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

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

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· The declaration of transparency from the corresponding author.

· Clinical trial registration, if applicable.

· Institutional review board (IRB) approval or exemption.

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

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

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

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

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

- 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).

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

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· 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.

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

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:

Introduction, Case Report, Discussion and References.

References

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

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

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

Examples

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

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

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

Tables and Figures

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

Units of Measurement and Abbreviations

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

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

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TURKISH JOURNAL OF OBSTETRICS AND GYNECOLOGY

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TURKISH JOURNAL OF OBSTETRICS AND GYNECOLOGY

EDITORIAL

Dear Colleagues,

We are excited to present you, valuable scientists, the first issue of 2022 of the Turkish Journal of Obstetrics and Gynecology with scientific studies. In this issue, there are high-quality publications both from our country and other countries.

We carry out meetings with our editors and section editors both online and face to face with the aim of improving our article evaluation process and publish high quality articles. In this context, we held our latest meeting during the 18th National Gynecology and Obstetrics Congress on 1-5 December 2021 in Antalya. Under the chairmanship of our journal's editor-in-chief, Eray Caliskan, MD, and with the participation of our co-editors and section editors, we made important decisions for the sustainability of our journal in publication and to maximize its scientific quality. Some of these decisions are article reviews and SPSS courses for the referees of our journal.

We plan to organize the 19th National Gynecology and Obstetrics Congress, our biggest national congress, on 18-22 May 2022 in Antalya. In line with our decision from the last congress, we plan to hold a session for Turkish Journal of Obstetrics and Gynecology.

We hope to see you at the 19th National Gynecology and Obstetrics Congress and with the new scientific issues of our journal.

Ercan Yilmaz, MD
Co-Editor in Chief

Clinical Investigation / Araştırma

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Brucellosis in pregnancy and its response to the changing immunoglobulin A: A prospective controlled study

Gebelikte bruselloz ve değişen immünoglobülin A yanıtı: Prospektif kontrollü çalışma

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Abstract

Objective: This study aimed to define the rare Brucella infection in pregnancy and its effects on immunoglobulins (Ig).

Materials and Methods: This prospective study has conducted Brucella screening using the Rose Bengal test on pregnant and non-pregnant outpatients who did not show any specific Brucella symptoms. The immunoglobulin levels were measured using the enzyme-linked immunosorbent assay. The study group consisted of pregnant women who were at 20 weeks or below gestation and applied to our hospital outpatient clinic for routine check-ups. The control group consisted of healthy patients who applied for routine controls.

Results: This study included a total of 584 participants, 293 of whom were controls and 291 were the study (pregnant) participants. The study revealed a 1.5% incidence of Brucella during pregnancy. In acute and chronic Brucella infection, lower levels of IgA response were observed in pregnant cases compared to the control group.

Conclusion: Brucella infection is a disease that can cause fetal problems, especially in endemic areas. The role of the altered IgA response in pathologies that are associated with Brucella infection stands out as a new target for disease pathophysiology.

Keywords: Brucella, immunoglobulin A, pregnancy

Öz

Amaç: Bu çalışma, gebelikte nadir görülen Brusella enfeksiyonunu ve immünoglobulinler (Ig) üzerine etkilerini belirlemek amacıyla planlandı.

Gereç ve Yöntemler: Bu çalışma prospektif olarak planlandı. Bu amaçla, hastanemiz polikliniğine başvuran 20. gebelik haftası ve altı gebeler spesifik Brusella semptomlarına bakılmaksızın Rose Bengal testi ile tarandı. İmmünoglobulin seviyeleri enzim bağlı immünosorbent deneyi ile ölçüldü. Kontrol grubu rutin kontroller için başvuran sağlıklı hastalardan oluşturuldu.

Bulgular: Bu çalışmaya 293'ü kontrol ve 291'i çalışma (gebe) olmak üzere toplam 584 katılımcı dahil edilmiştir. Çalışmada gebelikte Brusella görülme sıklığı %1,5 olarak bulunmuştur. Akut ve kronik Brusella enfeksiyonunda gebe olgularda kontrol grubuna göre daha düşük seviyelerde IgA yanıtı gözlendi.

Sonuç: Brusella enfeksiyonu özellikle endemik bölgelerde fetal problemlere neden olabilen bir hastalıktır. Brusella enfeksiyonu ile ilişkili patolojilerde değişen IgA yanıtının rolü, hastalığın patofizyolojisi için yeni bir hedef olarak öne çıkmaktadır.

