0.289), miscarriage rates (9.9% vs 7.3%; p=0.212), multiple pregnancy rates (2.3% vs 2.2%; p=0.299), endometrial thicknesses at the day of transfer (10.16±1.65 mm vs 10.13±1.84 mm; p=0.6), and mean number of transferred embryos (1.54±0.5 vs 1.59±0.49; p=0.326) were all comparable with the control group. No significant differences were observed within groups in terms of the number of transferred grade 1 and 2 embryos, mean number of embryos transferred, and distribution of transfers due to the days of embryos. Cycle outcomes and comparison of these findings are summarized in Table 2.

Table 1. Distribution and comparison of infertility etiologies among the groups

	Study group	Control group	p-value
Number of patients	131	923	
Mild male factor (n, %)	34 (26%)	261 (28.3%)	0.992
Anovulation (n, %)	31 (23.7%)	216 (23.4%)	
Tubal factor (n, %)	20 (15.3%)	128 (13.9%)	
Endometriosis (n, %)	12 (9.2%)	84 (9.1%)	
Combined (n, %)	9 (6.9%)	60 (6.5%)	
Unexplained (n, %)	25 (19.1%)	174 (18.9%)	

A cost analysis was performed by using the momentary retail prices of medications in Turkey at the time of manuscript preparation. The mean cost of medications in the adjuvant GH group per cycle including GH, gonadotropins, GnRH antagonists, and GnRH agonists was 377.7±131 USD. In the control group, the mean estimated cost per cycle was 359.2±953 USD. The mean cost of required medications were significantly comparable between the two groups (p=0.67).

Discussion

In this study, we evaluated the effects of GH co-treatment on IVF outcomes in women with expected normal ovarian response. Our results indicate significantly improved live birth delivery rates and clinical pregnancy rates in women with adjuvant GH administration.

A few studies have evaluated the outcomes of IVF cycles with GH supplementation in the normal population[14-17]. Younis et al.[17] conducted a prospective randomized study involving 21 women with normal ovulation who underwent the GnRH

Table 2. Comparison of cycle outcomes between the two groups

	Study group	Control group	p-value
Total dose of required gonadotropins	2325.67±643.22	2482.83±732.78	**0.013**
Stimulation lenght (days)	9.04±1.26	9.38±1.51	**0.036**
Estradiol levels on the day of triggering	1833.92±674.77	1586.37±742.91	**<0.001**
Progesterone levels on the day of triggering	0.52±0.33	0.59±0.31	**0.004**
Number of 2PN embryos per cycle	5.68±2.46	5.06±2.5	**0.003**
Number of embryos obtained			
Grade 1	181 (64.8%)	1261 (62.9%)	
Grade 2	90 (31.1%)	680 (33.9%)	0.586
Grade 3	8 (2.8%)	62 (3%)	
Number of embryos transferred (%)			
Grade 1	162 (80.2%)	1190 (81.2%)	0.774
Grade 2	40 (19.8%)	275 (18.8%)	
Fertilization rate per cycle	0.84±0.16	0.76±0.18	**<0.001**
Implantation rate	0.34 (69/202)	0.25 (371/1465)	**0.006**
Clinical pregnancy rate (no. of clicical pregnancies)	50.4% (66)	38% (351)	**0.008**
Live birth delivery rate (no. of live births)	41.8% (56)	32.2% (304)	**0.007**

agonist protocol with daily injections of 12 IU of GH. They found higher number of pregnancies, implantation rates, and estradiol levels in the GH supplementation group; however, none of these parameters have reached significance. Moreover, they reported comparable gonadotropin requirements and estradiol levels in women with and without GH supplementation[17]. Tapanainen et al.[16] assessed 19 women with 24 IU of GH supplementation. Although the study was not designed to evaluate live birth delivery rates, they reported two pregnancies in the placebo group and only one pregnancy in the GH supplementation group. They also reported comparable gonadotropin requirements among the groups, and compared with other studies, they found lower estradiol levels in women treated with GH[16]. The results of these two aforementioned studies might be affected by the small sample sizes[16,17]. Du et al.[14] retrospectively evaluated 556 women with infertility who underwent GnRH agonist cycles with 4.5 IU of GH supplementation. They found higher implantation rates and clinical pregnancy rates as well as higher embryo quality in women who received GH injections, but no differences were found in the required gonadotropin doses. Liu et al.[15] assessed 781 normal responders who underwent IVF cycles with GH supplementation of doses varying between 2 IU and 4 IU. They found a higher clinical pregnancy rate in the overall GH-administered group, but without significance. As they stratified patients according to the administered GH doses, women who received 4 IU of GH had the highest clinical pregnancy rates and required the lowest gonadotropin stimulation[15]. In our study, we found increased clinical pregnancy rates and live birth delivery rates in women with normal prognosis who were treated with 4 IU of adjuvant GH.GH directly and indirectly participates in the regulation of reproductive functions[22]. No consensus was established regarding the optimal GH doses required in infertility treatments. A study reported increased pregnancy rates in poor responders with GH supplementation doses as low as 0.5 IU[23]. An animal study indicated that GH might have bimodal inhibitory and stimulatory effects on various tissues[24]. For instance, Nakamura et al.[22] demonstrated that in the presence of FSH, GH enhances early reactions in steroidogenic pathways by increasing local insulin-like growth factor-1 (IGF-1) levels but inhibits FSH-induced aromatase via an IGF-independent way. In higher concentrations, the IGF-independent inhibitory effects of GH might surpass the stimulatory effects. Furthermore, in an animal study, Singh and Lal[25] mentioned the circadian effects of GH and showed increased ovarian steroidogenesis following GH injections in the morning but not in evening. The timing of GH injections within the day and doses might alter in vivo resultant effects of GH administration and contribute to different outcomes reported. In the present study, GH administrations were self-applied by the patient concomitant with gonadotropin injections. As a general adoption of practice, we recommend patients to execute injections between 6 pm and 8 pm.

Previous studies have demonstrated that GH enhances progesterone production by augmenting the effects and production of IGF-1[26]. Consistent with these findings, we found significantly higher progesterone levels in women with adjuvant GH administration.

Endometrial cells are known to express GH receptors, and most studies have indicated an increase in endometrial receptivity after GH administration[27]. We found comparable endometrial thicknesses between the study and control groups. Although endometrial thickness is somehow associated with pregnancy rates in general, it is a poor predictor of clinical pregnancies alone, implying the participation of other factors in endometrial receptivity at the molecular level[28]. Cui et al.[29] demonstrated that GH increases endometrial receptivity by increasing the expression of integrin-beta 3, a biomarker of endometrial receptivity, via IGF-dependent and IGG-independent pathways. To support these findings, we found significantly higher implantation rates in women who received GH supplementation despite the comparable number of transferred embryos and grades of transferred embryos between the study and control groups. Another possible explanation for the increased implantation rates in our study were the subtle improvements in embryo quality that morphological evaluations fail to demonstrate.

In a previous study, GH administration was shown to improve oocyte quality[30]. In the present study, the number of retrieved oocytes and M2 oocytes were not significantly different between the study and control groups. However, the number of 2PN embryos and fertilization rates were significantly higher in women with GH supplementation even if the grades of the obtained embryos were comparable between the groups. These findings might be a result of the increased oocyte quality in women who received GH injections.

Ovarian granulosa cells express GH receptors. GH is shown to potentiate the effects of FSH on granulosa cells and induce the proliferation of theca and granulosa cells[7]. In parallel with this information, we found significantly reduced gonadotropin requirements and stimulation lengths as well as significantly higher estradiol levels in the GH group in comparison with the control group.In the present study, we referred our study population as women with normal prognosis or women with expected normal ovarian response owing to our preference to the classification system suggested by the POSEIDON study group that utilized a more convenient approach to determine management strategies considering the prognosis of patients[19]. This choice of using the term "normal prognosis" or "expected normal ovarian response" was made to provide more functional data contributing to the clinical guidance in this group of patients.

Study Limitations

Despite the lack of comprehensive studies, some authors implicated that adult GH deficiency is fairly prevalent among women with infertility, based on their preliminary observations[31]. Therefore, they suggested measurements of IGF-1 and IGH binding protein-3 (IGFBP-3) levels before the initiation of GH supplementation as women with GH deficiency might be the exact subgroup of patients who benefit from GH supplementation. As a study limitation, data of IGF-1 and IGFBP-3 levels were not available in patient records. Thus, future studies of adjuvant GH administration including IGF-1 and IGFBP-3 levels or other diagnostic tests to detect adult GH deficiency could help clarify the effects of GH supplementation and the subgroup of patients who could benefit most.

Conclusion

Our study demonstrate that daily injections of 4 IU of GH significantly increased the live birth delivery rates, clinical pregnancy rates, implantation rates, number of 2PN embryos obtained, and fertilization rates in women with expected normal ovarian response who underwent GnRH antagonist cycles with dual triggering, which was probably due to the increased endometrial receptivity and increased oocyte quality. Further studies designed with prognosis-based approaches could provide more data to make robust recommendations.

Ethics

Ethics Committee Approval: Ethical approval was obtained from the Ethical Committee of Üsküdar University Faculty of Medicine (approval no: 61351342/April 2021-81).

Informed Consent: The need for informed consent was waived by the ethical committee due to the retrospective design.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

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

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

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MicroRNA let-7: A promising non-invasive biomarker for diagnosing and treating external genital endometriosis

Dış genital endometriozis teşhisi ve tedavisi için umut verici non-invaziv biyobelirteç olarak MikroRNA let-7

Darya A. Pokrovenko[1], Volodymyr Vozniuk[2], Mykhailo V. Medvediev[3]

[1]*Klinica Doctora Medvedeva, Dnipro, Ukraine*

[2]*Lviv Regional Public Health Center, Lviv, Ukraine*

[3]*Dnipro State Medical University, Department of Obstetrics and Gynecology, Dnipro, Ukraine*

Abstract

Objective: To evaluate the possibility of using microrna let-7 and mir-9 as non-invasive biomarkers for the diagnosis and treatment of external genital endometriosis.

Materials and Methods: We explored the samples of relatively healthy individuals and patients with endometriosis. All patients had undergone laparoscopic surgical treatment after clinical and laboratory examinations. We used RNA-GO to obtain total RNA from endometriosis samples excised by laparoscopic method. Next step involved reverse transcription for microRNA let-7 and mir-9. Correlation-regression analysis was performed using Mann-Whitney-Wilcoxon method. Subsequently, receiver operating characteristic analysis was conducted to determine the possibility of using let-7 microRNA for non-invasive detection of endometriosis. The results of the analysis in all groups were tested considering the normality of statistical distribution.

Results: Mann-Whitney analysis showed that the difference in mir-9 mRNA between the groups with and without endometriosis, as well as between the groups with more clinically and histologically severe and mild endometriosis, was statistically insignificant. In addition, a significant difference was noted regarding let-7 microRNA between the groups with and without endometriosis, as well as between the groups with more clinically and histologically severe and mild endometriosis. Comparison with cancer antigen-125 (CA-125) showed that let-7 microRNA was a more specific test than CA-125.

Conclusion: MicroRNA let-7 had the best parameters (sensitivity, specificity, and predictive value of positive and negative results) among the biomarkers studied. These biomarkers may be used for early and sometimes preclinical diagnosis of endometriosis.

Keywords: Genital endometriosis, microrna let-7, microrna mir-9, cancer antigen-125

Öz

Amaç: Dış genital endometriozis tanı ve tedavisi için mikro-RNA let-7 ve mir-9'un invaziv olmayan biyobelirteçler olarak kullanım olasılığını değerlendirmek.

Gereç ve Yöntemler: Nispeten sağlıklı bireylerin ve endometriozisli hastaların örneklerini araştırdık. Tüm hastalara klinik ve laboratuvar muayeneleri sonrasında laparoskopik cerrahi tedavi uygulandı. Laparoskopik yöntemle eksize edilen endometriozis hücre örneklerinden toplam RNA elde etmek için RNA-GO kullandık. Sonraki adım, mikroRNA let-7 ve mir-9 için ters transkripsiyon gerçekleştirmekti. Korelasyon-regresyon analizini yaptık ve Mann-Whitney-Wilcoxon yöntemini kullandık. Daha sonra, endometriozisin non-invaziv tespitinde let-7 mikroRNA kullanma olasılığını belirlemek için alıcı işlem karakteristikleri analizini gerçekleştirdik. Tüm gruplardaki analiz sonuçları, istatistiksel dağılımın normalliğinin değerlendirilmesi açısından test edildi.

Bulgular: Mann-Whitney analizi, endometriozisi olan ve olmayan hasta grupları arasında ve ayrıca klinik ve histolojik olarak daha şiddetli ve hafif endometriozisi olan gruplar arasında mir-9 mRNA açısından istatistiksel olarak anlamlı fark bulamadık. Aynı zamanda, endometriozisi olan ve olmayan hasta grupları arasında ve ayrıca klinik ve histolojik olarak daha şiddetli ve hafif endometriozisi olan gruplar arasında let-7 mikroRNA açısından anlamlı fark bulduk. Ca-125 ile karşılaştırma, let-7 mikroRNA'nın CA-125'e göre daha spesifik bir test olduğunu göstermiştir.

Sonuç: MikroRNA let-7, incelenen biyobelirteçler arasında en iyi parametrelere (duyarlılık, özgüllük, pozitif ve negatif prediktif değerler) sahiptir. Bu biyobelirteçler, endometriozisin erken ve bazen klinik öncesi teşhisi için kullanılabilir.

Anahtar Kelimeler: Genital endometriozis, mikro-RNA let-7, mikro-RNA mir-9, kanser antijeni-125

PRECIS: MicroRNA let-7 has the best parameters (sensitivity, specificity, and predictive value of positive and negative results) among the biomarkers studied, which may be used for early and sometimes preclinical diagnosis of endometriosis.

Address for Correspondence/Yazışma Adresi: Prof. Mykhailo V. Medvediev, Dnipro State Medical University, Department of Obstetrics and Gynecology, Dnipro, Ukraine
Phone: +380677378117 **E-mail:**medvedev.mv@gmail.com **ORCID ID:** orcid.org/0000-0002-0443-0572
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Introduction

Endometriosis has many symptoms that negatively affect the reproductive capability and quality of life of a woman[1]. This condition is characterized by the proliferation of endometrial tissue outside of the uterine cavity, which causes pelvic pain and infertility[2]. The prevalence of endometriosis varies between 5% and 10% in all women, 20%-25% in patients with gynecologic conditions, and 45%-50% among women with infertility. The true incidence of endometriosis is not exactly known[3]. Endometriosis is a common condition and occurs irrespective of ethnicity, race, socioeconomic conditions, and age[4]. The diagnosis of external genital endometriosis can help comprehend patient complaints as well as conduct a survey, ultrasound examination, and sometimes magnetic resonance imaging. Several recent advances have been made to understand the mechanisms underlying endometriosis; however, invasive methods, such laparoscopy, are still the "gold standard" of diagnosis[5]. The search for possible biomarkers for the diagnosis of endometriosis continues. Among non-invasive methods, microrna is a candidate for further research[6].

The microrna profile of blood and eutopic endometrium may provide important information in confirming diagnosis of endometriosis[7].

Owing to their good sensitivity and specificity, molecular genetic methods might improve the results of infertility treatment associated with endometriosis through earlier treatment, including surgery[8].

In addition, various research groups have high expectations regarding the role of microRNA in evaluating the effectiveness of drug therapy. In addition, microRNAs treatments have provided promising results for certain chronic diseases and cancers[9].

In our study, we selected microRNA let-7 and mir-9 as possible candidates. This is because of available data regarding the use of let-7 for treating diseases and use of mir-9 as microRNA, which induces apoptosis, thus playing a special role in the pathogenesis of endometriosis[10].

The goal of this study was to determine the possible role of microRNA let-7 and mir-9 in the non-invasive diagnosis of endometriosis.

Materials and Methods

We explored the samples from 86 patients: 1) control group with relatively healthy individuals and 2) endometriosis group, which was further divided into subgroups according to Altman Self-Rating Mania scale (ASRM) I-II stages and III-IV stages. All patients had undergone laparoscopic surgical treatment at a medical center with confirmation of diagnosis via histology after complete clinical and laboratory examinations. Indication for laparoscopy was infertility and/or dyspareunia and dysmenorrhea. The prevalence of endometriosis was assessed by the same surgical team according to ASRM requirements (surgeon: MM). Laparoscopy was performed using standard 5-mm instruments including bipolar dissector and disposable scissors according to ESGE recommendations.

The control group (Figure 1) included 24 patients who had no signs of endometriosis before examining the pelvic organs and peritoneum by laparoscopic intervention. Group I included 29 patients with ASRM I-II stage "minimal" and "mild" endometriosis with lesions on peritoneum and ovary, but with total points of 4-15. In contrast, group II included 35 women with ASRM III-IV stage "moderate" and "severe" endometriosis, with total points from 16 to >44[11].

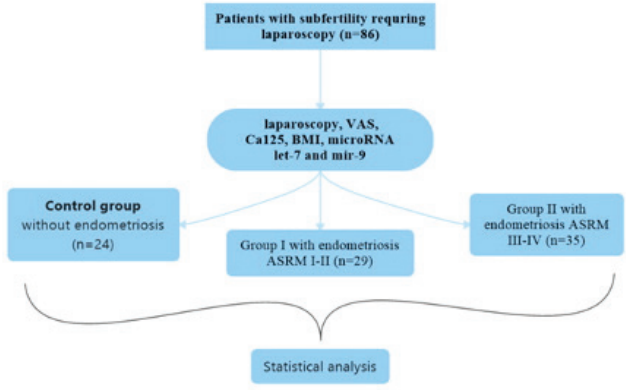

Figure 1. Study design

Except standard gynecological examination, vital sign assessment, body mass index (BMI), and serum cancer antigen-125 (CA-125) level determination were conducted for all patients at the Sinevo laboratory by enzyme-linked immunosorbent assay. Testing was performed using the Elecsys CA 125 II test system and Cobas 6000/Cobas 8000 analyzers from the Swiss company Roche Diagnostics. Method: Electrochemiluminescence immunoassay analyzer, in which the "antigen-antibody" reaction is initiated via an electric current, allows to control it with maximum selectivity and sensitivity. Principle of the method: First, patient sample is mixed with antibodies bound to biotin and antibodies bound to ruthenium, to form an "antigen-antibody" complex. After incubation of the mixture, paramagnetic microparticles with streptavidin (solid phase) are added. After the second incubation, the reaction mixture is transferred to the measuring cell of the apparatus, where under the action of the magnetic field, paramagnetic particles that are associated with the "antigen-antibody" complex are attracted to the electrode surface and non-antigen-bound antibodies are removed. Subsequently, an electric current is used to excite ruthenium and generate a signal that allows the detection of the "antigen-antibody" complex. The luminosity, measured photometrically, is equivalent to the concentration of the substance determined in the sample.

We evaluated the severity of pain syndrome in patients by visual analog scale (VAS) in millimeters (0: no pain; 100

mm: worst possible pain). Patients' complaints were divided into three groups: infertility, pain syndrome, and absence of complaints. For all patients, ultrasound pelvic examination was performed using transvaginal transducer (7-12 MHz) and convex transducer (3.5-8 MHz)[12].

MicroRNA Determination

We used RNA-GO to obtain high-quality total RNA from cell samples (NanoDrop-1000). Next step involved reverse transcription for microRNA let-7 and mir-9 and control U6 (Tap Man microRNA Reverse Transcription Kit). The results obtained were analyzed using the software 7500 Fast real-time polymerase chain reaction (PCR) (Applied Biosystems, USA). All data are presented as arithmetic data (mean values ± standard deviation).

Statistical Analysis

In total, 86 patients were included. Patient selection was based on the central limit theorem (CLT). A sample of 30 is the smallest sample size for which the CLT is still valid. The total sample size for the Manny-Whitney test at P (X > Y) =0.2065, like in our case, should be at least 40 observations. The statistical power was 0.806. For determining effect size, we used effect size Glass' Δ because standard deviations were significantly different between groups (12.22 vs 131.89). The effect size in our case was 0.822. We determined the normality of distribution of the studied values using the Shapiro-Wilk method and obtained the following results: statistics=0.455, p=0.000 (for alpha=0.05). Thus, it was possible to draw the conclusion about the "non normality" of data distribution in the sample by the main studied indicator (let-7 microRNA). The main results of demographic characteristics presented in Table 1, i.e., clinical laboratory and molecular genetic examinations of a prospective group of women with endometriosis, are also presented in Table 2.

To determine the correlation (r), we used the Spearman method (used for non-normal distributed data). In addition, we used Mann-Whitney-Wilcoxon method with Bonferroni correction (used for non-normal distributed data) to determine the significance of the difference between the mean values of three groups: a control group and two endometriosis groups.

In our investigation, ANOVA was not conducted owing to the small number of groups and the ability to compare them in turn. Next, we performed ROC analysis to determine the possibility of using let-7 microRNA for non-invasive detection of endometriosis. Area under the curves for let-7 microRNA and CA-125 for endometriosis were also compared (Table 3, Figure 1).

Results of all groups were tested with respect to the normality of the statistical distribution. Statistical analysis was performed using SPSS Statistics 17.0 (IBM Corporation, USA)[13].

This study was approved by the ethics committee (protocol number 3) and was conducted in accordance with the Helsinki declaration for clinical studies in humans. Informed consent for participation in the study was signed by all participants.

Table 1. Major demographic characteristics of patients

	ASRM score	Age	BMI	Level of pain (VAS score)
Mean	16.95	33.33	20.72	55.91
Std	**16.10**	**6.07**	**2.30**	**21.21**
Minimum	0	20	17	1
25%	0.5	28	18.5	40
50%	13	33	19.85	60
75%	34	38	22	70
Maximum	45	45	30.1	95

Std: Standard, BMI: Body mass index, VAS: Visual analog scale, ASRM: Altman Self-Rating Mania scale

Table 2. Main results of clinical laboratory and molecular genetic examinations of a prospective group of women with endometriosis

	CA-125	Microrna let-7	Microrna mir-9
Count	86	86	61
Mean	56.42	33.72	5.00
Std	**30.93**	**81.69**	**25.82**
Minimum	3	0.03	0
25%	26.25	0.88	0.04
50%	62.5	4.43	0.11
75%	82	17.77	0.56
Maximum	108	517.24	186.86

STD: Standard

Results

The investigated groups had comparable average indicators of patient age. In contrast, BMI had lower values in the endometriosis groups (22.35 in control group; 20.16 and 20.15 in endometriosis groups I and II, respectively). Forty patients in the endometriosis group had an endometrioid cyst (62.5%), with higher blood CA-125 levels, whereas the levels of let-7 did not depend significantly on the presence or absence of an endometriotic cyst. let-7 microRNA deviated considerably from the average value, and the scatter of values was considerably large (standard deviation: 81.69; average: 33.72). We evaluated the normality of data distribution in the sample using the Shapiro-Wilk test. This test was considered reliable for small samples containing up to 1000 values (we had 86). Shapiro-Wilk test result was as follows: statistics=0.455, p=0.000 (for alpha=0.05). Thus, it was possible to conclude regarding the incorrect distribution of data in the sample by the main studied indicator (let-7 microRNA).

There was a significant moderate negative correlation (-0.64) between let-7 microRNA and ASRM scores. In addition, the correlation between endometriosis (+/-) and let-7 microRNA was close to strong (-0.67), with a high degree of reliability and a negative character (Table 3).

The other indicator, mir-9, was found to be normally distributed (according to the Shapiro-Wilk test). mir-9 Was weakly correlated with ASRM scale. Mann-Whitney analysis showed that the difference in mir-9 mRNA between groups with and without endometriosis, as well as between groups with more clinically and histologically severe and mild endometriosis, was statistically insignificant. Thus, mir-9 mRNA was not an optimal candidate for diagnosing subclinical endometriosis (Figure 2).