Anahtar Kelimeler: Brucella, immünoglobulin A, gebelik

PRECIS: This study is a case-control study evaluating the relationship with brucellosis and IgA levels in pregnancy.

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Introduction

Brucellosis in pregnancy is a rare disease that is associated with various obstetric complications. Unpasteurized dairy product consumption, especially raw milk, soft cheese, butter, and ice cream, is the most common mode of transmission[1,2]. Human-to-human transmission due to blood transfusion, tissue transplantation, breastfeeding, sexual contact, congenital transmission, and hospital infection has also been reported in rare cases[3-6]. Brucellosis is an important health problem in endemic areas, such as South and Central America, India, the Mediterranean basin, the Balkans, and the Middle East. Turkey is among the endemic regions with an incidence of 25.7 cases per 100,000 population[7]. Brucellosis is rare in pregnancy, with an incidence in endemic areas from 1.3% to 12.2% 3-5[8-10]. Existing studies have examined brucellosis based on the collected data from the general population. Brucellosis in pregnancy is a rare condition, and most brucellosis cases are detected due to suspicions based on clinical findings. Brucellosis typically presents with an insidious onset of fever, malaise, night sweats (associated with a strong, peculiar, and musty odor), and arthralgia[11]. Pregnancy-specific findings are unclearly defined. However, the disease is stated to be associated with abortion, premature delivery, intrauterine fetal demise, congenital malformations, neonatal death, and low birth weight[12].

Demonstrating different specific classes or subclasses of antibody response in brucellosis has been suggested as useful in disease diagnosis and prognosis, as well as elucidating the differences between acute and chronic stages of brucellosis[13,14]. Patients with acute brucellosis have elevated Brucella-specific immunoglobulin (Ig) M alone. Patients with chronic brucellosis have elevated IgG and IgA antibodies only[15,16]. Changes in maternal immune regulation are observed during pregnancy. These changes do not constitute a case of generalized immunosuppression; however, they may include selective suppression and modulations. The process radically affects progesterone and estrogen, of which the levels change during pregnancy. Progesterone inhibits the synthesis of nitric oxide and tumor necrosis factor α by macrophages by causing Th2 polarization. At the beginning of pregnancy, a relatively strong Th1 response occurs, which provides the required inflammatory environment for implantation. These interactions determine the effect of some developing infections during this period in the fetus. However, the relationship between Brucella infection and pregnancy is unclearly known. This study attempted to evaluate specific IgM, IgG, and total IgA levels and obstetric results by performing Brucella screening in the first and second trimester in pregnant women who did not have any specific symptoms and applied to the hospital for routine control. This study aimed to evaluate the changing immune parameters, especially IgA levels, in brucellosis and contribute to the literature on this subject.

Materials and Methods

This study was prospectively conducted at Yozgat Bozok University Faculty of Medicine, Department of Obstetrics and Gynecology. The study group consisted of pregnant women who were at 20 weeks or below gestation and applied to our hospital outpatient clinic for routine check-ups. Patients under 20 weeks of age were included in the study to detect complications that are associated with early pregnancy. The control group consisted of non-pregnant patients between the ages of 18 and 40 years. Individuals with hypertension, diabetes, cancer with hematological and rheumatological diseases, and acquired immunodeficiency syndrome that may cause immunosuppression, as well as those who used drugs, such as glucocorticoids, were excluded from the study. Data on potential risk factors for brucellosis, including age, rural area residence, socioeconomic status, contact with animals, consumption of raw and pasteurized milk, and previous intrauterine fetal death, were collected through individual interviews before the study. Additionally, patients' body mass index (BMI), gravida parity, gestational age, systemic diseases, and used medications were recorded. Pregnancy loss that occur at ≤20 gestational weeks was defined as abortion, whereas >20th gestational week as in utero fetal death. Following that, blood samples were collected from the patients. All epidemiological, obstetric, clinical data, pregnancy outcome and newborn evaluations, and all laboratory results were recorded in a standardized manner. Patients with brucellosis were treated with cotrimoxazole or rifampin for at least 4 weeks.

Ethics committee approval 2017-KAEK-189_2018.02.27_01 and written consent from all participants were obtained.

Laboratory Evaluation

Blood samples are taken from the patients for serological tests and their serums were separated. The Rose Bengal tests and Coombs tests, which are used in the serological diagnosis of brucellosis from the obtained serum samples, were immediately studied, and the serum samples were stored at -20 °C until enzyme-linked immunosorbent assay (ELISA) tests were used to conduct ELISA IgG, IgA, and IgM studies.