Both the indicators, microRNA let-7 and ASRM points, had reliable negative correlation (-0.64). In addition, the indicators of endometriosis (+/-) and microRNA let-7 correlated with the nearest strong value (-0.67) owing to a high degree of reliability as well as a negative character (Table 3).

Mann-Whitney analysis revealed that microRNA let-7 was a reliable indicator between the group with and without endometriosis (Figure 3).

In addition, Mann-Whitney analysis demonstrated significant difference in let-7 microRNA between the groups of patients with and without endometriosis, as well as between groups with more clinically and histologically severe and mild endometriosis, making let-7 microRNA a clinically appealing biomarker than mir-9 mRNA.

According to the results of Prescott et al.[14] the sensitivity and specificity of serum CA-125 for the diagnosis of endometriosis

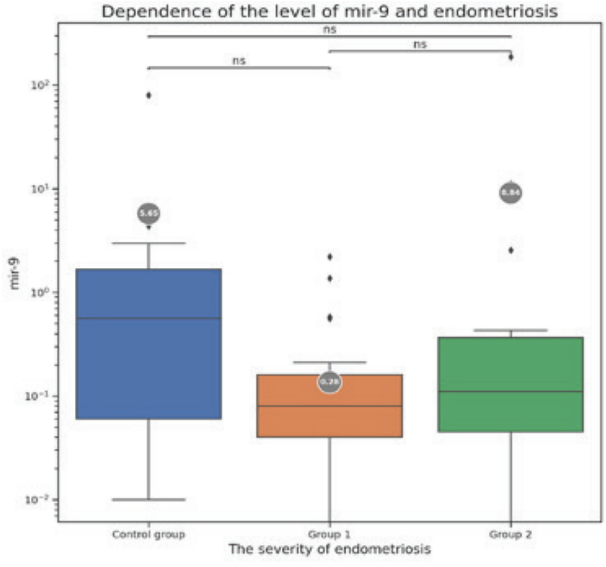

Figure 2. Level of mir-9 mRNA (± standard deviation and statistically significant using Mann-Whitney-Wilcoxon test, two-sided with Bonferroni correction) in groups of patients with mild endometriosis (group 1), more clinically and histologically severe endometriosis (group 2), and without endometriosis (control group)

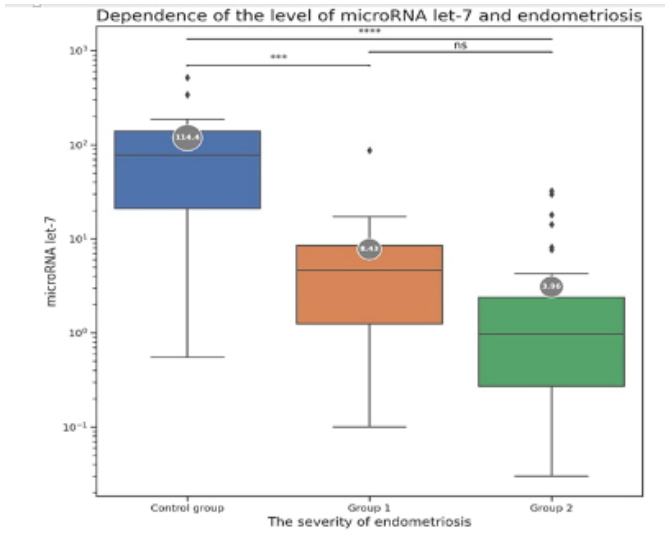

Figure 3. Level of microRNA let-7 (± standard deviation and statistically significant using Mann-Whitney-Wilcoxon test, two-sided with Bonferroni correction) in groups of patients with mild endometriosis (group 1), more clinically and histologically severe endometriosis (group 2), and without endometriosis (control group)

were 61.1% and 87.5%. Elevated CA-125 (>35 U/mL) was noted in 65/75 cases (86.70%) with advanced endometriosis, but in only 15/56 patients (26.8%) with minimal and mild endometriosis, respectively. Santulli et al.[15] demonstrated that serum CA-125 levels were significantly increased in women with severe forms of endometriosis, OMA, and DIE lesions. In addition, elevated serum CA-125 levels were associated with more severe and extended DIE lesions. CA-125 levels were not different from women without endometriosis' in women with superficial peritoneal lesions.

Based on promising data obtained from correlation analysis and group comparisons using Mann-Whitney test, we performed ROC analysis. The following indicators were determined in ROC analysis of microRNA let-7 and CA-125. For microRNA let-7, sensitivity was 92.563, specificity was 82.545, PPV was 93.912, and NPV was 79.234. Indicators of the classification model for CA-125 were as follows: sensitivity=86.105, specificity=76.422, PPV=91.397, and NPV=65.406. The ROC analysis revealed that the flat under curve for microRNA let-7 was higher than that for CA-125 (Figure 4).

Thus, microRNA let-7 has the best parameters (sensitivity, specificity, and predictive value of positive and negative results). Comparison with CA-125 using ROC analysis showed the presence of a larger AUC. This indicates great prospects for this marker for early and sometimes preclinical diagnosis of endometriosis.

Discussion

CA-125 is a glycoprotein biomarker used in women with endometriosis, and according to some authors, this could

Table 3. Spearman's rank correlation coefficient (r) for basic research indicators. Very weak: 0.00-1.99, weak: 0.20-399, medium: 0.40-0.599, strong: 0.60-0.799, very strong: 0.80-1.000. Significant level of correlation (***:p<0.01, **:p<0.05, *:p<0.1)

	Endometriosis (+/-)	ASRM score	Age	IMT	Level of pain (VAS scores)	CA-125	microRNA let-7	mir-9
Endometriosis (+/-)	1.0***	0.76***	-0.16	-0.42***	0.4***	0.59***	-0.67***	-0.32**
ASRM score	0.76***	1.0***	-0.05	-0.29***	0.5***	0.64***	-0.64***	-0.2
Age	-0.16	-0.05	1.0***	0.36***	0.03	-0.03	0.11	0.3**
IMT	-0.42***	-0.29***	0.36***	1.0***	-0.2*	-0.24**	0.31***	0.3**
Level of pain (VAS scores)	0.4***	0.5***	0.03	-0.2*	1.0***	0.41***	-0.28***	-0.24*
CA-125	0.59***	0.64***	-0.03	-0.24**	0.41***	1.0***	-0.37***	0.1
microRNA let-7	-0.67***	-0.64***	0.11	0.31***	-0.28***	-0.37***	1.0***	0.4***
mir-9	-0.32**	-0.2	0.3**	0.3**	-0.24*	0.1	0.4***	1.0***

ASRM: Altman Self-Rating Mania scale, VAS: Visual analog scale

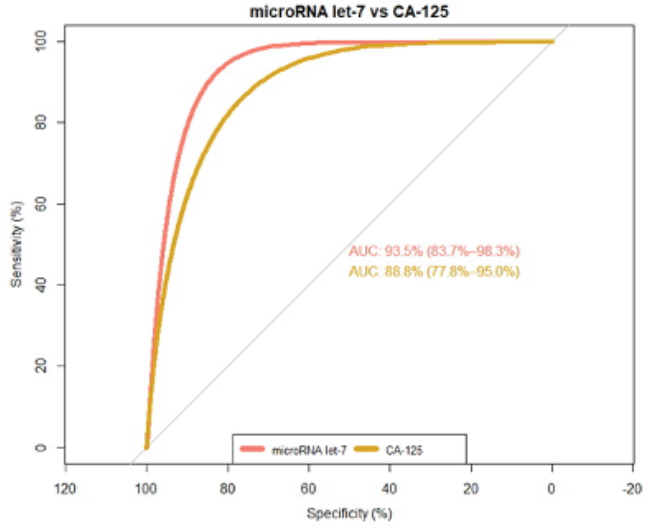

Figure 4. Sensitivity and specificity analyses for microRNA let-7 (AUC: 93.5%) and for CA-125 (AUC: 88.8%)

AUC: Area under the curve

be useful in practice among patients suspected to have endometriosis[16]. It has proven useful as an ovarian cancer biomarker for monitoring ovarian cancer therapy and early recurrences[17]. CA-125 levels have been found to be significantly higher in women with moderate or severe endometriosis, specifically for ovarian endometriomas and deeply infiltrating endometriosis[18]. A previous study analyzed serum CA-125 levels in 87 women aged 21-54 years suspected with endometriosis who had pelvic pain, dysmenorrhea, or dyspareunia. The mean age of the women was 32.22±6.91 years. The mean serum CA-125 level was 49.93±4.30 U/mL. There was a significant correlation between the endometriosis stage, lesion size, adhesion score, and preoperative CA-125 plasma concentration. The suggested preoperative serum cut-off levels in premenopausal and postmenopausal patients were 37 and 35 U/mL, respectively[19]. The search for non-invasive biomarkers of endometriosis has been ongoing since a long time. In a related study, researchers investigated the serum levels of CA-125, TNF, IL-1, IL-6, and IL-8. The data obtained were correlated with clinical symptoms and revised American Fertility Society score and stage as well as interpreted using Mann-Whitney U-test and ANOVA regression analysis. CA-125 levels were over the cut-off of 35 IU/L in 54% of patients (versus 8% of controls), averaging 67.5 (95% confidence interval: ±17.5). The sensitivity and specificity were 54% and 91%, respectively, with a p-value of <0.001 (statistically significant). For IL-6, 71% of cases and 87% of controls were above the cut-off of 2 pg/mL, with an average of 11.83±7. The sensitivity and specificity were 71% and 12%, respectively, but the difference was not statistically significant (p=0.071). Other tested serum markers had no discrimination value. ANOVA revealed a correlation between the severity of endometriosis and CA-125 (p=0.03) but not for IL-6[20]. These results have provided a reason for comparison of CA-125 as a non-invasive biomarker with certain specificity for endometriosis[21].

The important disadvantage is that the level of CA-125 could increase in inflammatory processes and oncological diseases, so its specificity is rather low[22]. In contrast, the determination of microRNA let-7 level is more promising for all phenotypes and at stages of endometriosis[23]. Chronic inflammation caused by the presence of fragments of ectopic endometrium and activation of myofibroblasts with the development of fibrous tissue leads to potential modifications of epigenetic programming, i.e., aberrant regulation of gene transcription and posttranslational regulatory mechanisms through non-coding RNA[24]. Previously, researchers have described ways to regulate H19/Let-7/IGF1R as well as the mechanisms that

contributes to endometrial dysfunction and is leading in the development of endometriosis and associated infertility. However, the method of determining H19 is very complex. H19 is quite unstable, and the determination of let-7 can be performed using real-time PCR and appropriate reagents for the isolation of miRNAs from biological substances[25]. Changes in epigenetics contribute to the development of resistance to progesterone and increased response to estrogen, which are the two key characteristics of women with endometriosis. Severity of fibrosis, i.e., the stage and spread of the disease, can potentially indicate the effectiveness of drug treatment[26]. The use of let-7 as a biomarker will help predict the response to drug therapy and choose the best treatment[27].

Conclusion

We believe that the measurement of microRNA let-7 is promising for routine use in patients with endometriosis. Considering data on microRNA let-7 as well as clinical data, we are planning an algorithm for diagnosis and management that will help identify the extent of endometriosis without laparoscopy at an earlier stage and help select an effective therapy for lower period of time. This approach could possibly decrease time to diagnosis, decrease surgery rate, and optimize overall endometriosis treatment results, including VAS score and fertility results.

Ethics

Ethics Committee Approval: This study was approved by the ethics committee (protocol number: 3) and was conducted in accordance with the Helsinki Declaration for clinical studies in humans.

Informed Consent: Informed consent for participation in the study was signed by all participants.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: D.A.P., M.V.M., Design: D.A.P., M.V.M., Data Collection or Processing: D.A.P., M.V.M., Analysis or Interpretation: V.V., Literature Search: D.A.P., M.V.M., Writing: D.A.P., M.V.M., V.V.

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

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The relationship between oxidative stress markers and endometrial hyperplasia: A case-control study

Oksidatif stres belirteçleri ile endometriyal hiperplazi arasındaki ilişki: Bir olgu kontrol çalışması

Engin Yıldırım[1], Can Türkler[2], Ümit Görkem[3], Ömer Yavuz Şimşek[4], Ercan Yılmaz[5], Hülya Aladağ[1]

[1]*Malatya Turgut Özal University Faculty of Medicine, Department of Obstetrics and Gynecology, Malatya, Turkey*

[2]*Erzincan Binali Yıldırım University Faculty of Medicine, Department of Obstetrics and Gynecology, Erzincan, Turkey*

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

[4]*Biruni University Faculty of Medicine, Department of Obstetrics and Gynecology, İstanbul, Turkey*

[5]*İnönü University Faculty of Medicine, Department of Obstetrics and Gynecology, Malatya, Turkey*

Abstract

Objective: Endometrial hyperplasia (EH) is considered an endometrial cancer precursor. This study aimed to determine the role of oxidative stress and thiol groups with antioxidant properties in EH pathogenesis.

Materials and Methods: In our prospective case-control study, participants were washed with 5 mL of saline before the endometrial biopsy. Endometrial washing fluid was taken into microtubules, and thiol and disulfide levels were analyzed using the Ellman reagent.

Results: A total of 108 patients were in the EH group and 84 patients in the control group. The total and native thiol levels were higher values in the control group (p<0.001, for both). Disulfide levels were higher in the EH group (p<0.001). Native/total thiol ratio and disulfide/total thiol ratio were higher in the EH group (p<0.001, for both). The analysis performed in the control group revealed a significant positive correlation between estradiol and disulfide levels (r=0.322, p=0.033). No significant correlation was found between estradiol and disulfide in the EH group.

Conclusion: Oxidative stress level was higher in the washing fluids of patients with EH and this stress plays a role in the EH etiology.

Keywords: Endometrial hyperplasia, oxidative stress, thiol, disulfide

Öz

Amaç: Endometriyal hiperplazi (EH) endometriyum kanseri prekürsörü olarak kabul edilir. EH patogenezinde oksidatif stresin rolünü ve antioksidan özelliği olan tiyol gruplarının rolünü saptamayı hedefledik.

Gereç ve Yöntemler: Prospektif olgu kontrol çalışmamızda katılımcılara endometriyal biyopsi yapılmadan önce 5 mL salin ile endometriyal yıkama yapıldı. Endometriyal yıkama sıvısı mikrotübüllere alındı, Ellman reagent kullanılarak tiyol ve disulfid düzeyleri analiz edildi.

Bulgular: EH grubunda 108, kontrol grubunda 84 hasta vardı. Toplam tiyol ve doğal tiyol seviyeleri kontrol grubunda daha yüksekti (her ikisi için p<0,001). Disulfid düzeyleri ise EH grubunda daha yüksekti (p<0,001). EH grubunda native/total tiyol oranı ve disulfid/total tiyol oranı daha yüksekti (her ikisi için p<0,001). Kontrol grubunda yapılan analizde estradiol düzeyleri ile disulfid düzeyleri arasında anlamlı pozitif ilişki saptandı (r=0,322, p=0,033). EH grubunda estriol ile disulfid arasında anlamlı korelasyon bulunamadı.

Sonuç: EH hastaların yıkama sıvılarında oksidatif stres düzeyi daha yüksek bulundu ve EH etiyolojisinde bu stres rol oynayabilir.

Anahtar Kelimeler: Endometriyal hiperplazi, oksidatif stress, tiol, disülfid

PRECIS: Oxidative stress level was found higher in the washing fluids of patients with endometrial hyperplasia and may play a role in the etiology of endometrial hyperplasia.

Address for Correspondence/Yazışma Adresi: Engin Yıldırım MD, Malatya Turgut Özal University Faculty of Medicine, Department of Obstetrics and Gynecology, Malatya, Turkey
Phone: +90 532 527 29 14 **E-mail:** dreyildirim@gmail.com **ORCID ID:** orcid.org/0000-0001-7937-4141

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Introduction

As a component of the PALM-COEIN classification, endometrial hyperplasia (EH) is one of the important pathological diagnoses in females with abnormal uterine bleeding (AUB) symptoms[1,2]. Its clinical importance is derived from its potential as an endometrial adenocarcinoma precursor[3]. EH is mainly caused by continuous and high estrogen exposure that was imbalanced with progesterone[4]. Endometrial estrogen exposure effects are not explained only with cellular proliferation. An example of this issue is the increased nitric oxide synthase activity in patients with endometriosis and adenomyosis with estrogen exposure in the etiology[5,6]. Previous studies showed that estrogens and estrogen metabolites play a prooxidant role and cause reactive oxidative species (ROS) formation[7]. ROS with estrogen stimulus cause cell genetic instability. Redox reactions formed by ROS stimulate intracellular signaling pathways and cause cell proliferation, cell migration, invasion, and malignant transformation[8].

Endometrial ROS balance is maintained with enzymatic and nonenzymatic antioxidative systems. Among these systems, thioredoxins are one of the disulfide-reducing proteins found in human endometrium, and its relationship with implantation was reported[9]. Thiol/disulfide homeostasis moves in the disulfide direction and binds to oxidant radicals in the first stage of oxidative damage. Oxidative products, such as reactive oxygen species formed in the organism, are reduced by transferring their excess electrons to compounds containing thiol while thiol groups are oxidized. Thiol group oxidation forms disulfide bonds. However, the reaction is reversible and the disulfide bonds formed are reduced back to thiol groups. Thus, dynamic thiol-disulfide homeostasis is achieved. Comparing the native thiol levels to the ratio of total thiol will show the changing dynamic -SH redox reactions more clearly[10].

Thiols are secreted as a response to oxygen radicals and protect the tissue against oxidative stress[11]. The amount of total thiol is free, protein-dependent, or reduced by glutathione. Thiol level is used as a marker that shows oxidative defense; Thiol/disulfide homeostasis plays an important role in maintaining many physiological processes and the disulfide balance form shifting is expected to be associated with oxidative stress[12]. As the association of oxidative stress and endometrium cancer was proven, increased cancer precursor lesions are expected[7]. Therefore, this study aimed to investigate the relationship of oxidative stress markers in females with EH and healthy females.

Materials and Methods

Study Design and Population

The present study was designed as a prospective case-control in nature and conducted at the Hitit University Hospital between March 2019 and April 2020. The study was approved by the Erzincan Binali Yıldırım University Clinical Research Ethics Committee and conducted following the Helsinki Declaration (approval number: 33216249-604.01.02-E.24314). Informed consent was obtained from all participants at the beginning of the study.

Females admitted to the hospital with AUB complaints were enrolled in the study. Firstly, detailed demographic data and medical history were recorded. The same clinician evaluated all participants using a pelvic examination and transvaginal sonography (Logiq P5, GE Healthcare, Milwaukee, USA).

The exclusion criteria included the presence of any reproductive tract structural abnormality leading to vaginal bleeding, any coexisting disease or drug usage affecting the reproductive tract, smoking, pregnancy, lactation, body mass index of >30 kg/m^2. Participants were between the ages of 18-55 years with any type of AUB pattern (<24 or >37-day interval, >7-day duration, or intermenstrual bleeding). The main indication for endometrial biopsy was determined as AUB. Endometrial biopsies were performed in cases where sonographically polypoid appearance, heterogeneous-cystic endometrial appearance, anemia, and AUB were long and severe. Thereafter, participants were classified into two main groups based on histopathological reports: (i) EH group composed of participants with histological EH diagnoses without atypia (n=108) and (ii) control group composed of women with normal histological diagnoses (n=84).

Specimen Collection and Analyses

The venous samples obtained from all participants in their early follicular phases of the menstrual cycle on days 2-4 after overnight fasting were collected into 5 mL serum separator tubes (BD Vacutainer, Becton Dickinson, New Jersey, USA). After 30 min for blood samples to clot, the samples were centrifuged at 1000×g for 20 min. The serum analyses for estradiol (E2), follicle-stimulating hormone (FSH), luteinizing hormone (LH), thyroid-stimulating hormone (TSH), and free thyroxine (fT4) were performed by an electro-chemiluminescence immunoassay method using an autoanalyzer (Cobas 6000, E 601 Roche Diagnostics, GmbH, Mannheim, Germany) on daily basis. The analyses of hematological parameters, including hemoglobin (Hb), neutrophil, lymphocyte, and platelet concentrations, were performed using an analyzer (Sysmex XE2100, TOA Medical Electronics, Kobe, Japan).

Participants were placed in a lithotomy position to obtain an endometrial sample. After a plastic flexible catheter (Medlab, Izmir, Turkey) passes through the cervical canal and fundal touch feeling was taken, a volume of 5 mL of saline solution was flushed into the uterine cavity[13,14]. Gentle suction was applied to recover the fluid back, and then approximately equal amounts of aspired solution were poured into microtubules to analyze the thiol and disulfide levels. Ellman reagent (5,5'-dithio-bis-2-nitrobenzoic acid, also known as DTNB) was used for spectrophotometric analysis to detect endometrial thiol and disulfide levels reacted with thiol molecules and formed a yellow complex with a maximum absorbance at 421 nm wavelength (Evolution™ 201 Bio, Thermo Scientific Inc., USA)[15,16]. Oxidative stress markers, including thiol, native

thiol, disulfide concentrations, and native thiol-to-total thiol, disulfide-to-total thiol, and disulfide-to-native thiol ratios, were calculated in each group.

Endometrial biopsies were evaluated in Hitit University Medical Faculty, Central Pathology Laboratory. Especially, the pathologists who specialized in gynecopathology were blinded from the pathological specimens of the study group.

Statistical Analysis

All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS version 23.0, IBM SPSS Inc. Chicago, IL, USA). Continuous variables were firstly assessed by the Shapiro-Wilks normality test for the statistical distribution normality. Eventually, no normally distributed data was found, and Mann-Whitney U test was used to compare the data in two study groups. According to the data distribution pattern, the descriptive statistics were presented as median (minimum-maximum). The nominal variables were presented as the number of cases and percentages. The correlation of oxidative stress markers was performed using Spearman's correlation test. A p-value of <0.05 was considered statistically significant.

Results

A total of 192 females were recruited for the study population. Demographic and biochemical characteristic comparisons in EH and control groups are presented in Table 1. The ages of the EH and control groups were statistically similar (p=0.360). No difference was found regarding the body mass index in both study groups, (p=0.068). As expected, the mean serum E2 level was higher in the EH group (p<0.001). FSH and LH levels did not statistically differ from each other (p=0.690 and p=0.441). The median serum TSH level was elevated in the EH group (p=0.002). In both groups, hematological parameters including Hb, lymphocyte, and platelet concentrations did not statistically differ from each other. In addition, no statistical difference was found between the neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio as inflammatory markers in the two groups (p=0.125 and p=0.435, respectively).