The Rose Bengal test (Biomedica, Turkey), Brucella Agglutination test with Coombs (Red Cell Biotechnology, Turkey), and Brucella IgM, IgA, and IgG (Novatec, Germany) tests by ELISA were studied with patient sera. Agglutination formation in the Rose Bengal test, titrations of 1/160 and above in the Coombs test, and Brucella IgM and IgG values of >11 Nova Tec Units (NTU) in the ELISA method were evaluated as positive.

Statistical Analysis

Statistical analysis was conducted using the Statistical Package for the Social Sciences program (version 20, SPSS, Chicago, IL). Data were expressed as mean ± standard deviation and percentages. Data distribution was assessed using the Kolmogorov-Smirnov test. With non-parametric numerical data, the Mann-Whitney U test was conducted, whereas the

Student t-test for parametric numerical data. Triple comparisons were made via the employment of the Kolmogorov-Smirnov test. Categorical data were compared using the chi-square test. P-values of <0.05 were accepted as statistically significant.

Results

This study included a total of 584 participants, of whom 293 were controls and 291 were the study (pregnant) participants. The mean age of the pregnant women was 27.3±6 years, and the mean BMI was 26.4±5.1. The mean abortion was 0.2±0.6. The mean age of the control group was 27.5±5.6 years and the mean BMI was 26.2±4.8. The mean abortion was 0.2±0.7, without statistically significant differences between the groups' mean values (p>0.05). Additionally, 48.5% of the pregnant women and 50.2% of the control group lived in rural areas, without significant differences between the groups (p>0.05). Moreover, no significant differences were found in the use of pasteurized milk between the groups (p>0.05). The mean week of gestation at the time of examination of the pregnant women was 11±3.7. Table 1 shows the demographic characteristics of the participants.

IgM results were positive in 16 (2.7%) participants, of whom 10 (3.6%) were pregnant and 6 (2.1%) were from the control group, without statistically significant differences between the groups (p=0.412). Additionally, 25 (4.3%) participants had positive IgG, of whom 10 (3.6%) were pregnant and 15 (5.3%) were from the control group, without significant differences in the results (p=0.148). The IgA result was 1.42±0.56 in pregnant women and 1.52±0.9 in the control group, without significant differences between the groups (p=0.612). The IgA concentration result was 8.46±4.77 in pregnant women and 10.47±6.27 in the control group, with significant differences between the groups (p<0.001) (Table 2).

IgA results and concentrations are shown in Table 3. Among the patients who had never encountered the disease, with negative IgM and IgG, a significantly lower IgA concentration was found

Table 1. Demographic characteristics

	Control group	Study group	OR	95% CI		p-value
				Lower	Upper	
Age (years)	27.5±5.6	27.3±6	0.49	-0.82	1.11	0.559
BMI (kg/m²)	26.2±4.8	26.4±5.1	0.42	-0.95	0.71	0.954
Gestational weeks	-	11±3.7	-	-	-	-
Parity	0.9±1	0.9±1	0.08	-0.14	0.19	0.642
Abortion	0.2±0.7	0.2±0.6	0.06	-0.12	0.10	0.625
Region of residence, n (%)	-	-	1.06	0.76	1.48	0.678
Rural	147 (50.2)	141 (48.5)	-	-	-	-
Urban	146 (49.8)	150 (51.5)	-	-	-	-
Use of pasteurized milk, n (%)	-	-	0.84	0.60	1.18	0.364
No	122 (41.6)	132(45.4)	-	-	-	-
Yes	171 (58.4)	159 (54.6)	-	-	-	-

Unless otherwise specified, results are presented as mean ± SD. CI: Confidence interval, BMI: Body mass index, OR: Odds ratio, SD: Standard deviation

Table 2. Participants' immunoglobulin levels and results

	All patients	Control group	Study group	p-value
IgG result, n (%)	-	-	-	0.148
Negative	559 (95.7)	268 (94.7)	270 (96.4)	-
Positive	25 (4.3)	15 (5.3)	10 (3.6)	
IgM result, n (%)	-	-	-	0.412
Negative	568 (97.3)	277 (97.9)	270 (96.4)	-
Positive	16 (2.7)	6 (2.1)	10 (3.6)	-
IgA	1.47±0.75	1.52±0.9	1.42±0.56	0.612
IgA Concentration	9.43±5.63	10.47±6.27	8.46±4.77	<0.001