Oxidative stress markers, including thiol, native thiol, disulfide concentrations, and native thiol-to-total thiol, disulfide-to-total thiol, and disulfide-to-native thiol ratios, were compared in both study groups, as presented in Table 2. The serum and

Table 1. Comparison of demographic and biochemical characteristics in endometrial hyperplasia and control groups

	EH Group (n=108, 55.9%) (Median. min-max)	Control Group (n=84, 44.1%) (Median. min-max)	p
Age (years)	45.0 (34.0-54.0)	44.0 (32.0-5.0)	0.360
BMI (kg/m²)	23.3 (20.3-28.7)	22.3 (18.3-29.4)	0.068
Gravida	3.0 (1-6.0)	3.0 (2-6.0)	0.05
Parity	3.0 (0-5.0)	3.0 (0-5.0)	0.833
Live	2.0 (0-5.0)	2.0 (0-4.0)	0.713
Abortus	0 (0-4.0)	0 (0-2.0)	**0.001**
Termination	0 (0-2.0)	0 (0-3.0)	**0.011**
Endometrial thickness (mm)	9.2 (5.6-10.3)	6.2 (4.9-8.3)	**<0.001**
E2 (pg/mL)	121.5 (80.5-169.7)	95.4 (71.8-141.3)	**<0.001**
FSH (IU/L)	9.3 (4.9-17.8)	9.4 (4.4-18.3)	0.690
LH (IU/L)	8.2 (3.7-13.6)	7.6 (3.9-13.6)	0.441
TSH (µIU/mL)	2.2 (0.9-4.1)	1.8 (0.8-3.2)	**0.002**
fT4 (ng/dL)	1.3 (0.7-2.4)	1.4 (0.7-2.4)	0.599
Hb (g/dL)	12.4 (7.7-14.7)	12.0 (8.6-14.6)	0.436
Neutrophil (10^3/µL)	3.3 (2.2-10.3)	4.4 (1.9-9.3)	**0.001**
Lymphocyte (10^3/µL)	2.4 (1.1-4.2)	2.4 (1.8-4.2)	0.198
Platelet (10^3/µL)	261.0 (119.0-429.0)	260.0 (152.0-373.0)	0.324
NLR	1.5 (0.8-6.8)	1.6 (0.6-3.9)	0.125
PLR	105.4 (401-231.4)	107.2 (66.9-1784)	0.435

EH: Endometrial hyperplasia, BMI: Body mass index, E2: Estradiol, FSH: Follicle-stimulating hormone, LH: Luteinizing hormone, TSH: Thyroid-stimulating hormone, fT4: Free thyroxine, NLR: Neutrophil-to-lymphocyte ratio, PLR: Platelet-to-lymphocyte ratio, Min: Minimum, Max: Maximum

native thiol levels were significantly decreased in the EH group (p<0.001, for both). However, serum disulfide level was higher in the EH group (p<0.001). Native thiol-to-total thiol, disulfide-to-total thiol and disulfide-to-native thiol ratios were elevated in EH group (p<0.001, p<0.001, and p<0.001, respectively).

The correlation analyses between oxidative stress markers and other study parameters were separately performed in both groups. Thiol levels were inversely correlated with TSH levels in the EH group (r=-0,288, p=0.023). Disulfide levels were positively correlated with TSH and inversely correlated with FSH and LH levels (r=0.375, p<0.001; and r=-0.206, p=0.032; r=-0.194, p=0.044, respectively). Disulfide/native thiol and disulfide/total thiol ratios were also positively correlated with TSH levels (r=0.398, p=<0.001; r=0,444 p=<0.001, respectively) (Table 3).

The correlation analysis of study parameters in the control group revealed that thiol levels were positively correlated with E2 levels and inversely correlated with TSH levels (r=0.322, p=0.033; r=-0.548, p<0.001, respectively). Native thiol levels were also inversely correlated with TSH levels (r=-0.533, p<0.001). Disulfide levels were positively correlated with E2 levels, but inversely correlated with TSH levels (r=0.399, p=<0.001; r=-0.259, p=0.011 respectively), as demonstrated in Table 4.

Discussion

Revealing the relationship was focused on between serum levels of oxidative stress markers and EH status in females with AUB complaints. In brief, native and total thiol levels of endometrial washing fluids were higher in the control group. Disulfide levels, native/total thiol levels, disulfide/total thiol levels, and disulfide/native thiol ratios were higher in the EH group.

This study also examined the correlation between the biochemical data and oxidative stress markers, which found a positive relationship between rising TSH levels and oxidative stress. Previous studies, parallel to our study results, revealed low disulfide and native thiol levels in patients with subclinical

Table 2. Comparison of oxidative stress markers in endometrial hyperplasia and control groups

	EH group (n=108, 55.9%) (Median. min-max)	Control group (n=84, 44.1%) (Median. min-max)	p
Thiol (mmol/L)	465 (336-1065)	696 (449-1218)	**<0.001**
Native thiol (mmol/L)	402 (302-991)	569 (212-1056)	**<0.001**
Disulfide (mmol/L)	87.6 (13.4-133.0)	31.5 (13.0-84.5)	**<0.001**
Native thiol/total thiol	92.9 (67.3-98.4)	84.2 (47.2-98.5)	**<0.001**
Disulfide/total thiol	16.9 (2.0-30.3)	4.2 (2.0-9.9)	**<0.001**
Disulfide/native thiol	20.3 (2.2-32.6)	5.04 (2.1-17.9)	**<0.001**

EH: Endometrial hyperplasia, Min: Minimum, Max: Maximum

Table 3. Correlation analysis of oxidative stress markers in endometrial hyperplasia group (n=108)

		E2	TSH	FSH	LH	NLR	PLR
Thiol (mmol/L)	**p**	**0.003**	**0.023**	0.579	0.295	0.749	0.947
	r	0.284	-0.288	0.054	0.102	0.031	0.006
Native thiol (mmol/L)	**p**	**0.001**	0.505	0.232	0.392	0.008	0.019
	r	0.306	0.065	-0.116	-0.096	0.253	0.226
Disulfide (mmol/L)	**p**	0.671	**<0.001**	**0.032**	**0.044**	0.673	0.212
	r	0.041	0.375	-0.206	-0.194	0.041	-0.120
Native thiol/total thiol	**p**	0.192	<0.001	0.169	**0.026**	**<0.001**	**<0.001**
	r	-0.127	0.433	-0.133	-0.214	0.459	0.441
Disulfide/total thiol	**p**	0.082	**<0.001**	0.475	0.076	0.771	0.681
	r	-0.169	0.444	-0.070	-0.171	0.028	-0.041
Disulfide/native thiol	**p**	0.117	**<0.001**	0.584	0.172	0.398	0.087
	r	-0.152	0.398	-0.053	-0.132	-0.082	-0.165

E2: Estradiol, FSH: Follicle-stimulating hormone, LH: Luteinizing hormone, TSH: Thyroid-stimulating hormone, NLR: Neutrophil-to-lymphocyte ratio, PLR: Platelet-to-lymphocyte ratio

Table 4. Correlation analysis of oxidative stress markers in the control group (n=84)

		E2	TSH	FSH	LH	NLR	PLR
Thiol (mmol/L)	**p**	**0.033**	**<0.001**	**0.012**	**0.008**	0.592	0.332
	r	0.322	-0,548	0.273	0.288	-0.059	0.107
Native thiol (mmol/L)	**p**	0.099	**<0.001**	0.371	0.207	0.194	0.827
	r	0.181	-0.533	0.099	0.139	-0.143	-0.024
Disulfide (mmol/L)	**p**	**<0.001**	**0.011**	**<0.001**	**<0.001**	0.101	0.697
	r	0.399	-0.259	0.410	0.347	0.186	-0.043
Native thiol/total thiol	**p**	**0.002**	0.913	**0.002**	**0.001**	0.385	0.473
	r	-0.340	0.012	-0.326	-0.351	0.099	-0.079
Disulfide/total thiol	**p**	0.063	0.488	**0.027**	0.135	**0.005**	0.876
	r	0.204	0.077	0.242	0.166	0.305	-0.017
Disulfide/native thiol	**p**	**0.021**	0.279	**0.014**	0.062	**0.042**	0.772
	r	0.257	0.119	0.268	0.206	0.223	-0.032

E2: Estradiol, FSH: Follicle-stimulating hormone, LH: Luteinizing hormone, TSH: Thyroid-stimulating hormone, NLR: Neutrophil-to-lymphocyte ratio, PLR: Platelet-to-lymphocyte ratio

hypothyroidism having high TSH values[17]. An inverse correlation was also found between the serum FSH, LH, and native/total thiol levels, and our results were consistent with similar negative correlations in previous studies[18].

Serum E2 level was demonstrated to have a positive correlation with disulfide, disulfide/total thiol, and disulfide/native thiol levels. In addition, E2 was negatively correlated with native to total thiol ratios. Previous studies had different implications for the relationship of E2 to oxidative markers. A study evaluating the 17b-E2 levels and oxidative balance revealed no significant correlation[19]. A study investigating pubertal gynecomastia revealed a negative correlation between E2 and thiol-disulfide[20]. The present study revealed lower total and native thiol levels in the endometrial washing fluid of females with EH. Disulfide, disulfide/total thiol, and disulfide/native thiol ratios were found higher in the EH group. These findings indicated that females with EH were exposed to high levels of oxidative stress. A previous study stated that EH and endometrial cancer is developed due to obesity-related oxidative stress[21]. Similar findings were found in other studies in females with uterine fibroids reporting high oxidative stress levels and decreased antioxidant capacity[22].

Impaired oxidative balance was reported in other gynecological pathologies. Total thiol levels were reported to decrease total thiol levels in endometriosis pathogenesis, which was concluded to reflect the decreased antioxidant capacity[23]. Antioxidant treatments are beneficial in endometriosis and endometrioid tumor treatments. Experimental animal studies reported that thiol-containing ligand and dinitrosyl iron complexes cease endometriosis-associated endometrioid tumors[24]. Other studies revealed the relationship between oxidative stress markers and endometrial polyps. One of these studies revealed that catalase, xanthine oxidase, and malondialdehyde levels were higher in patients with endometrial polyp[25].

Study Limitations

The main strength of our study was the assessment of the oxidative status in direct tissues, as it conducted examinations of tissue washing fluids in the EH group with healthy volunteers. To the best of our knowledge, no other study has evaluated the status of oxidative stress in endometrial wash fluid in the existing literature other than our study. Direct evaluation of the tissues or oxidative balance, especially in precancerous pathological changes, will accurately illuminate the etiopathogenesis. The present study possesses several limitations.

Conclusion

The present study found that total and native thiol levels were lower in patients with EH compared to that of the control group. Disulfide, disulfide/native thiol, and disulfide/total thiol levels were higher in the EH group. The oxidative stress level was higher in the endometrial washing fluids of patients with hyperplasia. Oxidative levels of patients with EH could be compared to patients with endometrial cancer, and studies could be conducted with more patients that could be more beneficial for clinical use. Therefore, our results need further validation in larger-sized studies to develop an accurate predictive test.

Ethics

Ethics Committee Approval: The study was approved by the Erzincan Binali Yıldırım University Clinical Research Ethics Committee and conducted following the Helsinki Declaration (approval number: 33216249-604.01.02-E.24314).

Informed Consent: Informed consent was obtained from all participants at the beginning of the study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: E.Y., Design: C.T., Data Collection or Processing: Ü.G., Ö.Y.Ş., H.A., Analysis or Interpretation: E.Yılm., Literature Search: E.Yılm., Writing: E.Y.

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

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Effects of the COVID-19 pandemic on obstetrics and gynecology residency training in Turkey

COVID-19 pandemisinin kadın hastalıkları ve doğum asistalık eğitimi üzerindeki etkileri

Göknur Topçu[1], İpek Betül Özçivit[2], Selçuk Erkılınç[3]

[1]*Kaçkar State Hospital, Clinic of Obstetrics and Gynecology, Rize, Turkey*

[2]*İstanbul University Cerrahpaşa-Cerrahpaşa Faculty of Medicine, Department of Obstetrics and Gynecology, İstanbul, Turkey*

[3]*Isparta City Hospital, Clinic of Gynaecologic Oncology, Isparta, Turkey*

Abstract

Objective: This study aimed to evaluate the effect of the coronavirus disease-19 (COVID-19) pandemic on obstetrics and gynecology residency in Turkey.
Materials and Methods: A 40-item questionnaire was prepared by the European Network of Trainees in Obstetrics and Gynecology. The survey included four parts, namely, workload, training aspects, trainees' health and safety, and women's and maternal health, conducted between April 2020 and September 2020. The submission of the questionnaire was voluntary.
Results: A total of 103 trainees from 28 cities responded to the survey. The mean duration of training was 2.5 years, and first- to fifth-year residents were included. In this study, 66 trainees (65.3%) were deployed in COVID-19 units, and the number of working hours was 84 hours per week. Moreover, 67% of the trainees reported insufficient outpatient clinic experience to meet education targets. Almost all trainees (101 of 103) trainees reported that the number of surgeries and/or elective surgeries decreased or were canceled. In addition, 63% and 68% of the trainees reported that their surgical skills were hindered by the reduced number of surgeries and dissatisfaction by not achieving a sufficient number of surgeries, respectively. Overall, 71% (n=73) were worried about their training. Only 45 trainees (43.6%) have raised their concerns to their program coordinators. Trainees in Turkey experienced a decrease in the workload during the first COVID-19 wave by 62% (n=64). In average, trainees worked nearly 30 h less than their usual workweek. Only 5% of the trainees (n=5) worked from home. Trainees claimed to have used sufficient personal protective equipment, and 66% (n=68) could keep their social distance in the hospital. The availability of health care was different between departments, and the family planning and reproductive medicine departments were the most affected.
Conclusion: The obstetrics and gynecology training in Turkey has significantly been affected by the COVID-19 pandemic.
Keywords: COVID-19, gynecology, obstetrics, residency, training, Turkey

Öz

Amaç: Bu çalışmanın amacı, koronavirüs hastalığı-19 (COVID-19) pandemisinin Türkiye'deki Kadın Hastalıkları ve Doğum uzmanlık eğitimine etkisini değerlendirmektir.
Gereç ve Yöntemler: Avrupa Kadın Doğum ve Hastalıkları Asistan Organizasyonu (ENTOG) tarafından 40 maddelik bir anket hazırlandı. Anket 4 bölümden (iş yükü, eğitim konuları, stajyerlerin sağlığı ve güvenliği, kadın ve anne sağlığı) oluşmuştur. Türkçe çevirisi Türkiye'deki asistanlara Nisan 2020 ile Eylül 2020 iletilmiştir. Ankete katılım gönüllülük esasına dayanmıştır.
Bulgular: Anketimizi 28 farklı şehirden toplam 103 asistan yanıtladı. Ortalama eğitim yılı 2,5 idi ve bu çalışmada tüm yıllardan asistanlar temsil edildi. COVID birimlerinde çalışma 66 asistan (%65,3) tarafından bildirildi. Haftada 84 saate kadar çalışıldığı belirlendi. Eğitim hedeflerine ulaşmak için yetersiz poliklinik deneyimi asistanların %67'si tarafından rapor edildi. Asistanların neredeyse tamamı (103 kişiden 101'i) ameliyat sayısının azaldığını veya iptal edildiğini bildirdi. Ameliyat sayısının azalması nedeniyle engellenen cerrahi beceriler ve yeterli sayıda ameliyat yapılmamasından kaynaklanan memnuniyetsizlik asistanların sırasıyla %63 ve %68'i tarafından bildirilmiştir. Asistanların %71'i eğitimleri konusunda endişeliydi. Sadece 45 asistan (%43,6) program koordinatörleri ile endişelerini paylaşabilmiş. Türkiye'deki asistanlar ilk COVID-19 dalgasında iş yükünde %62 (n=64) azalma yaşadı. Ortalama olarak, asistanlar normal çalışma haftalarına kıyasla neredeyse 30 saat daha az çalıştı. Asistanların sadece %5'i (n=5) evden çalışırken, yeterli

PRECIS: The obstetrics and gynaecology training in Turkey has significantly been affected by the COVID-19 pandemic. Decrease in the learning opportunities of trainees may lead to decrease in the quality of care.

Address for Correspondence/Yazışma Adresi: Göknur Topçu MD, Kaçkar State Hospital, Clinic of Obstetrics and Gynecology, Rize, Turkey
Phone: +90 536 608 16 13 **E-mail:** goknurtopcu@gmail.com **ORCID ID:** orcid.org/0000-0003-1362-6224
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kişisel koruyucu donanıma sahip olduklarını ve %66'sı (n=68) hastanede sosyal mesafelerini koruyabildiklerini bildirdi. Sağlık hizmetlerinin mevcudiyeti bölümler arasında farklılık gösterirken en çok aile planlaması ve üreme tıbbının etkilendiği bildirildi.
Sonuç: Türkiye'deki obstetrik ve jinekoloji eğitimi COVID-19 pandemisinden önemli ölçüde etkilenmiştir.
Anahtar Kelimeler: COVID-19, jinekoloji, obstetrik, asistan, eğitim, Türkiye

Introduction

Turkish Trainees in Obstetrics and Gynaecology (TTOG) is a member of the European Network for Trainees in Obstetrics and Gynaecology (ENTOG), which is an organization that aims to improve and harmonize training in obstetrics and gynecology across Europe with 35 member countries, ultimately improving women's health (ENTOG.eu 2020).

Coronaviruses, belonging to Nidovirales order, are enveloped non-segmented positive-sense RNA viruses. They are responsible for the severe acute respiratory syndrome coronavirus (SARS-CoV) outbreak in 2002, the ongoing middle East respiratory syndrome-related-CoV outbreak since 2012, and the COVID-19 outbreak (SARS-CoV-2) as declared by World Health Organization on March 11, 2020[1,2].

Since the announcement of the first positive case in Turkey, the COVID-19 pandemic has had serious consequences on the physical and mental health of both the people and the economy[3].

It has become the main reason for stress and anxiety among health workers because of the uncertainty of the long-term complications and the lack of treatment algorithms. Furthermore, the steady increase in the workload, fear of transmission, and prolongation of the pandemic are additional factors that lead to burnout syndrome and depression among health workers[4].

As of mid-August 2021, more than 6.02 million cases and 52,703 deaths have been recorded in Turkey[5]. To cope with this unpredictable situation, the Turkish healthcare system has been rearranged to optimize its resources, and serious precautions have been taken against the spread of infection, such as lockdowns and travel restrictions. With these modifications, nearly all hospitals in Turkey have turned into pandemic hospitals, where all elective appointments and surgeries have been canceled. All educational meetings and programs have been either canceled or shifted online.

This study aimed to evaluate the effect of the COVID-19 pandemic on the residency training programs of obstetrics and gynecology in Turkey. To the best of our knowledge, this is the first study from Turkey on the effect of COVID-19 on training programs.

Materials and Methods

Study Design

This cross-sectional exploratory survey study aimed to investigate the effect of the COVID-19 pandemic on the training and workload of Turkish trainees in obstetrics and gynecology and women's health through an online questionnaire survey.

Questionnaire

The online survey was prepared by using Google Forms consisting of 40 questions including multi-answer, multiple-choice, open-ended, and 10-point Likert scale items. The survey was divided into four parts: Part 1, workload; Part 2, training aspects in obstetrics and gynecology; Part 3, health and safety of the trainee; and Part 4, women's health and maternal health issues (including care given in obstetrics, family planning, reproduction, gynecology, and gynecologic oncology departments). The demographic data of the respondents, such as age, years spent in residency, city, and hospital type they are having their training, were collected at the beginning of the survey. The questionnaire was shared on the TTOG website, TTOG social media, TTOG mailing system, and through TTOG hospital-based representatives. Data were collected anonymously from April 29, 2020, to September 5, 2020, by residents after obtaining their informed consent. The principles stated in the Helsinki Declaration were followed. Trainees from all years were included. The obstetrics and gynecology training program in Turkey lasts for 4 years and can be extended for another year.

The survey enabled the trainees to compare their involvement in training activities (outpatient visits, diagnostic procedures, laparotomy, laparoscopic gynecological surgeries, etc.) between the pre-COVID-19 and COVID-19 periods.

Translation

The original version of the questionnaire was in English and then translated to Turkish to overcome the language barrier. Data collected in Turkish was not used in the ENTOG report. Trainees who have not completed the original English survey were asked to complete the Turkish version.

Statistical Analysis

Data normality was evaluated using the Kolmogorov-Smirnov test or Shapiro-Wilk tests. Continuous data were compared using independent samples t-tests or Mann-Whitney U tests. Categorical data were compared with the chi-square test. A p-value <0.05 was regarded as significant. Statistical analysis was performed using SPSS Statistics 21 (IBM Corp., Armonk, NY, USA).

Results

Characteristics

In total, 103 trainees from Turkey have completed our survey. The respondents' age ranged from 24 to 39 (mean, 29) years. The duration of training ranged from 3 to 56 (mean, 21.25) months. The largest proportion of the respondents was working

in Istanbul (22.3%). Obstetrics and gynecology training can be conducted either at a university hospital or a training and research hospital. The majority of the respondents were training at a university hospital (57.3%). National strategy against COVID-19 outbreak included partial quarantine (81.2%) in most of the cities. Local guidelines were followed in the management of patients with COVID-19 (98%). The national guidelines in the management of pregnant patients with COVID-19 were followed by 87% of the institutions. COVID-19 cases were managed by 66 of the respondents (65.3%) rather than obstetrics and gynecology cases. Insufficient personal protective equipment (PPE) was reported by 60 (59.4%) of the trainees. Nearly half of the respondents have received training regarding the use of PPE, and 57.4% of the respondents believed that they could still provide standard care in these circumstances (Table 1).

The respondents felt well-prepared in the management of patients with COVID-19. Physical and psychosocial health and feeling safe were rated as 4 and 6 of 10, respectively (Table 2).

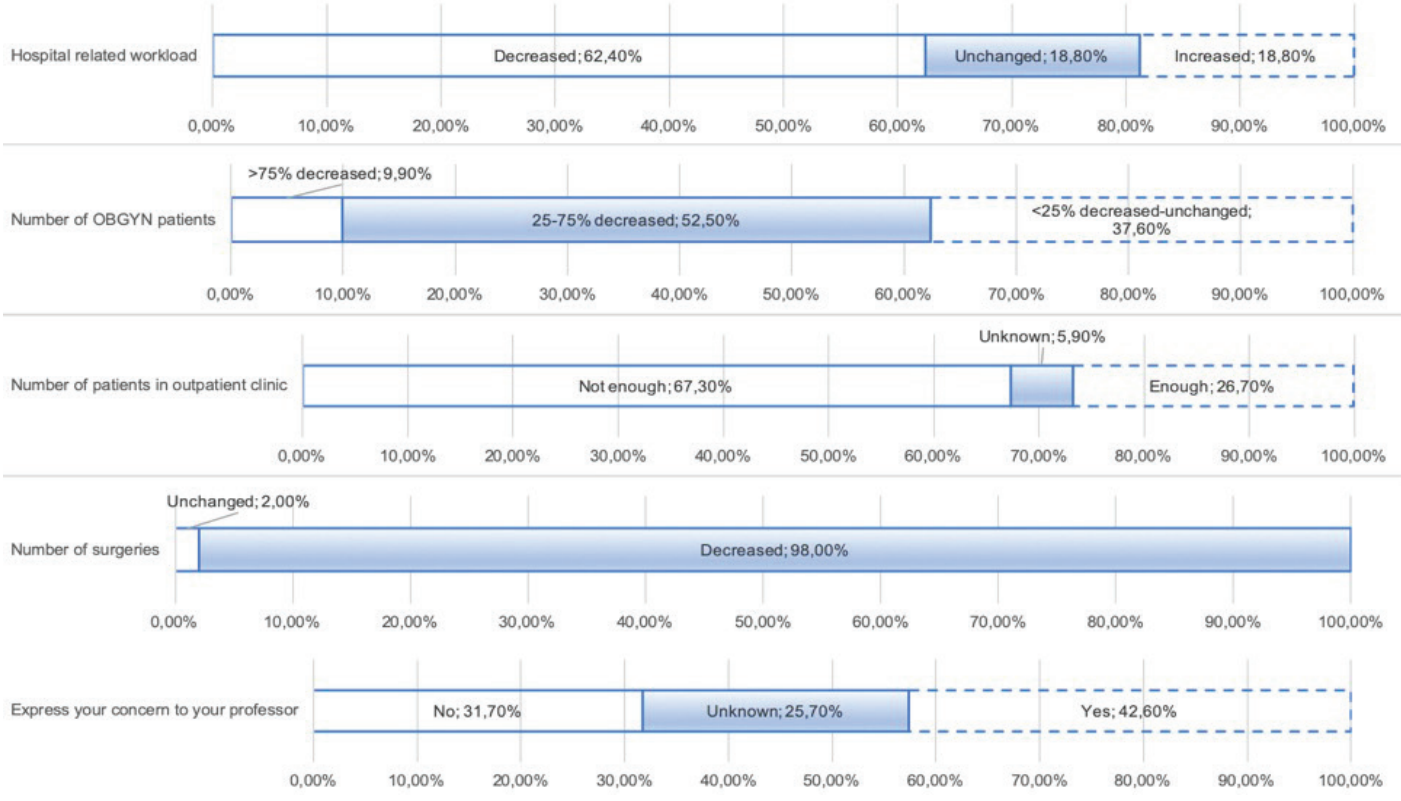
Survey Results

The workload decreased according to 63 (62.4%) respondents, while it remained unchanged or increased according to 18.8% and 18.8% of the respondents, respectively (Graph 1). The resting hours during and after work were different among trainees, as shown in Graph 2.

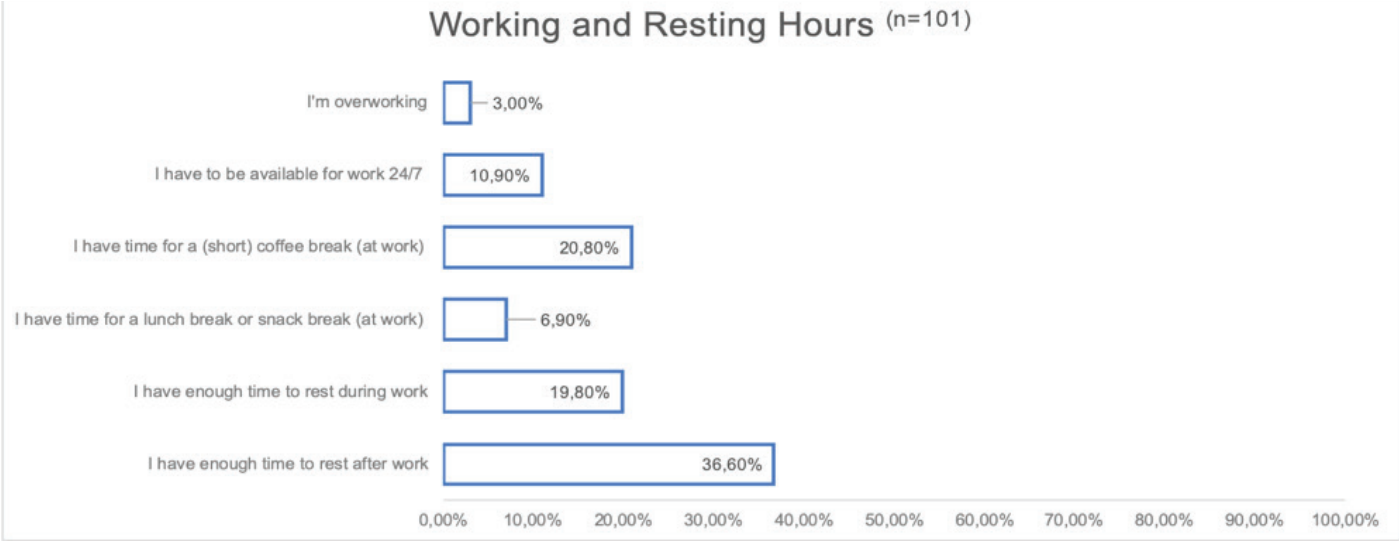
The number of obstetrics and gynecology patients has decreased during the COVID-19 pandemic. According to more than half of the respondents (53%-52.5%), the number decreased between 25% and 75% (Graph 2). The number of outpatients was not enough to meet training requirements according to 68 (67.3%) respondents. A decrease in the number of surgeries was reported by 99 (98%) respondents. Furthermore, while 72 (71.3%) residents were concerned about their education, only 43 (42.6%) had reported their concern to their professor/head of training/ head of department (Graph 2). The concern about the training was independent of the stage of training (p=0.258) (Table 3).

Regarding the health and safety of the trainees, protective measures were taken. Keeping social distance inside the hospital was reported possible by 68 (67.3%) respondents. The application of COVID-19 tests was possible in case of contact with patients with COVID-19 (40.6%) or in case of fever (10.9%) and minor symptoms (12.9%). Most of the respondents (67.3%) knew what to do when they started to exhibit COVID-19 symptoms (Graph 3). By contrast, 23.8% of the trainees reported no special instructions, and 61.4% reported that no attention was given for the provision of psychosocial support to the healthcare staff.

Trainees (46.5%) were assigned in COVID-19 intensive care units (ICUs) without any training. Insufficient training in the management of obstetric COVID-19 cases was reported by 76 (75.2%) respondents. We analyzed the duration spent at



Graph 1. Workload during the pandemic



Graph 2. Working and resting hours

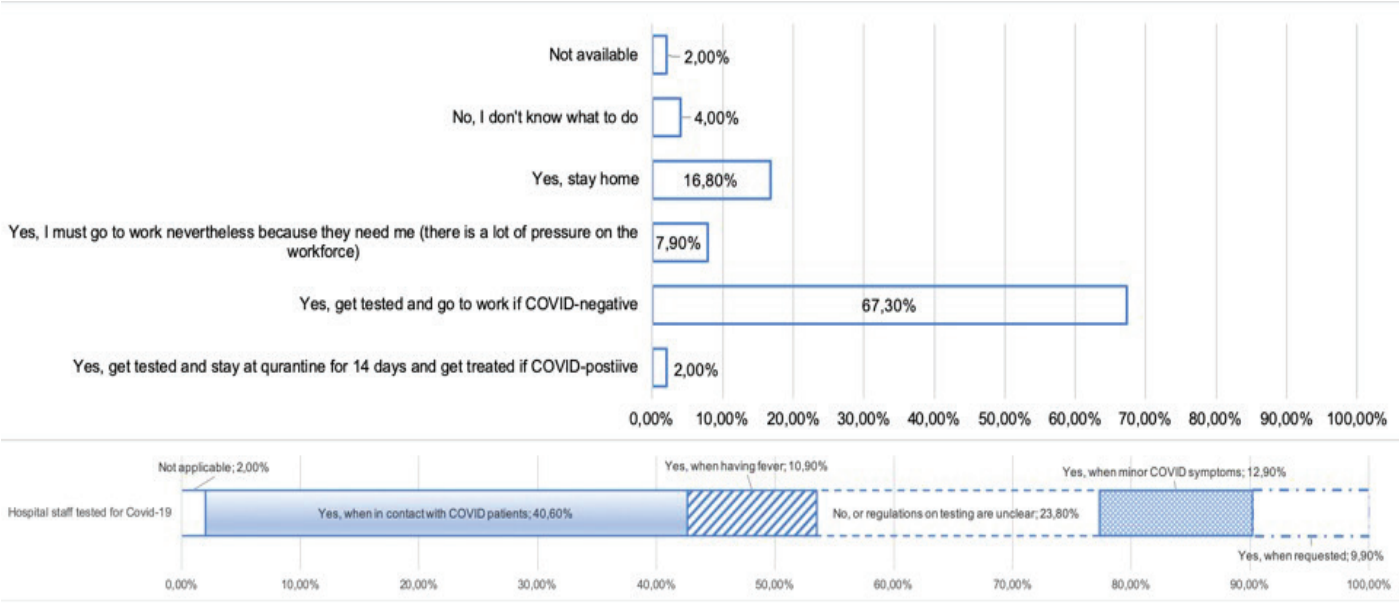
Table 1. Characteristics of the respondents

	Mean ± SD(n=103)
Age	29±4
Duration of training (months)	21.25±16
Feeling well-prepared in the management of COVID-19 cases (0-10)	4.33±2.23
Feeling healthy at the moment (physically and psychologically) (0-10)	6.24±2.15
Feeling safe at work (0-10)	4.02±2.16
	Overall N (%)(n=103)
City	
İstanbul	23 (22.3%)
Ankara	6 (5.8%)
İzmir	16 (15.5%)
Others	58 (56.3%)
Hospital type	
University hospital	59 (57.43%)
Training and research hospital	44 (43.6%)
Managed COVID-19 cases	
Yes	66 (65.3%)***
No	35 (34.7%)
Sufficient stocks of PPE	
Yes	60 (59.4%)
No	41 (40.6%)
National strategy	
Full quarantine	19 (18.8%)
Partial quarantine	82 (81.2%)
COVID-19 management guideline	
National	0 (0%)
Local	99 (98%)
None	2 (2%)
Pregnancy with COVID-19 management guideline	
National	2 (2%)
Local	88 (87.1)
None	11 (10.9%)
Providing standard care	
Yes	58 (57.4%)
No	41 (40.5%)
Not applicable	2 (2%)

COVID-19: Coronavirus disease-19

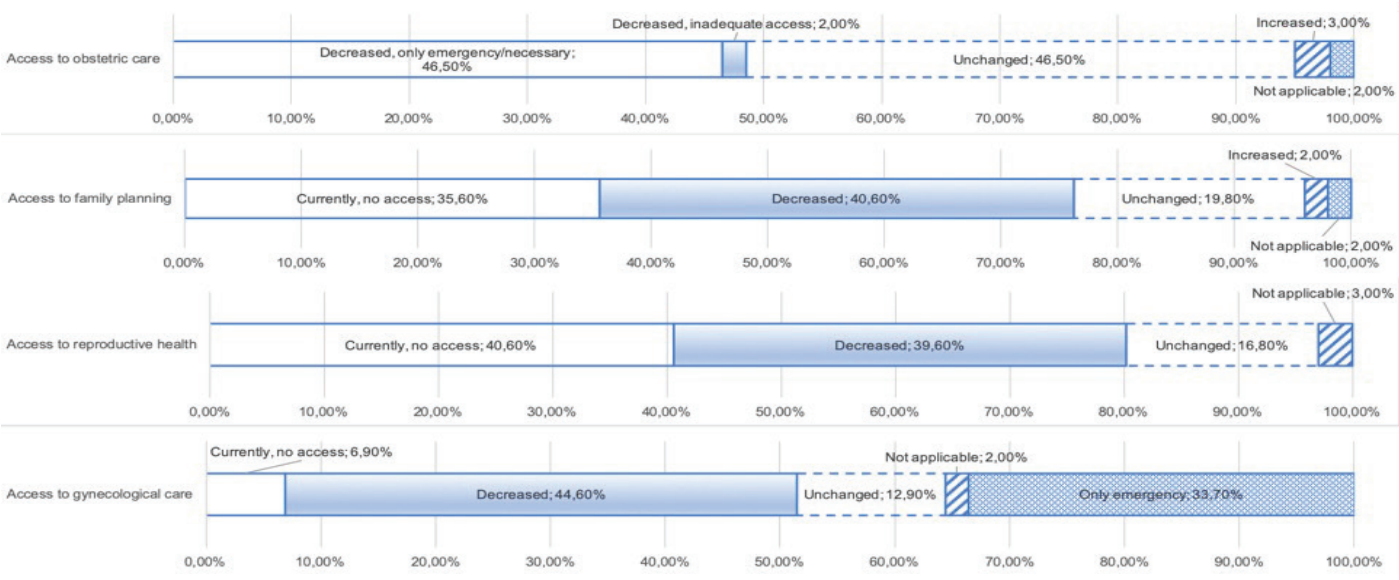
hospital according to the year of residency. Even though before the outbreak second-year residents were spending more time at the hospital (93.72 h/week) during the outbreak, first-year residents spent significantly more time than other residents (61.53 h/week). However, no significant difference was found in the durations spent in the hospital between years of residency before and during the outbreak (p=0.57 and p=0.674, respectively) (Table 2). First-year residents have worked in COVID-19 units significantly longer than other residents (p=0.003) (Table 3).

The COVID-19 pandemic has affected patient care as a result of all measures taken to reduce the spread of the virus. According to 47 (46.5%) respondents, access to obstetric care was not affected. However, according to the same number of residents, access to obstetric care has decreased and was only available for emergent cases. Access to family planning was decreased according to 41 (40.6%) respondents, while it was reported by 36 (35.6%) as unavailable. Access to reproductive medicine was reduced in 40 (39.6%) of the cases and was unchanged in 17 of them (16.8%). Access to general (benign) gynecological care was affected significantly and decreased according to 45 (44.6%) respondents, or only available for emergent cases according to 34 (33.7%) respondents (Graph 4).



Graph 3. Readiness for the COVID-19 pandemic

COVID-19: Coronavirus disease-19



Graph 4. Access to care during the pandemic

Discussion

Main Findings

This cross-sectional study aimed to determine the effect of the COVID-19 pandemic on obstetrics and gynecology training in Turkey. Our findings showed that the COVID-19 pandemic has caused a severe impairment and disruption in the educational program of obstetrics and gynecology residency in Turkey. Until now, no measures have been taken to compensate for the disruption. This study is important as this is the first study to evaluate the effect of the COVID-19 pandemic on the training programs in Turkey.

The COVID-19 pandemic had negatively affected the Turkish healthcare system, being overwhelmed by cases in addition to routine care. Educational activities in medicine have been affected as well[6].

The effect of the pandemic differed across the country, being more serious in crowded cities. Since most of the respondents were located in the biggest cities in Turkey, this survey may be regarded as representative of the current status of obstetrics and gynecology residency in Turkey during the pandemic.

Our results showed that approximately 70% of the trainees cared for patients with COVID-19. The first- and second-year trainees took on most of the burden during the pandemic in Turkey. Gynecologic care appeared to be more affected than obstetric

Table 2. Difference between the times spent at the hospital before and during COVID-19 outbreak and subdivisions according to the attended year of residency

Study variables	First-year resident	Second- year resident	Third-year resident	Fourth-year resident	Fifth-year resident	p-value§
During COVID-19 pandemics (hours ± *SD)*	61.53±19.5	59.68±20.5	54.1±16.7	57±26.23	54±25.46	0.57
Before COVID-19 pandemics (hours ± *SD)*	88.45±28.6	93.72±36.4	80.04±26.58	84.18±39.46	73±1.41	0.67

§p is calculated by analysis of variance, SD: Standard deviation, COVID-19: Coronavirus disease-19
**Significant at p<0.05 level

Table 3. Difference between working at COVID-19 units and concerns about the training according to the attended year of residency

Study variables	First year resident N (%)(n=38)	Second-year resident N (%)(n=25)	Third-year resident N (%)(n=25)	Fourth-year resident N (%)(n=11)	Fifth-year resident N (%)(n=2)	Overall N (%)(n=101)	p-value§
Working at COVID-19 units	32 (48.5%)	15 (22.7%)	13 (19.7%)	5 (7.6%)	1 (1.5%)	66 (65.3%)	0.003**
Concerned about the training	29 (40.3%)	19 (26.4%)	14 (19.4%)	10 (13.9%)	0 (0%)	72 (71.3%)	0.258

§p is calculated by chi-square test, COVID-19: Coronavirus disease-19
**Significant at p<0.05 level

care, which is not an elective care. Despite the decreased working hours of trainees relative to the pre-COVID era, the extra working time was not used for training activities. Although elective surgeries are ongoing in some centers in Turkey, most of the clinics ceased performing elective surgeries[7]. Moreover, given the reduced number of patients in outpatient clinics in urogynecology, reproductive medicine, and gynecology, the competency of trainees in these areas has been reduced. As a result, as reported by the respondents, the quality of surgical education has significantly decreased. The findings from our survey echo these situations. This issue needs to be addressed since the quality of care presented by future specialists who were trained during the pandemic has been badly affected. One possible solution to this problem is the addition of time lost during the pandemic to the routine curriculum. Increased didactic educational models, use of surgical simulators, and use of surgical videos may compensate for the gap in surgical education during the pandemic[8]. A revised national logbook must be developed for the COVID-19 era. Trainees need more support from their mentors since most of them complain about not being able to discuss their problems. Daily or weekly virtual meetings or educational courses may increase communication with the clinic and compensate for the shortcomings in education[9].

Emergent interventions in obstetrics and gynecology, such as emergent cesarean section or labor, make obstetrics and gynecology an emergent department. Therefore, when dealing with emergent interventions, trainees are faced with the risk of virus contamination from asymptomatic COVID-19 cases. High stress experienced during obstetrics and gynecology care increases the possibility of errors during interventions, even with fully equipped or trained staff. Our results revealed that the trainees felt unprepared to face hospital situations during the pandemic. Even though education in PPE use was given, PPE was insufficient in most cases. Trainees should receive instructions for the care of COVID-19 and obstetric COVID-19 cases before deployment to COVID-19 ICUs or obstetric clinics, which was not the case for our respondents. Even though the Ministry of Health announced a national algorithm for obstetric cases, educational meetings are needed to reinforce the application of these algorithms[10]. One possible advantage of this pandemic is that the trainees gained information and experience on how to handle such a health crisis. The prolongation of the pandemic may affect trainees in various ways; however, increased familiarity may help them handle problems faced during a pandemic.

As study strength, this is the first study that investigated obstetrics and gynecology training during the COVID-19 pandemic in Turkey. We included residents from all regions in Turkey and represented the current status. The main limitation of the study was the lack of subgroup analysis according to the years of training of the respondents, which may have contributed to the heterogenicity of the results. The negative effect of the pandemic on obstetrics and gynecology training in Turkey can be compared with the situation in Europe or in other specialties[11-16]. In Europe, 60% of the trainees were concerned about reaching the goals in their training; in Turkey and Italy, the rates of anxiety were higher, with 71.3% and 84%, respectively[11,12]. Unfortunately, unlike their colleagues in Europe (73%), only 42.6% of the trainees in Turkey had expressed their concern to their mentors, and limited arrangements were made to overcome these shortcomings in Turkey. Our respondents and trainees in Europe reported that the workload has decreased, and this emphasizes the burden of the obstetrics and gynecology training program in the pre-COVID era[12]. In addition, respondents reported a lack of psychosocial support (61.4%) compared with European trainees who received psychosocial support (65%). Insufficient

PPE was reported by 59.4% of our trainees, while their European colleagues had enough PPE supply. Our respondents reported that they were able to maintain social distancing inside the hospital compared with their European colleagues (67.3% vs 9%). Moreover, the application of COVID-19 testing and regulations on what to do with COVID-19 symptoms were clear to most of the trainees in Europe and Turkey[12]. Insufficient training on the management of COVID-19 cases in COVID wards or ICUs or obstetric clinics was reported at a similar rate by Turkish and European trainees (46.5% vs 50%, respectively). Turkish and European trainees reported feeling safe at a comparable rate (6 vs 6.4 of 10, respectively). The decrease in educational and surgical activities and number of patients was a worldwide problem during the pandemic, as was reported in Turkey[12,14,15]. Patient care was affected as a result of the measures taken. Obstetric care was not changed as reported by 46.5% of the trainees in Turkey compared with reports by 47% of the trainees in Europe. However, family planning, reproductive medicine, and gynecological care was reduced significantly in Turkey but was comparable with those in Europe[12].

Conclusion

The results of this study revealed an overall decrease in outpatient visits, elective surgeries, and educational activities of obstetrics and gynecology residents in Turkey during the COVID-19 pandemic. In this regard, the use of online platforms for educational meetings, providing simulation-based practices, and tele-mentoring of surgical procedures might help trainees complete their residency program without inadequacies.

Ethics

Ethics Committee Approval: Approval was obtained that there is no need for ethics commission approval for this study.
Informed Consent: Data were collected anonymously from April 29, 2020, to September 5, 2020, by residents after obtaining their informed consent.
Peer-review: Externally peer-reviewed.
Authorship Contributions
Critical Revision: S.E., Concept: G.T., Design: G.T., S.E., Data Collection or Processing: G.T., İ.B.Ö., Analysis or Interpretation: G.T., Literature Search: G.T., Writing: G.T., İ.B.Ö., S.E.
Conflict of Interest: No conflict of interest was declared by the authors.
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Review / Derleme

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Contained and uncontained morcellation in hysterectomy and myomectomy: A systematic review and meta-analysis

Histerektomi ve miyomektomide contained ve uncontained morselasyonun sistematik derlemesi ve meta-analizi

Greg Marchand[1], Ahmed Masoud[2], Ashley Christensen[3], Stacy Ruther[1], Giovanna Brazil[1], Alexa King[4], Hollie Ulibarri[1], Julia Parise[1], Amanda Arroyo[1], Catherine Coriell[1], Sydnee Goetz[1], Katelyn Sainz[1]

[1]*Marchand Institute for Minimally Invasive Surgery, Mesa, Arizona, USA*
[2]*Fayoum University Faculty of Medicine, Fayoum, Egypt*
[3]*Midwestern University College of Osteopathic Medicine, Glendale, Arizona, USA*
[4]*International University of Health Sciences, Basseterre, St. Kitts*

Abstract

We sought to analyze all high-quality studies available regarding the possible differences in contained and uncontained techniques for morcellation of fibroids and uteri. We systematically searched PubMed, Cochrane Central, Scopus, ClinicalTrials.Gov, MEDLINE and Web of Science from September 2010 to September 2020 for our search terms. We included studies that specifically enrolled patients undergoing power morcellation myomectomy or power morcellation hysterectomy procedures. In our search, we had no restriction to age, country, or publication date.

We extracted data related to study design, baseline characteristics of patients, and perioperative outcomes such as total operative time, total blood loss, and duration of hospital stay. We found no substantial difference in total operative time between contained power morcellation and uncontained manual morcellation myomectomy (p=0.52), but contained power morcellation had a significantly longer total operative time than uncontained power morcellation for hysterectomy and myomectomy [135.50 vs. 93.33 minutes, (p=0.003)]. Total blood loss was comparable for contained power morcellation versus uncontained manual morcellation myomectomy (p=0.32) and contained power morcellation versus uncontained power morcellation myomectomy or hysterectomy (p=0.91). Contained power morcellation and uncontained manual morcellation myomectomy had comparable hospital stay periods (p=0.5). Contained power morcellation leads to a longer operating time than uncontained power morcellation for both hysterectomy and myomectomy. No differences were found in comparisons of blood loss, operative time, or comparison to manual methods of morcellation.

Keywords: Power morcellation, manual morcellation, myomectomy, meta-analysis

Öz

Contained morselasyon için yeni sistemler geliştirilmiş olduğundan; biz bu çalışmada miyom ve rahim morselasyonu için uygulanan contained ve uncontained tekniklerdeki olası farklılıklara ilişkin mevcut tüm yüksek kaliteli çalışmaları analiz etmeye çalıştık.

Arama terimlerimiz için Eylül 2010'dan Eylül 2020'nin sonuna kadar PubMed, Cochrane Central, Scopus, ClinicalTrials.Gov, MEDLINE ve Web of Science'yi sistematik olarak taradık. Power morselasyon miyomektomisi veya power morselasyon histerektomisi prosedürleri uygulanan hastaları özel olarak kaydeden çalışmaları dahil ettik. Araştırmamızda yaş, ülke veya yayın tarihi ile ilgili herhangi bir kısıtlamamız yoktu. Çalışma tasarımı, hastaların temel özellikleri ve toplam ameliyat süresi, toplam kan kaybı ve hastanede kalış süresi gibi perioperatif sonuçlarla ilgili verileri çıkardık. Contained power morselasyon ve uncontained manuel morselasyon miyomektomisi (p=0,52) arasında toplam ameliyat süresi açısından anlamlı bir fark bulamadık, ancak histerektomi ve miyomektomi için contained power morselasyon uncontained power morselasyona göre daha uzun toplam ameliyat süresine sahipti [135,50 dakikaya karşı 93,33 dakika, (p=0,003)]. Toplam kan kaybı açısından contained power morselasyon ve uncontained manuel morselasyon miyomektomisi arasında (p=0,32) ve contained power morselasyon ile uncontained power morselasyon miyomektomisi veya histerektomisi arasında (p=0,91) fark yoktu. Hastanede kalış süresi açısından, contained power morselasyonu ve uncontained manuel morselasyon miyomektomisi benzer sonuçlara sahipti (p=0,5).