Unless otherwise specified, results are presented as mean ± SD. Ig: Immunoglobulin, SD: Standard deviation

in pregnant women than in the control group (p<0.001). The IgA concentration of the pregnant women who had the disease, with positive IgG and negative IgM, was lower than the control group, but without statistically significant differences between the groups (p=0.086). IgA concentrations in patients with positive acute infections (IgM positive) were lower in pregnant women than in the control group; however, no significant differences were found between the groups (p=0.233). Three patients were found to be both IgG and IgM positive, of whom two were pregnant and one was from the control group (data not shown). Table 4 shows the IgA results of patients with positive Coombs test. Accordingly, no statistically significant differences were found between the groups; however, IgA concentrations were increased as the titer increased.

Discussion

This study revealed that Brucella infection can be observed in pregnancy without causing any specific symptoms. Study results revealed a 1.5% incidence of brucellosis during pregnancy. This rate was 2% in the study population. No brucellosis-related maternal/fetal death and fetal anomaly were observed in this study. In acute and chronic Brucella infection, lower levels of IgA response were observed in pregnant cases compared to the control group. The evaluation of cases without infection revealed low IgA levels in pregnant women.

The main immunoregulatory effect in pregnancy is to protect the developing fetus, which is an allograft for the mother, from maternal immune responses. Th2 polarization develops as a useful aberration for fetal protection. The question remains as to how this results for Brucella, for which there is a primarily cellular immune response. The Th1/Th2 shift is important for successful pregnancy continuation and changes throughout the pregnancy. The required inflammatory environment for implantation is provided by the relatively dominant effect of Th1 in the first trimester, a sufficient combat environment for intracellular sample Toxoplasma gondii is created during this period, and this mechanism works in the prevention of disease-related abortion. Additionally, until the end of the second and third trimesters, the Th2 response remains dominant, and this parasite becomes difficult to eradicate, which makes it easier for the fetus to become infected. Previous studies revealed different findings on maternal and fetal outcomes of brucellosis in pregnancy. Some studies revealed an increased risk of abortion and congenital anomalies[17,18]. However,

Table 3. IgA levels in acute, chronic, and previous Brucella infection, and those without infection

		Control group		Study group		p-value
		Mean	SD	Mean	SD	
IgG (-)	**IgA**	1.50	0.85	1.42	0.56	0.606
	IgA concentration	10.32	5.98	8.49	4.83	0.000
IgG (-) IgM (-)	**IgA**	1.50	0.85	1.43	0.56	0.310
	IgA concentration	10.32	5.95	8.59	4.86	<0.001
IgG (+)	**IgA**	1.91	1.44	1.31	0.51	0.956
	IgA concentration	12.81	10.00	7.59	3.05	0.618
IG (+) IgM (-)	**IgA**	2.01	1.44	1.43	0.45	0.283
	IgA concentration	13.47	10.03	8.24	2.82	0.086
IgM (-)	**IgA**	1.52	0.89	1.43	0.55	0.758
	IgA concentration	10.49	6.24	8.58	4.81	<0.001
IgM (+)	**IgA**	1.34	1.20	0.92	0.33	0.588
	IgA concentration	9.16	7.82	5.25	1.87	0.233

Results are presented as mean ± SD. SD: Standard deviation, Ig: Immunoglobulin

Table 4. The relationship between Coombs titer and IgA levels in acute infection

	Coombs titer						p-value
	80		160		640		
	Mean	SD	Mean	SD	Mean	SD	
IgA	0.93	0.26	0.88	0.78	1.36	1.09	0.717
IgA concentration	5.66	1.97	5.85	4.75	8.34	7.51	0.943

SD: Standard deviation, Ig: Immunoglobulin

evidence on the relationship between Brucella and abortion in the literature remains insufficient[19], and brucellosis is estimated to cause fewer spontaneous abortions in humans than in animals due to the absence of Erythritol in the human placenta and fetus[20]. However, most studies lack a control group in their research designs. The current study revealed that total IgA levels and concentrations in the study group were lower than in the control group. Previous studies on Ig levels in pregnancy have revealed varying findings. Amino et al.[21] revealed that IgG, IgA, and IgM concentrations significantly decreases in the second and third trimesters, and the average values decrease in the second trimester as 18%, 13%, and 9%, respectively, and these findings were independent of maternal age, hyperemesis, ABO incompatibility, and the sex and weight of the baby at birth. Recent studies revealed that the change in IgA levels during pregnancy is "dynamic"[22,23]. When the changes in brucellosis cases were examined in the current study, the IgA levels in individuals who had active Brucella infections during pregnancy were at lower levels than those who did not have Brucella infections, although these changes were not statistically significant. Similarly, lower IgA levels were found in pregnant women who had the infection compared to those who did not. Results revealed that the IgA levels significantly decreased in individuals who had Brucella infections during pregnancy. This decrease continued after the patients recovered from brucellosis, which indicates that Brucella infections affect mucosal immunity-related IgA levels. Additionally, IgA concentrations were found to increase with increasing Coombs titers. This may be related to the transmission of the infection through the gastrointestinal tract or to the altered immune response during pregnancy. A conducted study on rats to elucidate the response of Brucella infection in the human body revealed low-level and short-term IgA antibodies in rats infected with Brucella abortus. Additionally, the study revealed that IgA antibody production is induced in infected rats, but its role in providing protection is unknown[24]. In a study evaluated all Ig changes in acute and chronic brucella infection in the normal population, anti-brucellosis IgG, IgM, IgA, IgE, IgG1 and IgG3 antibodies were increased in patients with acute brucellosis, while an increase in IgG, IgA, IgE and IgG4 was observed in patients with chronic brucellosis[25].

Brucellosis in pregnancy is described as a condition that should be primarily considered in areas where it is endemic and in cases with continuous fever and vaginal bleeding; however, the current study revealed that the disease can also occur in completely asymptomatic individuals. The biggest limitation of this study is that functional changes in antibody concentrations could not be completely excluded, which was also a limitation for many similar studies on different populations using different immunoassays. However, this is the first study to include a control group to better understand brucellosis and changes in IgA levels during pregnancy.

Conclusion

The changing immune response during pregnancy and the course of Brucella infection is unclearly known. The current study revealed lower levels of IgA response in pregnant cases with acute and chronic Brucella infection compared to the control group. This might be due to the relatively dominant effect of the inflammatory environment that Th1 is required for implantation in the first trimester. However, further studies with control groups are needed to examine the changing immune response and immunoglobulin levels in pregnancy. These studies can guide the evaluation and treatment of pregnancy-related outcomes, especially in regions where Brucella is endemic.

Ethics

Ethics Committee Approval: The study was approved by the Local Ethics Committee in Yozgat Bozok University Faculty of Medicine, with approval number 2017-KAEK-189_2018.02.27_01.

Informed Consent: Written consent from all participants were obtained.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: E.B., Design: D.A.K., E.B., Data Collection or Processing: E.Y.Ş., M.D.Ç., N.Y., Analysis or Interpretation: M.K., M.D.Ç., T.O., E.S.Y., Literature Search: D.A.K., Writing: D.A.K., E.B.

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

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

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Evaluation of dyslipidemia in preeclamptic pregnant women and determination of the predictive value of the hemato-lipid profile: A prospective, cross-sectional, case-control study

Preeklamptik gebelerde dislipideminin değerlendirilmesi ve hemato-lipid profilin prediktif değerinin belirlenmesi: Prospektif, kesitsel, olgu-kontrol çalışması

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Abstract

Objective: In this study, we examined the serum hematologic and lipid parameters of pregnant women with preeclampsia and an age- and gestational-age matched normotensive control group. We also compared the ratios of hemato-lipid parameters defined as systemic inflammatory markers and determined the predictive value of these values in preeclampsia.

Materials and Methods: All patients diagnosed with late-onset preeclampsia or severe preeclampsia between 34 and 40 weeks of gestation at Inonu University Faculty of Medicine between March 2019 and October 2020 were included.

Results: A total of 253 pregnant women were included in the study period. When the study groups were compared in terms of hematological and blood lipid profile; while serum lymphocyte, triglyceride, and total cholesterol levels were significantly higher in the preeclampsia group than in the control group ($p<0.001$, $p<0.001$, $p=0.013$, respectively); high-density lipoprotein (HDL)-cholesterol levels were found to be significantly lower ($p=0.017$). The cut-off value for the monocyte/HDL ratio in predicting severe preeclampsia was 16.65 with 59.0% sensitivity and 85.4% specificity [the area under the receiver operating characteristic 0.756, 95% confidence interval (CI) 0.681-0.821, $p<0.001$]. Multivariate analysis showed that the monocyte/HDL ratio was independently associated with both preeclampsia and severe preeclampsia [odds ratio (OR): 1.094; 95% CI 1.009-1.185 and OR: 1.731; 95% CI 1.218-2.459, respectively].