Address for Correspondence/Yazışma Adresi: Greg Marchand MD, Marchand Institute for Minimally Invasive Surgery, Mesa, Arizona, USA
Phone: +4809990905 **E-mail:** gm@marchandinstitute.org **ORCID ID:** orcid.org/0000-0003-4724-9148
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Hem histerektomi hem de miyomektomi için contained power morselasyon, uncontained power morselasyona kıyasla daha uzun ameliyat süresi ile ilişkili gibi görünmektedir. Manuel morselasyon yöntemleri arasında kan kaybı ve ameliyat süresi açısından hiçbir fark bulunmadı.
Anahtar Kelimeler: Power morselasyon, manuel morselasyon, miyomektomi, meta-analiz

Introduction

Uterine fibroids or myomas are some of the most common benign tumors of premenopausal women[1]. Patients may present with pain, abnormal uterine bleeding, pressure symptoms, or infertility[2,3]. The traditional approach to treat large uterine myomas involved abdominal myomectomy or hysterectomy. With the advent of minimally invasive laparoscopic techniques, there is a need for analysis of the different techniques for performing laparoscopic hysterectomy and myomectomy in the presence of large fibroids requiring some form of morcellation for removal[4]. Some studies have shown that laparoscopic myomectomy, versus open myomectomy, may have higher success rates and fewer complications when performed on appropriately selected patients[5]. Further described advantages of laparoscopic myomectomy include less postoperative pain, faster recovery time, shorter hospital stay, and higher probability of maintaining reproductive function[6].

In an attempt to solve the problems of specimen removal in laparoscopy, several techniques have been developed to break apart these tissues in order to deliver them through the small incisions that characterize laparoscopy, also known as morcellation. As a result, various techniques, such as laparoscopic power morcellation and manual morcellation have been developed for laparoscopic, vaginal, or mini-laparotomy (ML) use. These procedures can be performed with or without a specially designed bag to contain the shredded pieces which may result[7]. In unexpected malignancies, power morcellation can lead to the unintended spread of malignant cells. This has become of particular interest in recent years, as the contained power morcellation of unexpected leiomyosarcoma can result in spreading of the leiomyosarcoma and worsening of the patient's prognosis. This culminated in an Food and Drug Administration (FDA)-issued statement discouraging the use of power morcellation for myomectomy or hysterectomy in 2014, but this statement was later updated to allow the their usage only in a contained environment[8]. The FDA decision may have been a factor in pushing manufacturers of power morcellation devices to develop containment systems for avoiding cell spillage and unintentional implantation of malignant cells[9,10].

Prior to these events, contained laparoscopies in gynecology were mainly performed in ovarian cystectomy or oophorectomy to reduce the risk of dissemination of undetected malignant cells[11]. Currently, many studies have investigated the different outcomes of in-bag manual or power uterine morcellation and assessed new techniques to address the general drawbacks of morcellation[12-14].

In this analysis, we aimed at evaluating the contained power morcellation technique and compare its perioperative outcomes with the uncontained power and manual morcellation procedures. Unfortunately, at this time, there is insufficient follow-up data to support the aim of this study, including the discovery of future occult malignancy outcomes. Therefore, our aim at this time is limited to the analysis of the perioperative outcomes of these procedures. Moreover, we were unable to consider the effects of differences in morcellation techniques, closure methods, or the effects of administered intraoperative medications, such as vasopressin.

Materials and Methods

We conducted and drafted this study according to the steps described in "Cochrane handbook for systematic reviews of interventions" and the "Preferred reporting items for systematic reviews and meta-analyses (PRISMA)" guidelines[15,16].

Search Strategy and Data Collection

We searched the following electronic databases: PubMed, Cochrane Central, Web of Science, ClinicalTrials.Gov, MEDLINE and Scopus for all published studies up to September 1, 2020, published within the last 10 years (from September 1, 2011). We conducted our search using the following key terms: "Laparoscopy," "Celioscopy," "Peritoneoscopy," "Morcellation," "Fibroidectomy," "Myomectomy," and "Uterine Myomectomy." After removing duplicates using Endnote software, we screened all remaining citations for eligibility through two steps: First, screening the titles and abstracts and second, screening the full texts. We then included the studies matching our selection criteria. Moreover, we manually screened the references of the included studies for other related papers.

Selection Criteria

We included all studies that enrolled patients specifically undergoing power morcellation myomectomy or power morcellation hysterectomy. In our search, we had no restriction to age, country, or publication date. We excluded non-English studies, non-available studies, thesis, reviews, and individual case reports. We did not further sort included studies by selected techniques, closure methods, or intraoperative medications administered. All techniques of power morcellation or manual morcellation were assumed to be performed to the best of the ability of the surgeon, and other differences of technique, (with the exception of power vs. manual morcellation), were ignored.

Data Extraction

The following data was extracted:

1. Summary of the included studies and their elements such as study design, study arms, duration, number of patients, and conclusion.

2. Baseline characteristics of the enrolled patients including age, body mass index (BMI), gravidity and parity, number of nulliparous participants, history of abdominal surgeries, myoma characteristics, and myoma symptoms of pain, infertility, or abnormal uterine bleeding.
3. Perioperative outcomes including total operative time (defined as initial incision to skin closure), morcellation preparation time (defined as any time delegated by the surgeon to prepare in order to perform morcellation), total morcellation time (defined as the total time declared by the surgeon as needed to perform the morcellation portion of the procedure), total blood loss, and duration of hospital stay.
4. Data required to complete our quality assessment (assessment of bias).

Quality Assessment

We assessed the quality of the included studies using the National Institute of Health (NIH) quality assessment tools[17]. The tool for observational cohort studies composed of 14 questions to assess the risk of bias and confounders, while the tool for case series composed of 9 assessment questions. Assessment questions included the judgment of the clarity of the study question, the definition of the study population, the participation rate, the specification of the study inclusion and exclusion criteria, the sample size justification, the outcome measurement process, the sufficiency of timeframe and follow-up period, the precise definition and validity of the exposure and outcome measures, multiple measurements of the exposure, blinding of the outcome assessor, the loss of follow-up rate, and the potential confounding variables. These questions were answered by "yes," "no," "not applicable," "cannot determine," or "not reported." Then, each study was attributed a score to judge the overall quality as either "good," "fair," or "poor."

Data Synthesis and Analysis

Statistical analyses were performed using the RevMan version 5.4 software. Continuous outcomes were analyzed as mean difference (MD) and standard error. Pooled results were reported as MD and 95% confidence interval (CI) using the generic inverse-variance method under the random-effects model. We used I-squared and chi-squared statistics to assess heterogeneity among the studies. Indications of heterogeneity included I^2 values of ≥50% with chi-square p-values <0.10. In the case of heterogeneity, we resolved by performing a sensitivity analysis using the "leave-one-out" test. Some studies reported the results of myomectomy and hysterectomy patients as a single group, so the data from these studies were pooled and reported in a separate analysis.

Results

Literature Search and Characteristics of the Included Trials

Our search obtained 525 studies (232 from PubMed, 184 from Scopus, 100 from WOS, and 9 from Cochrane). After removing 49 duplicates and 452 citations by title and abstract screening, there were 24 papers that entered full-text screening and were further refined according to our criteria to eventually reach 12 articles (Figure 1)[10,18-28]. We ultimately included one case series and 11 cohort studies with 976 patients total. Among these, 714 patients underwent contained power morcellation, 213 underwent uncontained manual morcellation, and 49 underwent uncontained power morcellation. The mean age of the patients ranged from 31 to 49 years, and the mean BMI ranged from 22.2 to 32.1 across studies. A mean of 10% to 59% of patients among the studies had previous abdominal surgery and 18.5% to 65% of then had abnormal uterine bleeding. Summary of the included studies and baseline characteristics of the enrolled patients are found in Tables 1 and 2 respectively.

Results of Quality Assessment

According to the NIH quality assessment tool for observational cohort studies, all 11 included cohort studies were of fair quality[10,18-23,25-28]. According to the NIH quality assessment tool for case series, the included case series was of good quality[29]. For more details on all assessment questions of each study, refer to Supplementary Table S1 for cohort studies and Supplementary Table S2 for the case series study.

Study Outcomes

1. Total Operative Time (Min.)

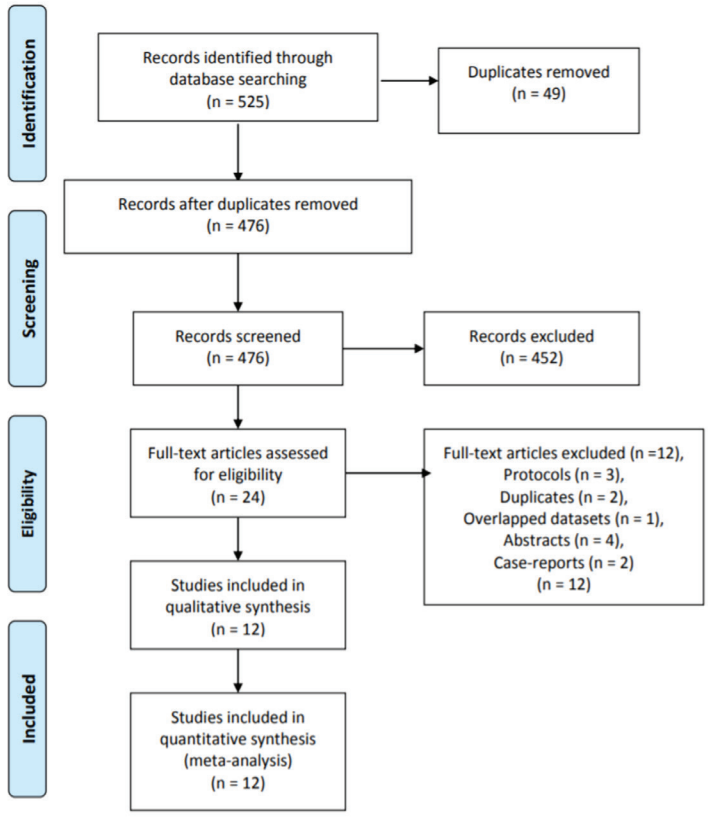


Figure 1. The PRISMA flow chart summarizing the literature search and including studies from each database

PRISMA: Preferred reporting items for systematic reviews and meta-analyses

Table 1. Summary of the included studies

Study ID	Patients	Study design	Duration	Sample size	Conclusion
Akdemir[27]	CPM	Prospective cohort	January 2014 to May 2014	30	The innovative technique could prompt the usage of a disposable latex glove for an enclosed morcellation that avoids piercing the enclosure container within the abdominal cavity, decreases the risks of bag perforation and leakage compared with previous contained power morcellation techniques.
Cohen[18]	CPM	Prospective cohort	2014 to 2015	89	Findings are consistent with prior work (Cohen 2014) demonstrating the feasibility of contained tissue extraction, however further refinement of this technique is warranted.
Cohen[28]	CPM	Prospective cohort	January 2014 to May 2014	73	Morcellation within an insufflated isolation bag is a feasible technique.
Kim[20]	CPM	Prospective cohort	September 2008 to October 2009	15	Single port transumbilical morcellation using a conventional electromechanical morcellator with or without a knife is feasible. Single port laparoscopic myomectomy is an alternative method with cosmetic advantage.
Paul[29]	CPM	Case series	November 2014 to January 2015	10	In-bag morcellation using this new bag is a feasible technique of morcellating uterine myomas in a contained manner.
Springborg[21]	CPM	Prospective cohort	June 2014 to September 2014	21	The presented improved contained morcellation technique is feasible in laparoscopic hysterectomy and myomectomy.
Vargas[19]	CPM & Uncontained power morcellation	Prospective cohort	November 2013 to April 2014	85	In-bag power morcellation results in perioperative outcomes comparable to the traditional laparoscopic approach. In this cohort, mean operative time was prolonged by 26 minutes within bag morcellation, but may potentially be reduced with further refinement of the technique.
Won[22]	CPM & Uncontained manual morcellation	Retrospective cohort	December 2014 to December 2016	58	In-bag power morcellation for SPA laparoscopic myomectomy is feasible and safe, minimizing the risks of open power morcellation. There were also no statistically significant differences in surgical outcomes.
Steller[23]	CPM	Retrospective cohort study	May 2014 to December 2015	187	Performing electromechanical power morcellation within the Espiner EcoSac 230 specimen bag was successfully performed in 187 patients with no bag-related complications. This method is feasible, reliable, and reproducible, even for a large specimen.
Boza[10]	CPM & Uncontained manual morcellation	Prospective cohort	December 2014 to January 2017	62	Both CPM and TVE can be used for safe retrieval of large myomas that are removed laparoscopically. Compared with CPM, TVE was associated with a shorter retrieval time, less postoperative pain, and less hospital costs.
Aoki[25]	CPM	Retrospective cohort	August 2015 to October 2015	12	Single-site in-bag morcellation performed with our new technique requires neither bag penetration nor piercing with a trocar and thus may prove beneficial for preventing spillage and dissemination of unwanted cells and tissue.
Trivedi[26]	CPM & Uncontained manual morcellation	Retrospective cohort	May 2012 to August 2018	720	In myomectomy group both conventional and in-bag laparoscopic morcellation were comparable in terms of duration of the surgery and blood loss. When all cases (hysterectomy and myomectomy combined) and cases of hysterectomy with large fibroids were studied, laparoscopic in-bag morcellation took less operative time and there was statistically significant difference in operative time. No case of leiomyosarcoma was found in our study of 720 cases of myomas or uterus with large myomas.

CPM: Contained power morcellation, SPA: Single port assisted, TVE: Transvaginal extraction

Table 2. Baseline characteristics of the included studies

Study ID	Arms	Sample size	Age	BMI	Gravida	Parity	Myoma size (cm)	Myoma weight (gm)	Total myoma number	Nulliparous*	History of abdominal surgery*	Infertility*	Pelvic pain*	Abnormal uterine bleeding*
Akdemir[27]	CPM	30	34.5 (4)	29.25 (4.75)	3.5 (2.5)	2.5 (1.5)	-	-	-	-	11 (37)	-	6 (30)	24 (30)
Cohen[18]	CPM	76	43 (8.53)	26.47 (5.93)	-	-	-	239.1 (229.7)	-	38 (50)	33 (43.4)	-	-	23 (30.3)
Cohen[28]	CPM	73	43 (4.3)	32.075 (6.583)	3.25 (1.5)	2.2 (0.83)	-	512 (238)	-	-	43 (58.9)	-	28 (38.4)	48 (65.8)
Kim[20]	CPM	15	38.3 (5.6)	22.8 (3.7)			6.1 (1.5)	-	1.6 (1.4)	6 (40)	2 (13.3)		6 (40)	4 (26.67)
Paul[29]	CPM	10	32.25 (5.076)	25 (2.38)	-	-		-	4 (2.38)	-	1 (10)	3 (30)	-	6 (60)
Springborg[21]	CPM	21	45.25 (3.75)	24 (2.5)	-	-	-	616.25 (296.25)	-	-	9 (42.9)	-	16 (76.2)	10 (47.6)
Vargas[19]	CPM	36	49.19 (9.12)	27.2 (5.74)	-	-	-	433.1 (360.2)	-	-	12 (333.3)	-	2 (5.5)	9 (25)
	Uncontained power morcellation	49	44.06 (8.93)	26.83 (5.5)	-	-	-	396.2 (328.3)	-	-	22 (44.9)	-	5 (10.2)	10 (20.4)
Won[22]	CPM	27	37 (7)	20.2 (2.7)	-	-	-	-	-	-	4 (14.8)	-	5 (18.5)	5 (18.5)
	Uncontained manual morcellation	31	39.75 (8.25)	23.1 (2.6)	-	-	-	-	-	-	9 (29)	-	3 (9.7)	7 (22.6)
Steller[23]	CPM	187	40 (6.093)	28.7 (7.42)	-	-	-	300 (329)	4.9 (4.57)	-	-	-	-	-
Boza[10]	CPM	31	38.5 (5.5)	23.2 (3.75)	-	-	8.5 (1.5)	188.7 (96.25)	3.5 (2)	16 (51.6)	4 (12.9)	4 (12.9)	3 (9.7)	18 (58)
	Uncontained manual morcellation	31	39.75 (6)	25.5 (5)	-	-	8.5 (1.5)	168 (97)	3 (2)	18 (58)	7 (22.6)	2 (6.5)	6 (19.4)	16 (51.6)
Aoki[25]	CPM	12	37.75 (4.446)	22.2 (2.486)	-	-	-	-	-	-	2 (16.67)	5 (41.67)	-	7 (58.3)
Trivedi[26]	CPM	196	33.5 (6.6)		-	-	7.2 (1.9)	400 (316.67)	2 (2.67)	-	-	-	-	-
	Uncontained manual morcellation	151	31.0 (4.6)		-	-	7.2 (2.3)	350 (333.33)	2 (2.83)	-	-	-	-	-

*: Data are presented as N (%). All other data presented as mean (SD), SD: Standard deviation, CPM: Contained power morcellation, BMI: Body mass index

1.1 Myomectomy-only data

Six studies[10,22,26,27,29,30] with analyzable data reported this outcome, with a total of 522 myomectomy patients; 309 patients underwent contained power morcellation, and 213 patients underwent uncontained manual morcellation. The pooled data showed an increase in total operative time in the contained power morcellation group [MD=116.66, 95% CI (102.38, 130.93)] compared to the uncontained manual morcellation group [MD=104.81, 95% CI (71.23, 138.40)]. There was no substantial differences in operative time between the two groups (p=0.52) (Figure 2).

The pooled results for both contained power morcellation (p<0.00001, I^2=90%) and uncontained manual morcellation (p<0.00001, I^2=97%) were heterogeneous, and could not be resolved.

1.2 Hysterectomy and myomectomy data

Five studies[18,19,21,25,28] with analyzable data reported this outcome, with a total of 485 myomectomy or hysterectomy patients; 436 patients underwent contained power morcellation, and 49 patients underwent uncontained power morcellation. The pooled data showed an increase in total operative time for contained power morcellation [MD=135.50, 95% CI (110.23, 160.76)], over the uncontained power morcellation groups [MD=93.33, 95% CI (80.76, 105.90)]. The test for subgroup difference confirmed a statistically shorter operation time with the uncontained power morcellation (p=0.003) (Figure 3). The contained power morcellation subgroup was heterogeneous (p<0.00001, I^2=92%), could not be solved and was not applicable to the other subgroup since it represented a single study arm.

2. Morcellation Preparation Time (Min.)

2.1 Myomectomy-only data

Two studies[27,29] with analyzable data reported this outcome, with a total of 40 myomectomy patients all undergoing contained power morcellation. The results showed an average morcellation preparation time of 9.89 minutes [95% CI (5.12, 14.65)] (Supplementary Figure S1). Pooled data were heterogeneous (p<0.0008, I^2=91%), and could not be resolved.

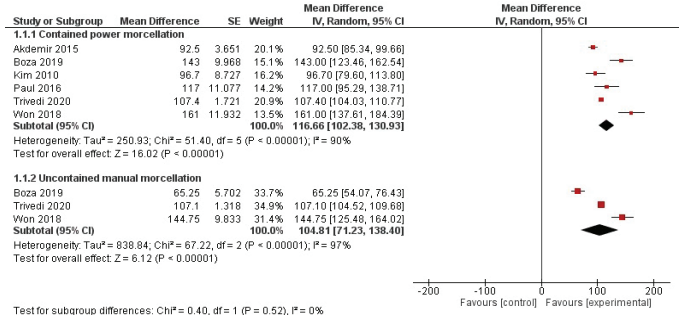


Figure 2. Forest plot of the total operative time (min.) comparison between contained power morcellation versus uncontained manual morcellation myomectomy

CI: Confidence interval

2.2 Hysterectomy and myomectomy data

Two studies[21,25] with analyzable data reported this outcome, with 51 myomectomy or hysterectomy patients all undergoing contained power morcellation surgery. The results showed an average morcellation preparation time of 15.83 minutes [95% CI (4.26, 27.39)] (Supplementary Figure S2). Pooled data were heterogeneous (p<0.00001, I^2=96%), and we could not solve the heterogeneity.

3. Total Morcellation Time (Min.)

3.1 Myomectomy-only data

Two studies[27,29] with analyzable data reported this outcome, with 40 myomectomy patients who underwent contained power morcellation. The results showed an average morcellation time of 29.74 minutes, [95% CI (21.29, 38.19)] (Supplementary

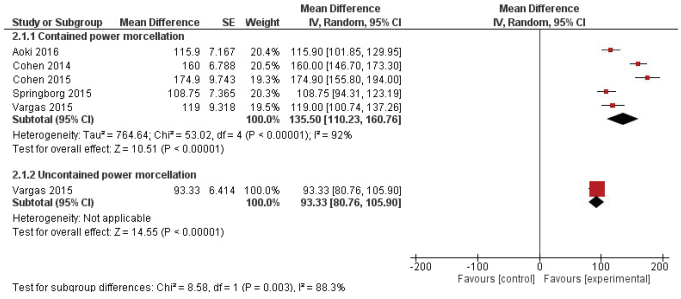
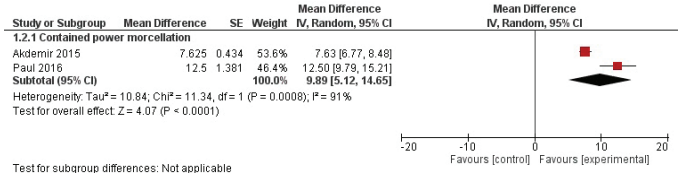


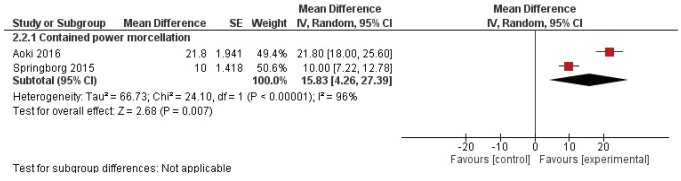
Figure 3. Forest plot of the total operative time (min.) comparison between contained power morcellation versus uncontained power morcellation myomectomy or hysterectomy

CI: Confidence interval



Supplemental Figure S1. Forest plot of the morcellation preparation time (min.) for contained power morcellation myomectomy

CI: Confidence interval



Supplemental Figure S2. Forest plot of the morcellation preparation time (min.) for contained power morcellation hysterectomy or myomectomy

CI: Confidence interval

Figure S3). The data were heterogeneous (p<0.03, I^2=78%), and we could not solve the heterogeneity.

3.2 Hysterectomy and myomectomy data

Two studies[18,25] with analyzable data reported this outcome, with 119 myomectomy or hysterectomy patients who underwent contained power morcellation. The results showed an average morcellation time of 32.80 minutes, [95% CI (26.42, 39.18)] (Supplementary Figure S4). Pooled data were homogenous (p<0.16, I^2=50%).

4. Total Blood Loss (mL)

4.1 Myomectomy-only data

Five studies[10,22,26,27,29] with analyzable data reported this outcome, with 507 myomectomy patients; 294 patients underwent contained power morcellation, and 213 patients underwent uncontained manual morcellation. The pooled data showed an increase in total blood loss in the contained power morcellation group [MD=143.12, 95% CI (105.37, 180.87)] compared to the uncontained manual morcellation group [MD=111.32, 95% CI (62.06, 160.59)]. There was no difference in this outcome between the two groups (p=0.32) (Figure 4). Pooled data were heterogeneous for both contained power morcellation (p<0.00001, I^2=92%) and uncontained manual morcellation (p<0.00001, I^2=97%), and we could not solve the heterogeneity.