Conclusion: This study demonstrated that serum triglyceride and total cholesterol levels were significantly higher and serum HDL-cholesterol levels were significantly lower in pregnant women with late-onset preeclampsia compared to normotensive pregnant women. Additionally, this study revealed that the measurement of monocyte/HDL ratio in the pregnant population could be a useful clinical tool for predicting preeclampsia.

Keywords: Dyslipidemia, pregnancy, HDL cholesterol, monocytes, preeclampsia

PRECIS: We evaluated the hemato-lipid profile of pregnant women with preeclampsia, and determined the predictive value of the ratios of hematological and lipid parameters in preeclampsia.

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Öz

Amaç: Bu çalışmada preeklamptik gebeler ile preeklampsi ile komplike olmayan gebelerin serumlarında hematolojik ve lipid parametreleri değerlendirildi. Ayrıca sistemik enflamatuvar marker olarak da tanımlanan hemato-lipid parametrelerin oranları karşılaştırılıp bu oranların preeklampsideki prediktif değeri belirlendi.

Gereç ve Yöntemler: Çalışmaya İnönü Üniversitesi Tıp Fakültesi Hastanesi'nde 01.03.2019-01.10.2020 tarihleri arasında gebeliğin 34-40. haftasında geç başlangıçlı preeklampsi ve şiddetli preeklampsi tanısı alan ve çalışma kriterlerine uygun tüm hastalar alınmış olup katılımcıların serum örneklerinde rutin laboratuvar testlerinin yanında lipid profili analizi yapılıp, gebelerin perinatal ve neonatal sonuçları kaydedildi.

Bulgular: Çalışma periyodu içinde toplam 253 gebe çalışmaya dahil edildi. Çalışma grupları hematolojik ve kan lipid profili açısından karşılaştırıldığında; preeklampsi grubunda kontrol grubuna göre serum lenfosit, trigliserid ve total kolesterol seviyeleri anlamlı olarak yüksek iken (sırasıyla p<0,0001, p<0,001, p=0,013); yüksek yoğunluklu lipoprotein (HDL)-kolesterol düzeyleri anlamlı olarak düşük saptandı (p=0,017). Hematolojik ve lipid parametrelerinin oranları değerlendirildiğinde kontrol grubu ile karşılaştırıldığında monosit/HDL oranı ve monosit/lenfosit oranlarının hem preklampsi hem de şiddetli preeklampsi grubunda anlamlı olarak yüksek olduğu saptandı (sırasıyla p=0,007, p<0,0001 ve p=0,021, p<0,0001). Şiddetli preeklampsi prediksiyonunda monosit/HDL oranı için cut-off değeri %59,0 sensitivite, %85,4 spesifite ile 16,65 [alıcı çalışma karakteristiğinin altındaki alan 0,756, %95 güven aralığı (GA) 0,681-0,821, p<0,0001] saptandı. Multivariate analiz monosit/HDL oranının bağımsız olarak hem preeklampsi hem de şiddetli preeklampsi ilişkili olduğunu gösterdi [sırasıyla risk oranı (OR): 1,094; %95 GA 1,009-1,185 ve OR: 1,731; %95 GA 1,218-2,459].

Sonuç: Bu çalışma geç başlangıçlı preeklampsi saptanan gebelerde normotansif gebelere göre serum trigliserid ve total kolesterol düzeylerinin anlamlı oranda yüksek, serum HDL-kolesterol düzeylerinin ise anlamlı oranda düşük olduğunu göstermekle birlikte gebe popülasyonunda monosit/HDL oranı ölçümünün preeklampsi gelişiminin prediksiyonu açısından yararlı olabileceğini ortaya koymuştur.

Anahtar Kelimeler: Dislipidemi, gebelik, HDL kolesterol, monosit, preeklampsi

Introduction

Preeclampsia is a complex systemic disease specific to human pregnancies that increase maternal and fetal morbidity and mortality in developed and developing countries. It complicates 5-10% of all pregnancies and is diagnosed by the detection of proteinuria and/or end-organ dysfunction with hypertension [blood pressure (BP) ≥140/90 mmHg] beginning after 20 weeks of gestation in a previously normotensive woman[1]. The pathophysiology of preeclampsia includes both maternal and fetal/placental factors. Many biomarkers have been studied to predict the development of pre-eclampsia[2,3]. Implantation of the embryo and development of the placenta that includes the trophoblast invasion are essential points for a healthy pregnancy[4]. Because of abnormal spiral artery invasion and impaired trophoblast function, the inflammatory process begins and causes alterations in angiogenic factors that proceed to placenta-mediated diseases, including preeclampsia in pregnancy. Also, it has been shown that inappropriate trophoblastic invasion and placentation, which affect the pathophysiology of preeclampsia, cause a systemic inflammatory response by releasing reactive oxygen species and cytokines from the placenta into the maternal circulation due to placental ischemia/hypoxia[5].

Circulating monocytes express tissue factors in inflammatory or pro-thrombotic conditions and change them to the procoagulant phenotype. It has been shown that high-density lipoprotein (HDL) can inhibit the expression of tissue factors in monocytes by preventing p38 activation and inhibiting phosphoinositide 3-kinase[6]. Additionally, it has been suggested that HDL neutralizes the pro-inflammatory and pro-oxidant effects of monocytes by inhibiting the migration of macrophages and increasing the oxidation of low-density lipoprotein (LDL) by promoting the outflow of accumulated cholesterol from cells in the vascular wall. Furthermore, HDL has also been shown to protect endothelial cells from inflammation and oxidative stress by controlling the activation of monocytes and the proliferation of monocyte precursor cells[7]. Many studies have shown that high monocyte count and low HDL cholesterol levels may be associated with inflammation and oxidative stress, and it has been reported that monocyte/HDL cholesterol ratio can be used as a new prognostic marker in many cardiovascular diseases, especially in atherosclerosis and metabolic syndrome[8,9]. However, there is no study in the literature evaluating the monocyte/HDL-cholesterol ratio, which is defined as a systemic inflammatory marker in many studies in preeclamptic patients.

Since the inflammatory response has been suggested to be an important process in preeclampsia, many researchers have investigated the change in leukocyte count to find the relationship between leukocyte counts and preeclampsia. They have found that leukocyte counts increase, especially in patients with preeclampsia and severe preeclampsia[10,11]. Moreover, the neutrophil count is higher in preeclamptic pregnant women than healthy ones. Researchers have found that severe inflammation in preeclampsia often accompanies neutrophil activation and develops simultaneously with clinical symptoms in these patients[12]. Some investigators have suggested that in the preeclamptic group, neutrophils and lymphocytes release various inflammatory cytokines to activate inflammatory cells and immune response, leading to endothelial dysfunction. Therefore, neutrophil and lymphocyte levels can be used as predictive markers of preeclampsia[13]. However, many hematological parameters such as neutrophil count and lymphocyte count in adults are affected by geographic location, nutritional characteristics, racial characteristics, and many other factors. To date, several studies have been conducted on predictive markers of preeclampsia, but unfortunately, only a few have been found to be significant.

In this study, we aimed to evaluate the hematological and lipid parameters in the serum of pregnant women with late-onset preeclampsia and those normotensive control groups, to compare the rates of these parameters defined as systemic inflammatory markers, and to evaluate the predictive value of these rates in preeclampsia.

Materials and Methods

Ethical Committee approval was obtained from the Inonu University School of Medicine Clinical Research Ethics Committee for the study, and the researchers committed to comply with the World Medical Association Declaration of Helsinki (including improvements added in 2013) for the conduct of medical research on human subjects throughout the study (approval number: 2019/56). All participants gave their written informed consent prior to their inclusion in the study. In this prospective, cross-sectional, case control study, lipid profile analysis was conducted in addition to routine laboratory tests in serum samples of all patients diagnosed with late-onset preeclampsia and severe preeclampsia at 34-40 weeks of pregnancy between 01.03.2019 and 01.10.2020 in the Inonu University Faculty of Medicine Department of Obstetrics and Gynecology. The perinatal and neonatal outcomes of the participants were recorded. The study's control group consisted of age and gestational age-matched normotensive pregnant women who applied to our clinic in the same period.

All pregnant women who met the following criteria were enrolled in this study: (i) Singleton viable pregnant women between 18 and 45 years old; (ii) 34^{+0}- 40^{+0} weeks of gestation (gestational age confirmed by first-trimester ultrasonography); (iii) Body mass index between 19.5-40.0 kg/m^2; (iv) Normal fetal anatomy.

The exclusion criteria were as follows: (i) Multiple pregnancy; (ii) Major fetal anomalies (fatal anomalies or require prenatal and postnatal surgery); (iii) Chromosomal anomalies, genetic syndromes, and macroscopic placental anomalies; (iv) Fetal death; (v) Patients with eclampsia, ablatio placentae, disseminated intravascular coagulation; (vi) Presence of maternal systemic disease that may affect the serum lipid profile (previously known dyslipidemia, diabetes mellitus, chronic liver disease, renal failure, hypo- hyperthyroidism, cardiovascular diseases, autoimmune diseases, cancer, active bacterial or viral infections, smoking or alcohol use); (vii) Drug use (corticosteroids, non-steroidal anti-inflammatory drugs, antilipidemic and immuno-suppressive drugs).