4.2 Hysterectomy and myomectomy data

Six studies (18,19,21,23,25,28) with analyzable data reported this outcome, with 485 myomectomy or hysterectomy patients; 436 patients underwent contained power morcellation, and 49 patients underwent uncontained power morcellation. The pooled data showed an increase in total blood loss in the contained power morcellation group [MD=119.62, 95% CI (85.28, 153.96)] compared to the uncontained power morcellation group [MD=116.10, 95% CI (62.14, 170.06)]. The test for subgroup difference showed no difference between the two groups (p=0.91) (Figure 5). The data for the contained power morcellation subgroup was heterogeneous (p<0.00001, I^2=85%), and we could not solve the heterogeneity, while heterogeneity did not apply to the uncontained power morcellation subgroup because it represented a single study arm.

5. Duration of Hospital Stay (Days)

5.1 Myomectomy-only data

Five studies[10,20,22,27,29] with analyzable data reported this outcome, with 175 myomectomy patients; 113 patients underwent contained power morcellation, and 62 patients underwent uncontained manual morcellation. The pooled data showed an increase in the duration of hospital stay in the contained power morcellation [MD=1.86, 95% CI (1.17, 2.54)] over the uncontained manual morcellation groups [MD=2.10, 95% CI (1.86, 2.35)]. Both groups had comparable hospital stay duration (p=0.5) (Figure 6). Pooled data were heterogeneous for contained power morcellation (p<0.00001, I^2=97%) and uncontained manual morcellation (p<0.12, I^2=58%), and we could not solve the heterogeneity.

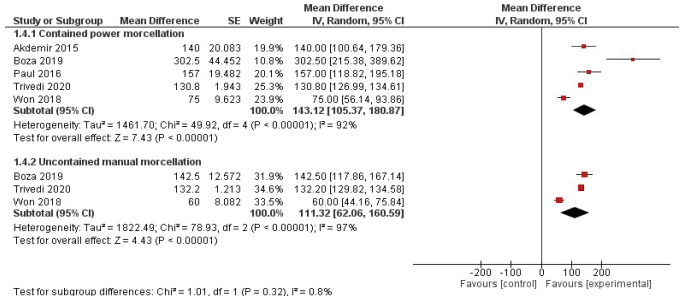


Figure 4. Forest plot of the total blood loss (mL) comparison between contained power morcellation versus uncontained manual morcellation myomectomy

CI: Confidence interval

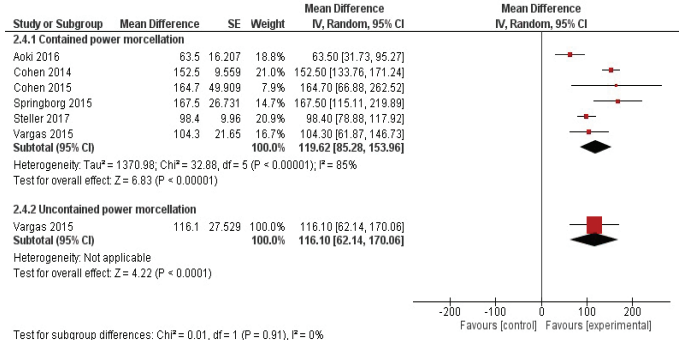
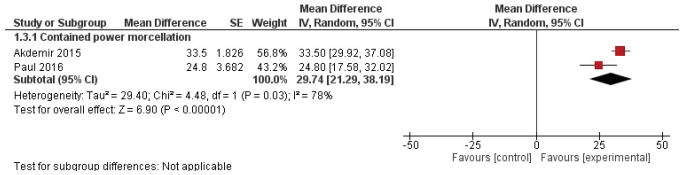


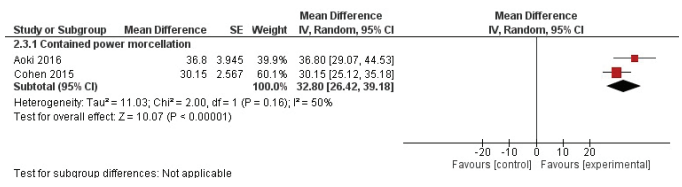
Figure 5. Forest plot of the total blood loss (mL) comparison between contained power morcellation versus uncontained power morcellation myomectomy or hysterectomy

CI: Confidence interval



Supplemental Figure S3. Forest plot of the total morcellation time (min.) for contained power morcellation myomectomy

CI: Confidence interval



Supplemental Figure S4. Forest plot of the total morcellation time (min.) for contained power morcellation hysterectomy or myomectomy

5.2 Hysterectomy and myomectomy data

Two studies[21,28] with analyzable data reported this outcome, with 94 myomectomy or hysterectomy patients who underwent contained power morcellation. Pooled data showed an average hospital stay duration of 0.74 days, [95% CI (0.25, 1.23)] (Figure 7). Pooled data were also heterogeneous (p<0.00001, I^2=95%), and this could not be solved.

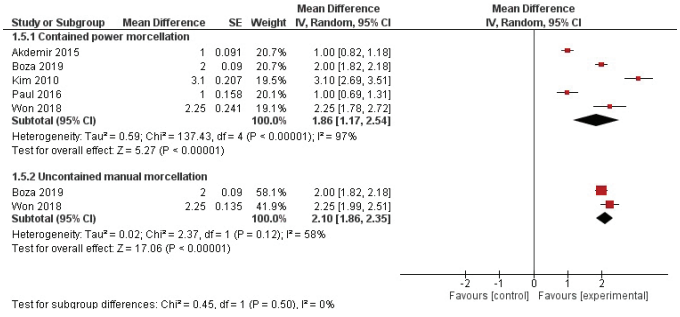


Figure 6. Forest plot of the duration of hospital stay (days) comparison between contained power morcellation versus uncontained manual morcellation myomectomy

CI: Confidence interval

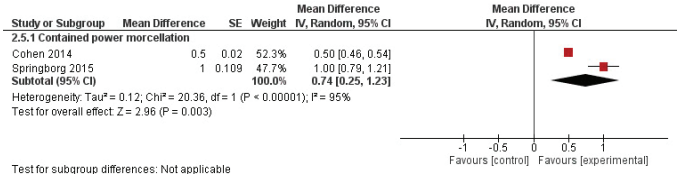


Figure 7. Forest plot of the duration of hospital stay (days) for contained power morcellation myomectomy or hysterectomy

CI: Confidence interval

Discussion

Contained power morcellation and uncontained power morcellation for myomectomy and hysterectomy had similar total blood loss, but uncontained power morcellation had a shorter total operative time for both procedures. Contained power morcellation and uncontained manual morcellation myomectomy had similar total operation time, total blood loss, and hospital stay duration. These findings confirm that contained power morcellation is a feasible procedure, but likely does involve an increase in operation time.

In 1979, Semm[30] reported the first laparoscopic myomectomy (LM), which aimed to minimize complications due to abdominal myomectomy or hysterectomy. Laparoscopy enabled surgeons to remove multiple myomas during one procedure[31,32]. Then morcellation emerged to overcome the limitations related to myoma size, making it possible to extract myomas reaching 20 cm or greater laparoscopically[33]. Morcellation approaches varied over time and several studies investigated different techniques, including *in situ* as well as incisional morcellation techniques[34-38].

A retrospective cohort study compared perioperative outcomes associated with electronic power morcellation (PM), manual vaginal morcellation, and manual morcellation via ML in 297 cases, including 137 myomectomies[7]. They reported no significant differences between the techniques of the three morcellation methods, but the operative time was longer with the ML. Therefore, the three techniques are considered feasible options for LM.

The FDA statement largely precludes morcellation that is both uncontained and uses a power source, but it does not specifically discourage the use of techniques that exclude one or the other[8]. Therefore, there are confounding views as to whether PM should be used with containment or rather simply replaced with a manual method. A recent review concluded that different factors could correlate with sarcoma dissemination and also recommended PM use only in premenopausal women undergoing myomectomy, only after an endometrial biopsy was obtained[39]. A meta-analysis of 176 premenopausal women who underwent LM compared intraoperative and postoperative outcomes between in-bag PM and uncontained manual morcellation but no significant difference was found between the methods due to the low-quality evidence used[40]. Although PM is often criticized for possible injuries, it is worth mentioning that no intraoperative complications were reported in most of our included studies[21-23,25-27,29]. Also, the advantages of PM regarding cosmetic effect[20] and easier management of large myoma[23] are worth noting. Only two of the included studies reported no intraoperative complications with manual morcellation[22,26]. Three intraoperative complications were reported in the included studies for contained[18,19] and uncontained PM myomectomy or hysterectomy[19].

Given that PM is discouraged without a containment system, different techniques are emerging to cope with available resources in low-income countries. For example, Akdemir et al.[27] described the feasibility of using a surgical glove instead of an expensive bag for laparoscopic morcellation in Turkey.

Strengths

Our study is the first meta-analysis to compare contained PM with uncontained manual morcellation. We extracted all analyzable data and included all available observational studies with variations in sample sizes. The Cochrane handbook and PRISMA guidelines were followed throughout this manuscript. According to quality assessment tools, most included studies were of moderate quality.

Study Limitations

These include the observational design of included studies, the high heterogeneity that could not be solved in most outcomes, and the small sample size of some included studies. As a result, we were forced to perform an indirect analysis due to insufficient data for direct comparison. In addition, variations in surgical techniques, which could vary from surgeon to surgeon if not surgery to surgery, could introduce some error into our

analysis. This could include the injection of vasoconstricting agents, or differences in tissue closure techniques. This would likely have the largest effect on the surgeon's operating time.

There is a critical need for more well-designed randomized controlled trials with larger samples and more accurate measurements in order to determine the efficacy and safety of contained PM, in relation to various perioperative and postoperative outcomes. It would be particularly useful if future studies could investigate novel techniques used to practice contained morcellation and manage large myomas, and could include comparisons of intraoperative medications, such as vasopressin, which has the potential to significantly decrease blood loss and operative times.

Conclusion

Contained PM myomectomy has similar total operation time, total blood loss, and hospital stay duration compared with uncontained manual morcellation myomectomy. Also, it has similar total blood loss as uncontained PM for myomectomy or hysterectomy. However, contained PM seems to have a longer total operation time than uncontained PM.

Ethics

Peer-review: Internally peer-reviewed.

Authorship Contributions

Concept: A.K., G.M., A.M., A.C., G.B., K.S., Data Collection or Processing: A.C., G.B., H.U., A.A., S.G., J.P., C.C., Analysis or Interpretation: A.M., Tables: A.C., Figures: A.A., H.U., Supervision: S.R.

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Supplementary Table S1. Risk of bias assessment of the included cohort studies

ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Score
Akdemir, 2015[27]	Yes	Yes	Yes	Yes	No	Yes	No	No	Yes	No	Yes	No	No	No	11
Cohen, 2015[18]	Yes	Yes	Yes	Yes	No	Yes	No	Yes	Yes	No	Yes	No	No	No	10
Cohen, 2014[28]	Yes	Yes	Yes	Yes	No	Yes	No	Yes	Yes	No	Yes	No	No	No	11
Kim, 2010[20]	Yes	Yes	Yes	Yes	No	Yes	No	No	Yes	No	Yes	No	No	No	10.5
Springborg, 2015[21]	Yes	Yes	Yes	Yes	No	Yes	No	Yes	Yes	No	Yes	No	No	No	11
Vargas, 2015[19]	Yes	Yes	Yes	Yes	No	Yes	No	Yes	Yes	No	Yes	No	No	No	11.5
Won, 2018[22]	Yes	Yes	Yes	Yes	No	Yes	No	No	Yes	No	Yes	No	No	No	11.5
Steller, 2017[23]	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	No	Yes	No	No	No	11.5
Boza, 2019[10]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	No	Yes	No	No	No	11.5
Aoki, 2016[25]	Yes	Yes	Yes	Yes	No	Yes	No	Yes	Yes	No	Yes	No	No	No	10.5
Trivedi, 2019[26]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	No	No	No	11.5

1: Was the research question or objective in this paper clearly stated?
2: Was the study population clearly specified and defined?
3: Was the participation rate of eligible persons at least 50%?
4: Were all the subjects selected or recruited from the same or similar populations (including the same time period)? Were inclusion and exclusion criteria for being in the study prespecified and applied uniformly to all participants?
5: Was a sample size justification, power description, or variance and effect estimates provided?
6: For the analyses in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured?
7: Was the time frame sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed?
8: For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (e.g., categories of exposure, or exposure measured as continuous variable)?
9: Were the exposure measures (independent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?
10: Was the exposure(s) assessed more than once over time?
11: Were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?
12: Were the outcome assessors blinded to the exposure status of participants?
13: Was loss to follow-up after baseline 20% or less?
14: Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)?

Supplementary Table S2. Risk of bias assessment of the included case series study

ID	1	2	3	4	5	6	7	8	9	Score
Paul, 2015[29]	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	8

1. Was the study question or objective clearly stated?
2. Was the study population clearly and fully described, including a case definition?
3. Were the cases consecutive?
4. Were the subjects comparable?
5. Was the intervention clearly described?
6. Were the outcome measures clearly defined, valid, reliable, and implemented consistently across all study participants?
7. Was the length of follow-up adequate?
8. Were the statistical methods well-described?
9. Were the results well-described?

Review / Derleme

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Effect of single- and double-layer cesarean section closure on residual myometrial thickness and isthmocele - a systematic review and meta-analysis

Tek ve çift katmanlı sezaryen kapatmanın rezidüel miyometrial kalınlık ve istmosel üzerine etkisi - sistematik bir gözden geçirme ve meta-analiz

Greg J Marchand[1], Ahmed Masoud[2], Alexa King[3], Stacy Ruther[1], Giovanna Brazil[1], Hollie Ulibarri[1], Julia Parise[1], Amanda Arroyo[1], Catherine Coriell[1], Sydnee Goetz[1], Ashley Christensen[4], Katelyn Sainz[1]

[1]*Marchand Institute for Minimally Invasive Surgery, Mesa, Arizona, USA*

[2]*Fayoum University Faculty of Medicine, Fayoum, Egypt*

[3]*International University of Health Sciences, Basseterre, St. Kitts*

[4]*Midwestern University College of Osteopathic Medicine, Glendale, Arizona, USA*

Abstract

Objective: To determine the incidence of isthmocele, its effect on residual myometrial thickness (RMT), and other complications of Cesarean delivery (CD) in relation to single- and double-layer CD closure. We searched PubMed, SCOPUS, Web of Science, ClinicalTrials.gov, MEDLINE and Cochrane Library for relevant clinical trials assessing the use of single- and double-layer uterine closure in patients undergoing cesarean sections from inception through to March 2021.

Materials and Methods: Our population was women undergoing cesarean section with uterine closure by any double-layer method, compared with those undergoing uterine closure through a single-layer method. RMT (in mm) was measured at 6 weeks, niche/isthmocele existence at 6 weeks, RMT (in mm) at 6-24 months and niche/isthmocele existence at 6-24 months. In order to present the highest quality evidence, we only included clinical trials in our analysis. To perform this review, we reported dichotomous outcomes using percent and total, while continuous outcomes were reported using mean ± standard deviations, and relative 95% confidence intervals using the inverse variance method.

Results: We found that the RMT in the double-layer closure group was significantly higher at six weeks [mean difference (MD)=-0.43 (-0.77, -0.09)], (p=0.01) and at 6-24 months of follow-up [MD=-1.27 (-2.28, -0.25)], (p=0.01). The incidence of isthmocele in the two groups, as well as the other investigated outcomes were similar across the different groups.

Conclusion: High-quality evidence shows that double-layer closure results in a higher RMT compared with a single-layer closure, despite no significant difference in isthmocele formation.

Keywords: Cesarean section closure, single-layer closure, double-layer closure, isthmocele cesarean section, cesarean scar defects

Öz

Amaç: Tek ve çift katmanlı sezaryen kapatma ile ilişkili olarak istmosel insidansının ve istmoselin rezidüel miyometrial kalınlık (RMT) ve diğer sezaryen komplikasyonları üzerindeki etkisinin değerlendirilmesi amaçlanmıştır. PubMed, SCOPUS, Web of Science, ClinicalTrials.gov, MEDLINE ve Cochrane Library'de, sezaryen ameliyatı geçiren hastalarda tek ve çift katmanlı uterus kapatmanın kullanımını değerlendiren klinik araştırmalar için bunların kullanılmaya başlanmasından Mart 2021'e kadarki süreçte arama yaptık.

Gereç ve Yöntemler: Bu çalışmada; sezaryen uygulanan ve herhangi bir tek katmanlı yöntemle uterusu kapatılan kadınlarla herhangi bir çift katmanlı yöntemle uterusu kapatılan kadınlar kıyaslandı. Ölçtüğümüz sonlanımlar arasında; 6. haftadaki rezidüel miyometrium kalınlığı (mm olarak), 6. haftadaki niş/istmosel varlığı, 6-24 aydaki rezidüel myometrium kalınlığı (mm olarak) ve 6-24 aydaki niş/istmosel varlığı yer almaktaydı. En yüksek kalitede kanıt sunmak için analizimize yalnızca klinik çalışmaları dahil ettik. Bu incelemeyi gerçekleştirmek için yüzde ve toplam kullanarak ikili sonlanımları analiz ettik. Sürekli sonlanımlar ise ters varyans yöntemi ile ortalama fark, standart sapma ve göreceli %95 güven aralıkları kullanılarak değerlendirildi.

Address for Correspondence/Yazışma Adresi: Greg J Marchand MD, Marchand Institute for Minimally Invasive Surgery, Mesa, Arizona, USA
Phone: +4809990905 **E-mail:** gm@marchandinstitute.org **ORCID ID:** orcid.org/0000-0003-4724-9148
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Bulgular: Çift katmanlı kapama grubundaki RMT'nin 6. haftada [MD=-0,43 (-0,77, -0,09)], (p=0,01) ve 6 ila 24 aylık takipte anlamlı olarak daha yüksek olduğunu bulduk (MD=-1,27 [-2,28, -0,25]), (p=0,01). İstmosel insidansı ve araştırılan diğer sonlanımların herhangi biri açısından iki grup arasında hiçbir fark görülmedi.
Sonuç: Yüksek kaliteli kanıtlar, çift katmanlı kapatmanın, tek katmanlı kapatmaya kıyasla daha yüksek RMT ile sonuçlandığını gösterirken, istmosel oluşumu açısından anlamlı bir fark yok gibi görünmektedir.
Anahtar Kelimeler: Sezaryen kapatma, tek katman kapatma, çift katman kapatma, isthmosel, sezaryen, sezaryen skar defektleri

Introduction

Cesarean delivery (CD) accounts for 38% of total deliveries worldwide, with an expected increase in the future[1,2]. Although CD can often be an unavoidably life-saving option for neonates, it is known to cause a variety of short- and long-term complications[3,4]. The short-term complications include abnormal uterine bleeding, pain, infection, and thromboembolic complications. Long-term complications include complicated future pregnancies, including the risk of uterine scar dehiscence and rupture, pathology involving placental adherence to the scar (accreta and percreta), and incidence of ectopic pregnancy within the scar[3-5]. Several authors have recently investigated the connection of two specific complications of CD, namely isthmocele formation and a reduced residual myometrial thickness (RMT) in the area of the uterine scar, and their relationship with serious complications such as uterine scar dehiscence and uterine rupture in future pregnancies. Isthmocele formation has also been associated with pelvic pain and abnormal uterine bleeding in the non-pregnant state[5].

The "isthmocele," was first described by Hugh Morris in 1995[6], and refers to the scar due to a CD as visualized on a sagittal plane ultrasound. The isthmocele is often referred to as a "niche" because of the predictably triangular shape of the defect in the uterine myometrium, resembling a pouch on the anterior wall of the uterine isthmus[7]. This finding is a result of myometrial discontinuation or thinning at the site of the previous incision[8]. At time of ultrasonography, an isthmocele appears as a triangular anechoic area at the site of the incision and may best be visualized by saline contrast hysterosonography[7,9].

Several authors have attempted to classify the severity of an isthmocele. Many have done so by measuring the reduction in wall thickness or according to the residual (or remaining) myometrial thickness (RMT) at the site of the scar. Authors have also postulated that measurements of the RMT may have predictive value in regards to the risk of uterine rupture during delivery in patients with previous CD[10,11]. This postulation holds that a lower RMT may indicate a weaker uterine scar, and thus a higher likelihood of uterine rupture or dehiscence with subsequent pregnancies[11,12].

There are no clear findings as to how often CD results in the formation of an isthmocele, nor which CD closures are most at risk for this phenomenon[9]. It is possible that this incidence would depend largely on the method used to assess uterine thickness[10]. Despite this, most authors agree that the prevalence of isthmocele is on the rise[11-13]. Furthermore, several authors have linked risk factors to its occurrence[3]. These include multiple CDs as the major risk factor, duration of labor (prior to CD) and the position of the incision (lower uterine segment or contractile portion) on the uterus[14].

The incidence of isthmocele is becoming a serious issue and many authors have suggested an increased incidence of serious complications of pregnancy following the development of an isthmocele[12,15]. In addition to uterine rupture, authors have described an isthmocele as being related to the development of placenta previa, accreta, scar dehiscence, and ectopic pregnancy[12,15,16].

Recently, many authors have postulated that the closure technique at the time of CD play a key role in the development of an isthmocele[17-19]. The assumption is that different techniques may affect the healing of the scar and result in different RMT values. These include techniques resulting in the physical approximation of less tissue, as well as in irregularities in the closure, leading to the development of the isthmocele[19]. As closure techniques vary from institute to institute as well as from surgeon to surgeon, there is no consensus of the superiority of one technique over others. Several authors, however, demonstrated that a single-layer closure may result in a higher incidence of isthmocele formation, when compared with double-layer techniques[19,20].

This lack of consensus has led us to focus on the comparison between different closure techniques in the formation of the isthmocele and its possible pathologic sequelae. Thus, we sought to investigate this phenomenon, with the possibility that high-quality evidence may exist to aid in the decision of which CD closure method may be able to prevent the incidence of isthmocele, and possible sequelae.

In this systematic review and meta-analysis, we aimed at assessing the correlation between sonographic characteristics of an isthmocele (especially RMT) and the incidence of maternal complications, especially uterine rupture. We further sought to analyze if the choice of CD closure technique (specifically single- or double-layer closure) affects the formation of isthmocele and the possible maternal complications.

Methods

This meta-analysis was performed following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)[21] and the guidelines reported in the Cochrane Handbook for Systematic Reviews of Interventions[22].

Literature Search

We searched six databases: Web of Science, SCOPUS, Cochrane CENTRAL, ClinicalTrials.Gov, MEDLINE and PubMed, from

inception until March 2021. We adopted the following search strategy with no restrictions on date of publication or language: [(double-layer far-far-near-near) OR (FFNN) OR (single-layer continuous locked) OR (SLL) OR (continuous single-layer unlocked) OR (continuous locked single-layer) OR (double-layer sutures)] AND [(isthmocele) OR (cesarean scar defect) OR (uterine scar deficiency) OR (uterine niche) OR (uterine pouch) OR (cesarean)].

Eligibility Criteria

We included all the studies that met the following criteria: **(i) Population:** women undergoing cesarean section, **(ii) Intervention:** uterine closure by any double-layer closure, **(iii) comparator:** uterine closure by any single-layer closure, **(iv) Outcomes:** the primary outcome was RMT (in mm) at 6 weeks, niche/isthmocele existence at 6 weeks, RMT (in mm) at 6-24 months and niche/isthmocele existence at 6-24 months. Other outcomes included the number of patients needing additional sutures, estimated number of additional suture throws required, blood loss (mL), change in hemoglobin or hematocrit level, postoperative hemoglobin or hematocrit value, maternal infectious morbidity, postpartum fever, number of patients needing a blood transfusion, and the incidence of postoperative endometritis. **(v) Study design:** we included only clinical trials. Our exclusion criteria were: (1) uncontrolled clinical trials, (2) studies that did not report data or measures for our selected outcomes, or (3) studies with no available full text.

Screening of Results

We exported the results of the search using Endnote X8.0.1 (Build 1044), with the removal of duplicates performed automatically by the software. After that we screened the studies manually in two steps, title and abstract screening followed by a full text screening.

Data extraction and Analysis

After screening, we extracted the data from the selected studies and categorized it into three main groups:

1) Baseline and demographic data of patients in each study, including age (in years), incidence of nulliparity, gestational age at CD (in weeks), BMI (in kg/m^2), preterm delivery, prior cesarean deliveries and operative time (in minutes).

2) Data for analysis including outcome values of RMT (in mm) at 6 weeks, niche/isthmocele existence at 6 weeks, RMT (in mm) at 6-24 months, niche prevalence at 6-24 months, number of patients needing additional sutures, estimated number of additional suture throws required, blood loss (mL), change of hemoglobin level, hematocrit value, maternal infectious morbidity, postpartum fever, number of patients needing a blood transfusion and incidence of postoperative endometritis. In addition to the previous two categories, we extracted the data required to assess the risk of bias using the seven domains according to Cochrane's risk of bias tools[23].

Data Analysis

We used Review Manager Software (RevMan 5.4.1) to analyze the data. We analyzed dichotomous outcomes using percent and total, while continuous outcomes were displayed through the mean difference (MD), standard deviations (SD), and relative 95% confidence intervals using the inverse variance method. The two tests used to measure inconsistency among the studies were the I-squared test (I^2) and the p-value of chi-square test. In accordance with recommendations from the Cochrane Handbook, outcomes with I^2>50%, p<0.1 were considered heterogeneous, while outcomes with I^2<50%, p>0.1 were considered homogeneous[23]. Homogenous data were analyzed using a fixed-effect model, while heterogeneous outcomes were analyzed using the random-effect model.

Quality Assessment

We evaluated the quality of this systematic review and meta-analysis using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) guidelines. According to the Cochrane risk of bias (ROB) tool for clinical trials, we performed the ROB assessment for all included studies according to the following categories: 1) proper randomization, 2) blind allocation of the included patients into each group, 3) blinding of patients only (single-blinding), blinding of both personnel and participants (double-blinding), or a complete lack of blinding, 4) Attrition bias, 5) Selection bias 6) Assessor's awareness of the outcome (blinded or not), 7) Other bias. Using these categories, we also assessed the total ROB for all included studies using the same tool.

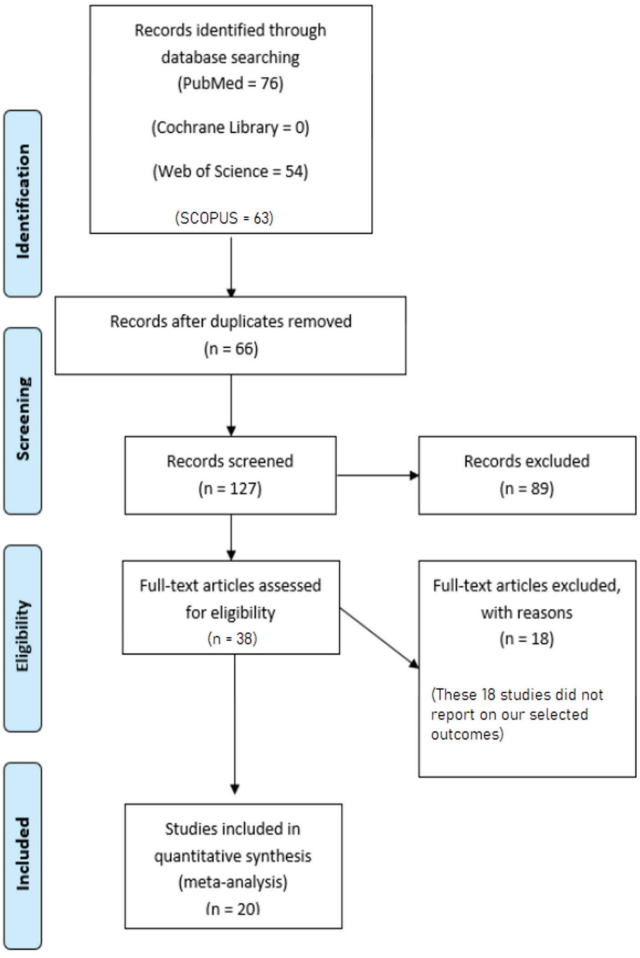
Results

Summary of Included Studies

Supplementary Figure S1 shows a PRISMA flow diagram of our literature search. In our study, we performed an analysis of 8799 patients from twenty studies[18,23-42]. A total of 4406 patients experienced a single-layer closure for their cesarean section, and 4393 patients experience a double-layer closure. The mean age of the single-layer closure group was 29.1±4.7 years, while that of the double-layer closure group was 29.09±5.05 years. Table 1 show a detailed summary of the included participants, including their demographic data, incidence of nulliparity, gestational age at CD (in weeks), BMI (in kg/m^2), incidence of preterm delivery, number of prior CD, and incidence of multiple births.

Results of Risk of Bias Assessment

The result of the ROB assessments yielded an overall low ROB, according to Cochrane's tool. Following randomization, all studies were at low risk of randomization, except for Hayakawa et al.[18], whose is trial was not randomized. As for the allocation concealment, all studies reported adequate allocation concealment; therefore, they were judged as a low ROB, except Hayakawa et al.[18] and Batioğlu et al.[30], which

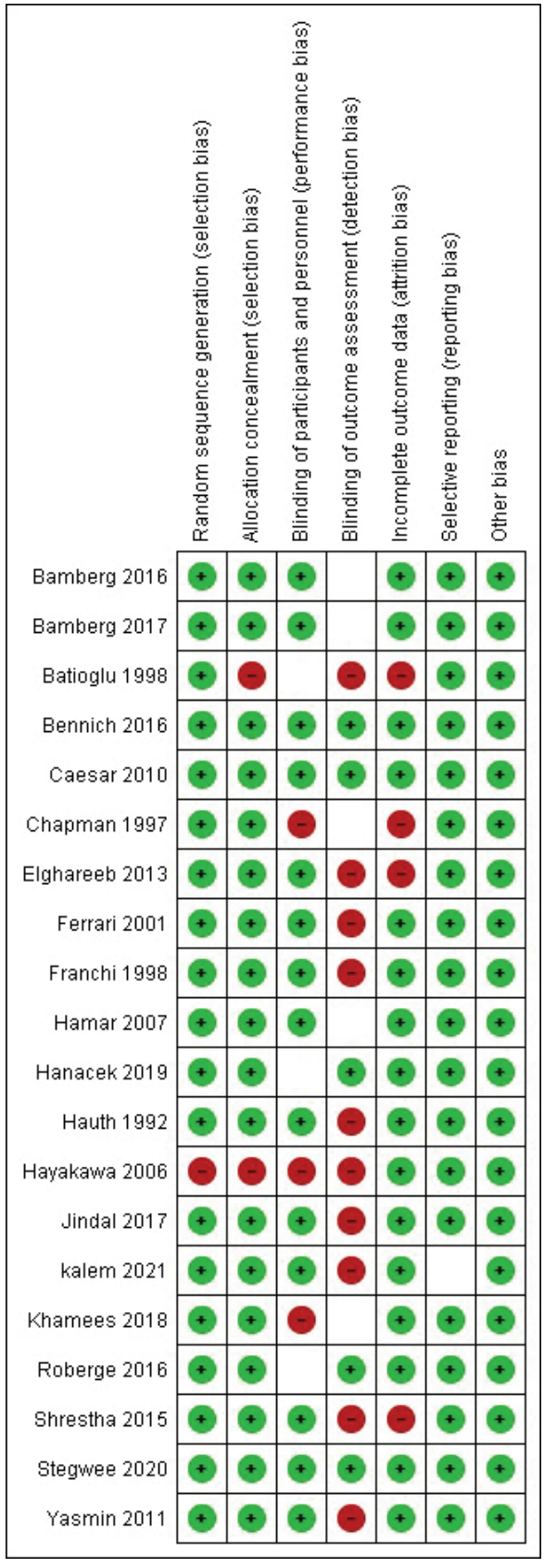


Supplementary Figure S1. The PRISMA flow diagram of our literature search

reported inadequate allocation concealment. The majority of the included studies were blinded, with the exception of three studies[27,30,36] that did not report enough data about blinding of the participants and personnel. As a result, these three studies were judged to be at an unclear ROB, and three additional studies[18,31,35] were not blinded at all. All studies were at high ROB with regards to the blinding of the assessors with the exception of five studies[34,33,35,40,41] that had insufficient data, and five studies[27,29,36,39,42] that were judged to be low risk. The remaining domains of the Cochrane tool were all at low ROB, except four studies[30-33,37] found to be at high risk of attrition bias, and one study[28] that showed unclear data in the category of selective reporting. A summarized illustration of the risk of the assessed bias of the included trials is found in Supplementary Figures S2A and S2B. Supplementary Table S1 shows the detailed ROB assessment.

Patients Needing Additional Suturing:

Seven studies demonstrated patients requiring additional suturing as an outcome[33,36,37,39,41-43,45] and showed that there was no significant difference between the two groups [RR=1.02



Supplementary Figure S2B. Results of our assessment of bias of the included studies

Table 1. Detailed summary of the included participants and their demographic data

Study ID	Sample size, n		Age (years), mean (SD)		Nulliparity, n (%)		Gestational age at cesarean (weeks), mean (SD)	
	Single	Double	Single	Double	Single	Double	Single	Double
Jindal 2016	27	27	30.8 (4)	31.1 (6.4)	22 (80.8)	20 (74.1)	39.2 (0.6)	39.1 (0.5)
Jindal 2017	157	129	31.9 (5.7)	30.3 (6.5)	59 (37)	48 (37)	37.6 (2.4)	37.3 (2.3)
Bennich 2016	35	38	30.3 (4.5)	30.5 (5.5)	nr	nr	38.7 (0.6)	38.9 (0.7)
Jindal 2001	83	75	31.7 (4.8)	30.7 (4.8)	nr	nr	38.3 (1.54)	38.2 (2.1)
Franchi 1998	149	150	29.5 (5.1)	30.6 (4.7)	83 (55.7)	77 (51.3)	36.7 (2.8)	37 (2.4)
Hanacek 2019	149	175	31.3 (3.7)	31.7 (3.7)	nr	nr	40 (1.5)	40.3 (0.7)
Hayakawa 2006	50	51	31.1 (5)	31.4 (5.5)	nr	nr	36.9 (2.6)	36.6 (3.1)
Kalem 2021	68	70	29.25 (6.27)	28.94 (5.17)	nr	nr	38.50 (2.7)	39.40 (3.6)
Caesar 2010	1438	1496	30.6 (5.9)	30.6 (5.9)	989 (67)	1027 (69)	39 (2)	39.1 (1.9)
Jindal 2011	30	30	nr	nr	nr	nr	nr	nr
Jindal 2016	157	129	31.9 (5.7)	30.3 (6.5)	59 (37)	48 (37)	37.6 (2.4)	37.3 (2.3)
Stegwee 2020	1144	1148	32 (4.7)	32.1 (4.6)	764 (76.3)	764 (76.2)	38.9 (1.3)	38.9 (1.3)
Khamees 2018	26	12	nr	nr	nr	nr	nr	nr
Jindal 2017	188	169	23.2	24.5	nr	nr	38.1 (1.5)	37.8 (1.8)
Shrestha 2015	25	25	26.04 (5.06)	23.92 (4.32)	21 (84)	17 (68)	38.36 (2.21)	38.92 (1.35)
Elghareeb 2013	75	75	28.84 (3.4)	28.36 (3.2)	nr	nr	39.11 (0.7)	39.16 (0.7)
Batioglu 1998	63	55	28 (4)	30 (4.2)	nr	nr	40 (1.2)	39 (1.3)
Hamar 2007	15	15	30 (7)	25 (7)	11 (73)	8 (53)	39.3 (0.5)	38.6 (0.9)
Chapman 1997	70	75	24	nr	nr	nr	39 (3.7)	37 (5.2)
Hauth 1992	457	449	24.2	24.6	nr	nr	38	37.8

Study ID	BMI, kg/m^2, mean (SD)		Preterm delivery, n (%)		Prior cesarean deliveries, n (%)		Operative time (minutes)	
	Single	Double	Single	Double	Single	Double	Single	Double
Jindal 2016	25.1 (4.7)	23.5 (3.9)	nr	nr	nr	nr	25.1 (4.7)	23.5 (3.9)
Jindal 2017	24.5 (4.9)	25.6 (6.2)	38 (24)	25 (19)	66 (42)	57 (44)	nr	nr
Bennich 2016	24.6 (4.8)	24.1 (3.5)	nr	nr	nr	nr	23.7 (4.7)	25.3 (4.2)
Jindal 2001	22.81 (4)	21.85 (4)	nr	nr	nr	nr	31.6 (1.38)	44.4 (1.44)
Franchi 1998	Nr	nr	57 (38.3)	48 (32)	23 (15.4)	18 (12)	33.75 (13.75)	52.5 (22.5)
Hanacek 2019	22.7 (3.6)	22.2 (3)	nr	nr	nr	nr	nr	nr
Hayakawa 2006	26.2 (3.9)	26.2 (3.9)	nr	nr	nr	nr	nr	nr
Kalem 2021	26.04 (2.37)	25.90 (2.28)	nr	nr	nr	nr	36.91 (6.2)	35.71 (7.7)
Caesar 2010	Nr	nr	nr	nr	nr	nr	36.2 (11.6)	38.3 (11.8)
Jindal 2011	Nr	nr	nr	nr	nr	nr	40.06 (2.98)	41.07 (3.8)
Jindal 2016	24.5 (4.9)	25.6 (6.2)	38 (24)	25 (19)	66 (42)	57 (44)	35.8	36.1 (10)
Stegwee 2020	26.4 (4.6)	26.6 (4.8)	133 (13.2)	142 (14)	nr	nr	38.9 (11.7)	42.8 (11.2)
Khamees 2018	Nr	nr	nr	nr	nr	nr	33.2 (3.1)	37.8 (3.4)
Jindal 2017	Nr	nr	nr	nr	nr	nr	51.4 (6.3)	52.6 (4.5)
Shrestha 2015	Nr	nr	nr	nr	nr	nr	nr	nr
Elghareeb 2013	Nr	nr	nr	nr	nr	nr	43.86 (7.1)	47.7 (5.9)
Batioglu 1998	Nr	nr	nr	nr	7 (11.1)	6 (10.9)	36.54 (16.15)	39 (17.3)
Hamar 2007	Nr	nr	nr	nr	nr	nr	55 (15)	58 (12)
Chapman 1997	Nr	nr	9 (14)	19 (25)	nr	nr	nr	nr
Hauth 1992	Nr	nr	139 (30)	137 (30)	126 (28)	99 (22)	43.8	47.5

BMI: Body mass index, Kg: Kilograms, M: Meters, NR: Not reported

(0.95, 1.11)], (p=0.58). The pooled analysis was homogeneous (p=0.16); I^2=36%, as seen in Figure 1.

Number of Additional Suture Throws Required:

Only three studies[28,39,42] reported the number of additional suture throws required as an outcome. Their overall MD was similar across the two groups [MD= -0.77 (-2.45, 0.91)], (p=0.37). The pooled analysis was heterogeneous (p=0.01); I^2=99% as seen in Figure 2. We resolved the heterogeneity by the exclusion of one of the studies (Ferraria et al.)[25] (p=0.7); I^2=0%. The pooled analysis after this exclusion showed no significant difference between the two groups [MD=0.00 (-0.06, 0.06)], (p=0.9) also seen in Figure 2.

Blood Loss (in mL):

Ten studies[21,27,28,36,38,39,41-43,45] reported blood loss outcomes. Their overall MD was similar across the two groups [MD= -12.56 (-47.06, 21.94)], (p=0.48). The pooled analysis was heterogeneous (p=0.01; I^2=84%) as seen in Figure 3. We could not resolve the heterogeneity by subgroup analysis or the "leave-one-out" method.

Change in Hemoglobin Level:

Three studies[28,29,31] reported data on the change in hemoglobin level. Their overall MD was similar across the two groups [MD=0.03 (-0.11, 0.17)], (p=0.65). The pooled analysis was homogeneous (p=0.42); I^2=0%, as seen in Figure 4.

Hematocrit:

Three studies[28,33,36] reported the postoperative hematocrit level as an outcome. Their overall mean difference was similar between the two groups [MD= -0.07 (-0.98, 0.85)], (p=0.89).

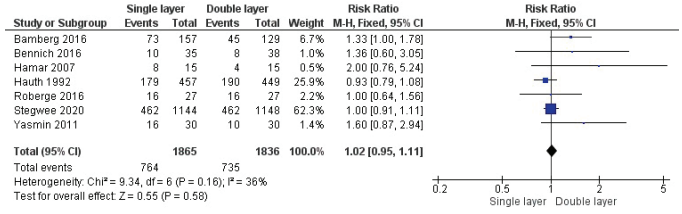


Figure 1. Analysis of the outcome of patients needing additional suturing

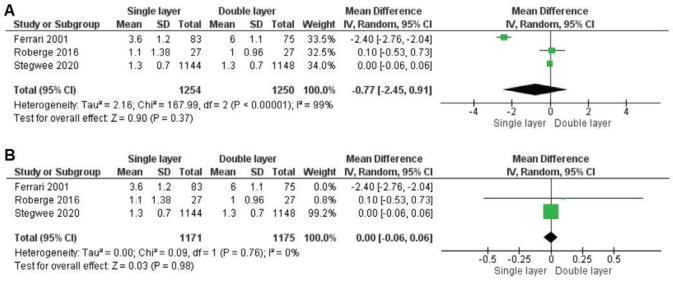


Figure 2A. Analysis of the outcome of the number of additional suture throws required. **2B.** Analysis of the outcome of the number of additional suture throws required, but after excluding one study to solve heterogeneity

The pooled analysis was homogeneous (p= 0.98); I^2=0%, as seen in Figure 5.

Maternal Infectious Morbidity:

Maternal infectious morbidity was reported as an outcome by four studies[27,32,34,36]. The overall risk ratio showed that there was no significant difference in maternal infectious morbidity between the two groups [RR=1.00 [0.86, 1.16)], (p=0.96). The pooled analysis was homogeneous (p=0.5); I^2=0%, as seen in Supplementary Figure S3.

Postpartum Fever:

Postpartum fever was reported as an outcome by seven studies[21,27-29,32,33,43]. The overall risk ratio showed no significant difference in postpartum fever between the two groups [RR= 0.77 (0.54, 1.08)], (p=0.13). The pooled analysis was homogeneous (p=0.31); I^2=15%, as seen in Supplementary Figure S4.

Number of Patients Requiring Transfusion:

Six studies[27,29,32,34,37,43] reported the number of patients requiring blood transfusion as an outcome. The overall risk ratio showed that there was no significant difference in this outcome between the two groups [RR= 0.96 (0.69, 1.32)], (p=0.78). The pooled analysis was homogeneous (p=0.83); I^2=0%, as seen in Supplementary Figure S5.

Postpartum Endometritis:

Five studies[27,29,32-34,37,43] demonstrated the incidence of postoperative endometritis as an outcome. The overall risk ratio showed that this outcome was not significantly different between the two groups [RR=1.15 (0.93, 1.43)], (p=0.19). The pooled analysis was homogeneous (p=0.85); I^2=0%, as seen in supplementary Figure S6.

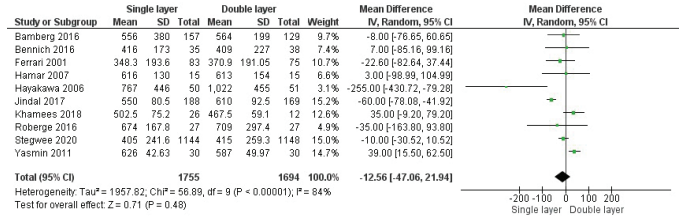


Figure 3. Analysis of the outcome of total blood loss

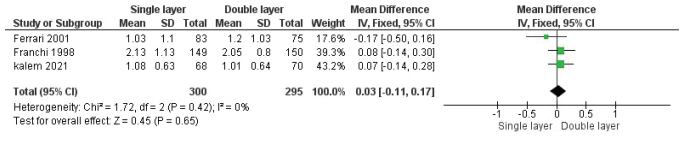


Figure 4. Analysis of the outcome of change of hemoglobin level

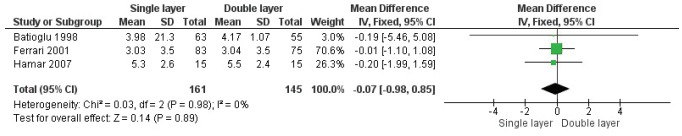
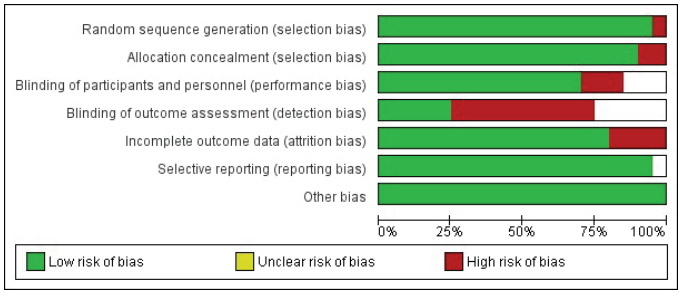


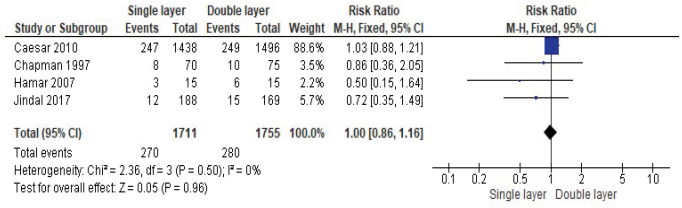
Figure 5. Analysis of the outcome of postoperative hematocrit

RMT (in mm) at 6 Weeks:

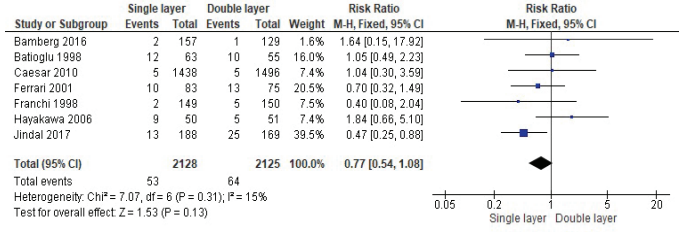
Nine studies[35,36,38-40,42-45] reported the RMT (in mm) at 6 weeks as an outcome. The overall mean difference showed that there was a significant difference in RMT between the two groups [MD= -0.71 (-1.31, -0.12)], (p=0.02). The pooled analysis was heterogeneous (p=0.01; I^2=79%) as seen in supplementary Figure S7A. We resolved the heterogeneity by excluding one study [El-Gharib et al.][32] (p=0.17); I^2=32%. The pooled analysis after the exclusion still showed a significant difference in RMT between the two groups [MD=-0.43 (-0.77, -0.09)], (p=0.01) as seen in Supplementary Figure S7B. The RMT (in mm) at 6 weeks of the single-layer closure group was significantly less than that in the double-layer closure group.



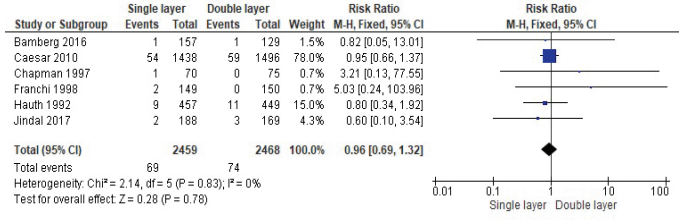
Supplementary Figure S2A. Graphical representation of the risk of bias assessment



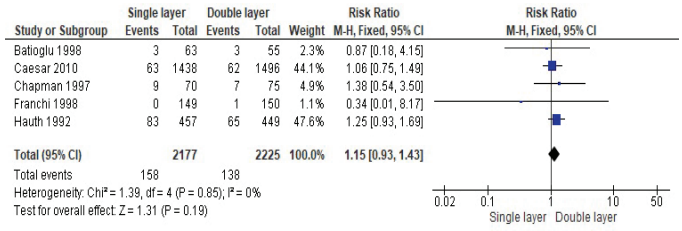
Supplementary Figure S3. The incidence of maternal infectious morbidity



Supplementary Figure S4. The incidence of postpartum fever



Supplementary Figure S5. Analysis of the number of patients needing blood transfusions



Supplementary Figure S6. The incidence of endometriosis

Incidence of Uterine Niche/Isthmocele at 6 Weeks:

Nine studies[21,27,30,34,38,41,42,44,45] reported the incidence of a uterine niche/isthmocele at 6 weeks as an outcome. The overall risk ratio showed that there was no significant difference in this outcome between the two groups [RR=1.00 (0.95, 1.05)], (p=0.93). The pooled analysis was homogeneous (p=0.15; I^2=34%) as seen in Supplementary Figure S8.

RMT (in mm) at 6-24 Months:

The RMT (in mm) at 6-24 months was reported as an outcome by five studies[30,31,43-45]. The overall MD showed a significant difference in RMT between the two groups [MD= -1.27 (-2.28, -0.25]), (p=0.01). The pooled analysis was heterogeneous (p=0.01; I^2=93%) as seen in Supplementary Figure S9. We could not solve the heterogeneity by subgroup analysis or the "leave-one-out" method. The RMT (in mm) at 6-24 months of the single-layer closure group was significantly less than the thickness of the double-layer closure group.

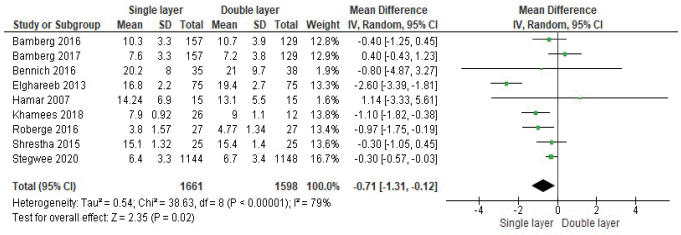
Incidence of Uterine Niche/Isthmocele at 6-24 Months:

The incidence of a uterine niche/Isthmocele at 6-24 months outcome was reported as an outcome by four studies[30,31,44,45]. The overall risk ratio showed no significant difference in this outcome between the two groups [RR=1.19 (0.89, 1.60)], (p=0.24). The pooled analysis was heterogeneous (p=0.01; I^2=88%) as seen in Supplementary Figure S10A. We resolved the heterogeneity by the exclusion of one study [Kalem et al.][28] (p=0.18); I^2=41%. The pooled analysis after the exclusion showed no significant difference between the two groups [RR= 1.07 (0.96, 1.19)], (p=0.23) as seen in Supplementary Figure S10B.

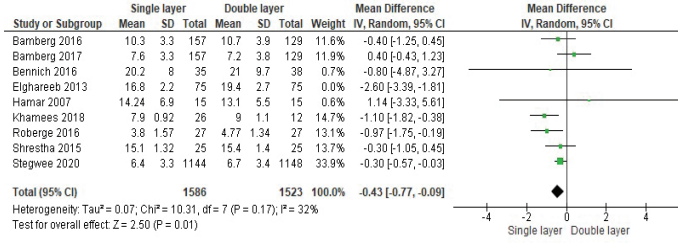
Discussion

In this meta-analysis, we included 8799 patients from 20 clinical trials. We found that the RMT in the double-layer closure group was significantly higher at 6 weeks follow-up and at 6-24 months follow-up compared to that in the single-layer group (p=0.01). Interestingly, there was also no significant difference in the incidence of uterine niche or isthmocele regardless of the closure used, at both postoperative 6 weeks and 6-24 months. There was also no significant difference between the two groups regarding the other measured outcomes: the need for additional suturing, the number of additional suture throws

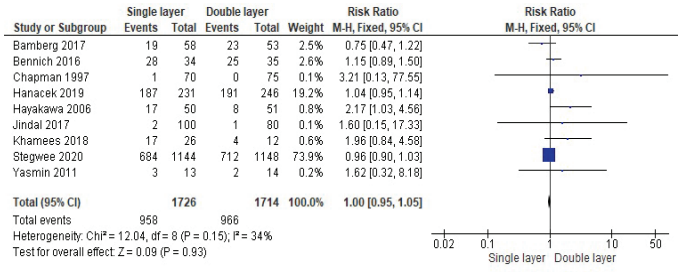
required, change in hemoglobin level, postoperative hematocrit level, maternal infectious mortality, postoperative endometritis, postpartum fever, or patients needing a blood transfusion.



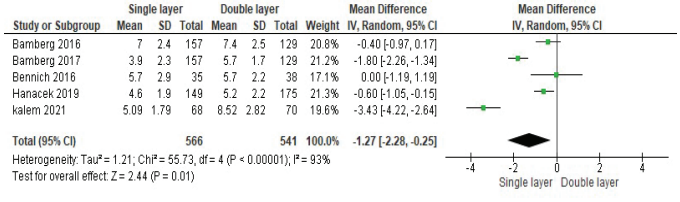
Supplementary Figure S7A. Analysis of residual myometrium thickness (mm) at 6 weeks



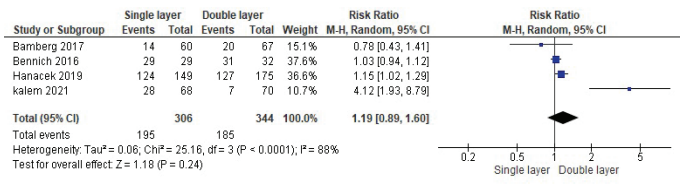
Supplementary Figure S7B. Analysis of residual myometrium thickness (mm) at 6 weeks outcome



Supplementary Figure S8. Analysis of niche/isthmocele prevalence at 6 weeks



Supplementary Figure S9. Analysis of residual myometrium thickness (mm) at 6-24 months



Supplementary Figure S10A. Analysis of niche prevalence at 6-24 months

Regarding recent meta-analyses on this topic, Stegwee et al.[39] found that double-layer closure of the uterus was superior to single-layer closure as far as RMT and overall healing is concerned in their 2017 analysis, which is consistent with our results. Their study also found an overall decreased RMT with single-layer sutures, and a higher incidence of dysmenorrhea in the single-layer closure group. As with our analysis, isthmocele prevalence was the same in groups, as was the incidence of uterine dehiscence or rupture. Stegwee's meta-analysis included observational studies and was not limited to RCTs, and therefore they were able to include longer term outcomes such as incidence of uterine rupture with future pregnancies.

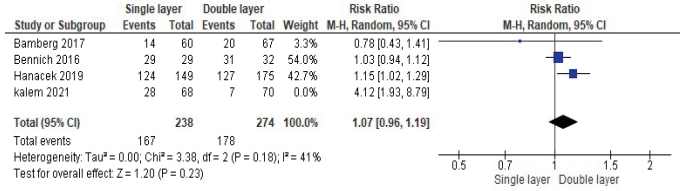
Moreover, in their multicenter double-blinded RCT, conducted by the same authors two years later, Stegwee et al.[46] reaffirmed the superiority of the double-layer closure over the single-layer techniques regarding different outcomes. However, they found out that single-layer closure was associated with shorter operative time, lower isthmocele/niche prevalence, and reduced postoperative pain[44].

Secondary to the limited availability of high-quality data on this topic, we could not address the association between a reduced RMT or isthmocele and future pregnancy complications. Other studies including lower quality data have attempted to answer this question. The majorities of these trials have concluded that the lower the RMT, the higher the risk of uterine scar defect, and this may be more pronounced in the presence of an isthmocele[10-12,44]. As stated, we did not have high-quality data to confirm or deny these findings.

Generally, an isthmocele is asymptomatic and is incidentally diagnosed. If symptomatic, however, it may manifest with abnormal uterine bleeding, postmenstrual spotting, dysmenorrhea, pelvic pain, and even infertility[9]. Treatment of an isthmocele, including medical and surgical treatments up to and including hysterectomy have been suggested by many authors, with no clear consensus in the literature[45,46]. Management with birth control pills, hysteroscopy, laparoscopy, vaginal procedures and hysterectomy have also been discussed[46,47].

Strengths

Our meta-analysis has many strong points. We conducted this study in strict adherence to the Cochrane handbook[25]. In addition, we included only randomized controlled trials and excluded all observational studies (especially retrospective designs). This ensured the strongest levels of evidence according



Supplementary Figure S10B. Analysis of niche prevalence at 6-24 months

to the GRADE guidelines. Also, we tried to cover more than one follow-up period, which we feel gave more comprehensive evidence regarding clinical outcomes. In addition, this meta-analysis that we have completed in March of 2021, includes many late breaking clinical trials[27,28,30,38,47]; that have not yet been included in any other analysis, to the knowledge of our authors. Finally, the majority of studies we included showed a low ROB in nearly all the assessed domains.

Study Limitations

The major limitation was the lack of reported outcomes regarding long-term follow-up, particularly regarding future pregnancy outcomes and the incidence of uterine rupture. We sought that including the latest RCTs would provide sufficient data to analyze these outcomes. At this time this high-quality data on this topic from RCTs does not exist. The second weakness was a higher than expected heterogeneity in some of the reported outcomes. As a result, some outcomes could not be resolved by sensitivity analysis. This may affect the clinical application of the reported results. This is likely secondary to low sample, and relatively high dropout rates in some trials. We recommend further research on different techniques of uterine suturing and closure compared to RMT and isthmocele formation, and long-term follow-up relating to future pregnancy outcomes for these patients. We await the reexamination of these data when more evidence exists.

Conclusion

Double-layer closure showed higher RMT compared with single-layer closure. However, both closure techniques showed no significant difference regarding the incidence of uterine isthmocele (or niche) or other outcomes. Surgeons can predict higher RMT, but not a lower incidence of isthmocele if using a double-layer technique. High-quality data from RCTs regarding how lower RMT and isthmocele are associated to future pregnancy outcomes and the incidence of uterine rupture does not currently exist as we look forward to future RCTs on this subject.

Ethics

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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

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Supplementary Table S1. Risk of bias assessment

Study	Randomization	Allocation concealment	Blinding of participants and personnel	Blinding of outcome	Attrition bias	Selective reporting	Other bias
Bamberg 2016	low	low	low	unclear	low	low	low
Bamberg 2017	low	low	low	unclear	low	low	low
Bennich 2016	low	low	low	low	low	low	low
Ferrari 2001	low	low	low	high	low	low	low
Franchi 1998	low	low	low	high	low	low	low
Hanacek 2019	low	low	unclear	low	low	low	low
Hayakawa 2006	high	high	high	high	low	low	low
Kalem 2021	low	low	low	high	low	unclear	low
Caesar 2010	low	low	low	low	low	low	low
Yasmin 2011	low	low	low	high	low	high	low
Roberge 2016	low	low	unclear	low	low	low	low
Stegwee 2020	low	low	low	low	low	low	low
Khamees 2018	low	low	high	unclear	low	low	low
Jjindal 2017	low	low	low	high	low	low	low
Shrestha 2015	low	low	low	high	high	low	low
Elghareeb 2013	low	low	low	high	high	low	low
Batioglu 1998	low	high	unclear	high	high	low	low
Hamar 2007	low	low	low	unclear	low	low	low
Chapman 1997	low	low	high	unclear	high	low	low
Hauth 1992	low	low	low	high	low	low	low

Case Report / Olgu Sunumu

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Mayer-rokitansky-kuster-hauser syndrome with presacral schwannoma presenting as a pelvic mass: A literature review and case report

Pelvik kitle olarak prezente olan presakral schwannomlu mayer-rokitansky-küster-hauser sendromu ve literatürün gözden geçirilmesi

Raziye Narin[1], Sefa Özyazıcı[2], Mehmet Ali Narin[3], Sevtap Seyfettinoglu[3], Elife Aşut[4]

[1]*University of Health Sciences Turkey, Adana City Training and Research Hospital, Clinic of Obstetrics and Gynecology, Adana, Turkey*
[2]*University of Health Sciences Turkey, Adana City Training and Research Hospital, Clinic of General Surgery, Adana, Turkey*
[3]*University of Health Sciences Turkey, Adana City Training and Research Hospital, Clinic of Gynecological Oncology, Adana, Turkey*
[4]*University of Health Sciences Turkey, Adana City Training and Research Hospital, Clinic of Pathology, Adana, Turkey*

Abstract

A 32-year-old woman was admitted to our gynecology outpatient clinic with primary amenorrhea, a pelvic mass, and pain. Sonographic examination and magnetic resonance imaging revealed an approximately 124×103 mm heterogeneous mass. Moreover, laparotomy revealed fibrotic uterine bands with normal ovaries, tubes, and a solid retroperitoneal lesion. On the second postoperative day, the mass was removed, and the patient was discharged with full recovery. Microscopic examination of the pelvic mass confirmed the diagnosis of schwannoma. To the best of our knowledge, this is the first report on the co-occurrence of Mayer-Rokitansky-Küster-Hauser syndrome and schwannoma, without the presence of any other pathology.
Keywords: MRKH syndrome, schwannoma, pelvic mass

Öz

Pelvik kitle olarak prezente olan presakral schwannomlu Mayer-Rokitansky-Küster-Hauser sendromu (MRKHS). MRKH'li hastalar sıklıkla ekstragenital anomaliler, özellikle ürolojik ve iskelet anomalileri sergiler. Hastamız, schwannomanın eşlik ettiği ilk MRKHS olgusudur. Otuz iki yaşında kadın hasta jinekoloji polikliniğimize primer amenore, pelvik kitle ve ağrı şikayetleri ile başvurdu. Sonografik değerlendirme ve manyetik rezonans görüntülemede, yaklaşık 124×103 mm'lik heterojen bir kitle görünümü mevcuttu. Laparotomide fibrotik uterin bantlara eşlik eden normal overler, tubalar ve sert bir retroperitoneal lezyon izlendi. Kitle ekstirpe edildi ve postoperatif ikinci günde hasta şifa ile taburcu edildi. Pelvik kitlenin mikroskobik incelemesi schwannoma tanısını doğruladı. Bilgilerimize göre bu iki malformasyonun herhangi bir ek patoloji olmaksızın birlikte ortaya çıktığı ilk olgudur.
Anahtar Kelimeler: MRKH sendromu, schwannoma, pelvik kitle

Introduction

Vaginal agenesis, also known as Mullerian aplasia or Mayer-Rokitansky-Küster-Hauser (MRKH) syndrome, is characterized by congenital absence of the vagina and variable uterine development. It results from agenesis or hypoplasia of the Mullerian duct system; however, the underlying etiology remains unknown. The incidence of MRKH syndrome has been estimated to be 1 in every 4.500 female births[(1)]. Most of the cases appear to be sporadic; however, cases having a family history of this disease have also been described. The first clinical signal is generally primary amenorrhea in patients who have a normal female phenotype, a normal 46 XX karyotype, and normal and functioning ovaries with no signs of androgen excess[(2)]. Furthermore, external examination of these patients reveals completed puberty with normal secondary female sexual characteristics (pubic hair and breast development: Tanner stage 5) and normal external genitalia.

Schwannomas are rare tumors of ectodermic origin that grow from the neural sheath and are usually found in the neck and

Address for Correspondence/Yazışma Adresi: Prof. Raziye Narin, University of Health Sciences Turkey, Adana City Training and Research Hospital, Clinic of Obstetrics and Gynecology, Adana, Turkey
Phone: +90 322 455 90 00 **E-mail:** ryencilek@yahoo.com.tr **ORCID ID:** orcid.org/0000-0001-5352-878X
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extremities. These tumors commonly arise from the cranial nerves as acoustic neurinomas, but they are extremely rare in the pelvis and retroperitoneal area (less than 0.5% of reported cases), unless they are associated with von Recklinghausen disease (type 1 neurofibromatosis)[3]. Schwannomas are mostly benign in nature and are more common in adult females between the age of 20 and 50 years, with a male-to-female ratio of 2:3. Preoperative biopsy examinations can be challenging, and immunohistochemistry is necessary for the correct diagnosis of a schwannoma[4]. Further, a low rate of mitosis and the absence of atypical mitotic figures and nuclear hyperpigmentation characterize a benign lesion.

To the best of our knowledge, this is the first report on the co-occurence of MRKH syndrome and schwannoma in adulthood, without any other pathology, such as genitourinary system abnormalities, skeletal anomalies, or deafness.

Case Presentation

A 32-year-old virgin female patient was admitted to our gynecology clinic with primary amenorrhea and pain. Her family history was unremarkable. Abdominal examination revealed a rigid and fixed mass extending to the level of the umbilicus, with no visceromegaly or ascites. During the pelvic examination, the external genitalia were normal, but there was vaginal agenesis (Figure 1A). In addition, breast development was normal (Tanner stage 5). Ultrasound imaging revealed a 124×103 mm heterogeneous mass, and an abdominal magnetic resonance scan revealed a 130×100 mm mass lesion located in the presacral region and compressing the bladder posteriorly. However, the patient had no complaints related to defecation or miction. Furthermore, all laboratory findings were normal (follicle stimulating hormone: 7.9 mIU/mL, luteinizing hormone: 14.5 mIU/mL, estradiol: 200 pg/mL, Ca-125: 12.2 U/mL, CA 19-9: 8.0 U/mL, CA 15-3: 7.4 U/mL, and hemoglobin: 14 g/dL).

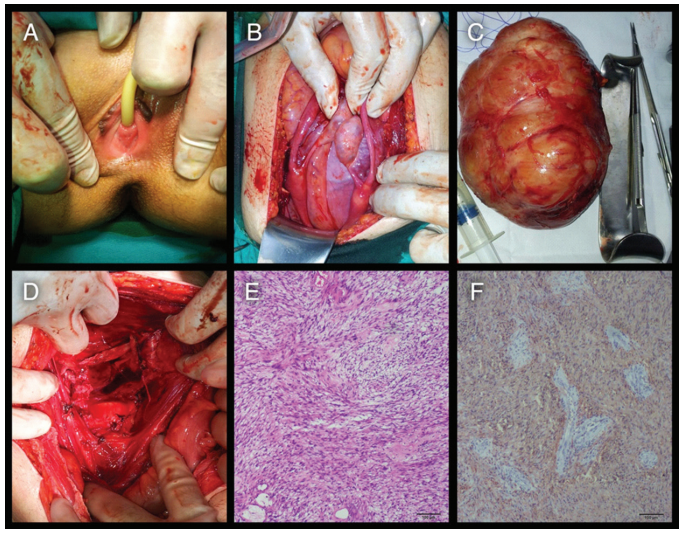

Figure 1. External genitalia (A), internal genitalia and the pelvic mass (B), appearance of specimen (C), retroperitoneal area after mass removal (D), histological appearance of schwannoma, spindle cell neoplasm composed of cellular and hypocellular areas (H&E 100x) (E), and S100 immunostaining of Schwann cells immunohistochemistry 100x (F)

The patient underwent laparatomy under general anesthesia. The tubes and ovaries were normal, but the uterus was seen as two distinct tubular-shaped fibrotic bands (Figure 1B). Behind the internal genital organs, a solid and fixed mass was discovered (Figure 1B). This mass was extirpated by a general surgeon (Figure 1C, D). There was no complication in the postoperative period and she was discharged on the second postoperative day.

A schwannoma was discovered during the pathological examination of the specimen (Figure 1E, F). Immunohistochemical markers showed a diffuse positivity of S-100, actine (-), desmin (-), Kİ-67: 3%, and CD34 (-).

The patient was asked to sign a consent form for publication.

Discussion

Women with MRKH syndrome have a normal female karyotype, with normal ovaries and ovarian functions, and thus they develop normal secondary sexual characteristics (e.g., breasts, axillary hair, and pubic hair). However, these women have a congenital absence of the uterus, cervix, and upper part of the vagina.

In the literature search we conducted using the keywords "MRKH syndrome" and "pelvic mass," very few case reports were found. In most of these cases, the pelvic mass was found to be a leiomyoma. They reported that despite the low probability of having fibroids without a uterus, fibroids should not be ruled out in such patients[5,6].

It is known that urinary system anomalies are frequently observed in patients with MRKH syndrome. Karimbayev et al.[7] presented a 14-year-old patient with MRKH syndrome and pelvic ectopic kidney as a pelvic mass in their study.

Bae et al.[8] reported the first known case of cancer of the supernumerary ovary in a patient with MRKH syndrome, and they proposed that although both ovaries were confirmed to be normal in the patient with MRKH syndrome, ovarian neoplasm should be considered in the diagnosis of a pelvic mass.

Juusela et al.[9] presented two rare pathologies, bilateral Sertoli cell tumors of the ovary and MRKH syndrome, which developed concomitantly in the same patient.

In a study carried out by Kawano et al.[10], bilateral Mullerian remnants with functioning endometrium and a pelvic mass that was considered an endometriotic cyst were revealed on magnetic resonance imaging. Table 1 shows the author's name, publication year, and the diagnosis of accompanying pelvic masses in patients with MRKH syndrome[11-20].

To the best of our knowledge, this is the first case in which MRKH syndrome and schwannoma are seen together. MRKH syndrome is mainly sporadic, but it may be an inherited disorder. The genetic defects responsible for MRKH syndrome remain unclear. Furthermore, a recent study investigated male

Table 1. Author's name, publication year, and the diagnosis of accompanying pelvic masses in patients with MRKH syndrome

Author	Publication year	Diagnosis
Papa et al.[11]	2008	Leiomyoma
Lanowska et al.[12]	2009	Leiomyoma
Fletcher et al.[13]	2012	Leiomyoma
Bae et al.[8]	2013	Cancer of the supernumerary ovary
Kawano et al.[10]	2014	Endometrioma
Girma and Woldeyes.[14]	2015	Leiomyoma
Hasegawa et al.[15]	2015	Leiomyoma
Narayanan et al.[16]	2015	Leiomyoma
Dimitriadis et al.[17]	2016	Mitotically active leiomyoma
Karimbayev et al.[7]	2018	Pelvic ectopic kidney
Juusela et al.[9]	2018	Bilateral ovarian Sertoli cell tumors
Jokimaa et al.[18]	2020	Leiomyoma
Romano et al.[19]	2021	Leiomyoma
Ibidapo-Obe et al.[20]	2021	Leiomyoma

MRKH: Mayer-Rokitansky-Küster-Hauser

microchimerism as a possible cause but found no evidence to support this finding[21]. Schwannomas may occur spontaneously or in the context of a familial tumor syndrome, such as neurofibromatosis type 1[22]. Retroperitoneal schwannomas are extremely rare tumors that are difficult to diagnose preoperatively[23].

Besides MRKH syndrome and retroperitoneal schwannoma, our patient had no other conditions. Moreover, genetic studies could not be carried out. Therefore, we do not know if there is a common genetic defect that can cause both disorders. However, since the presented case is unique in the literature, it appears unlikely that a common genetic defect exists.

Treatment for MRKH syndrome involves a combination of psychosocial support and correction of anatomical defects, such as the creation of a functional vagina and transplantation of a uterus. In our case, since the patient is not married and her primary complaint was pelvic pain, we only surgically removed the mass. She was relieved after the surgery, and since the pathological diagnosis was benign schwannoma, no further treatment was required. At a 6-month postoperative follow-up, the patient was completely asymptomatic, and computed tomography scan imaging revealed no mass.

Conclusion

We present a unique case of MRKH syndrome with a schwannoma. To treat pelvic masses with Mullerian congenital anomalies, accurate evaluation and informed consent are required prior to surgery.

Ethics

Informed Consent: The patient was asked to sign a consent form for publication

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

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

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

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

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