Procedure

All patients diagnosed with late-onset preeclampsia or severe preeclampsia at 34-40 weeks of pregnancy in the Gynecology and Obstetrics Clinic of Inonu University School of Medicine and who delivered in our center between 01.03.2019 and 01.10.2020 were included in the study. Preeclampsia was diagnosed in a pregnant woman with a systolic BP of 140 mmHg and/or diastolic BP of 90 mmHg in two BP taken four hours apart beginning after the 20th week of pregnancy in addition to the presence of proteinuria and/or end-organ dysfunction findings. Proteinuria was diagnosed when the quantity of protein in 24-hour urine exceeded 300 mg, or when it was considered unacceptable to wait for the results of protein analysis in 24-hour urine, the existence of protein in urine protein analysis with a dipstick was +2, and/or a protein/creatinine ratio of 0.3 in spot urine was used for detecting proteinuria. Signs of end-organ damage dysfunction was defined as the presence of thrombocytopenia (<100X10^3 mcL), liver dysfunction (doubling of blood transaminase levels from average concentration), presence of kidney failure (serum creatinine above 1.1 mg/dL, or doubling of creatinine levels in the absence of other renal diseases), the presence of pulmonary edema, the presence of either cerebral or visual symptoms. Severe preeclampsia was diagnosed when the systolic BP was 160 mmHg and above and/or the diastolic BP was 110 mmHg and above on two measurements at least 4 hours apart in a pregnant woman who met the criteria for preeclampsia or when end-organ dysfunction was noted. In the presence of non-severe preeclampsia, patients were followed up with weekly maternal and fetal monitoring unless there was an indication for delivery before the 37th week of pregnancy. As long as there was no deterioration in fetal or maternal status during the follow-up examinations, delivery was planned at 37 weeks of pregnancy. In the presence of severe preeclampsia at the 34th gestational week and above, delivery was scheduled as soon as the maternal condition was stabilized. Patients diagnosed with severe preeclampsia were hospitalized, and emergency hypertension treatment, eclampsia prophylaxis (loading and maintenance magnesium sulfate therapy), and antenatal corticosteroids (12 mg betamethasone intramuscularly in two doses, 24 h apart) were administered according to standard protocols. When vaginal delivery is not contraindicated, labor induction was performed according to standard protocols. Venous blood samples were collected after 12 h of fasting in the prenatal period. Total cholesterol (TC), triglyceride, and HDL cholesterol values were analyzed with the original reagent by Abbott Architect C8000 system (Abbott Diagnostics, USA), and HDL cholesterol was analyzed by direct enzymatic method without precipitation. LDL cholesterol was calculated using the Friedewald formula (TC= LDL-cholesterol+ HDL-cholesterol + Triglyceride/5).

Age (year), gravida, parity, body mass index (kg/m^2), systolic BP (mmHg), diastolic BP (mmHg), leukocytes (mm^3), neutrophils (mm^3), lymphocytes (mm^3), monocytes (mm^3), hemoglobin (g/dL) of all patients in the study and control groups, platelet (mm^3), blood urea nitrogen, creatinine (mg/dL), aspartate aminotransaminase (u/L), alanine aminotransferase (u/L), lactate dehydrogenase (u/L), uric acid, 24-hour urine protein (mg), triglyceride (mg/dL), TC (mg/dL), LDL cholesterol (mg/dL), HDL cholesterol (mg/dL), monocyte/HDL ratio, neutrophil/HDL ratio, neutrophil/lymphocyte, monocyte/lymphocyte,

platelet/lymphocyte, LDL/HDL, gestational week at the time of serum sample collection, gestational week at birth, type of delivery, birth weight, APGAR 1 minute, APGAR 5 minute, cord blood pH, cord blood base deficit, neonatal intensive care unit requirement parameters were recorded.

Power Analysis: The sample size calculation presented in the study was based on the fact that the effect that creates an increase in the monocyte/HDL ratio of 1.3 (2.4 standard deviations) in pregnant women complicated with pre-eclampsia was considered statistically significant. It was revealed that at least 61 volunteers in each group were required to detect this difference at 80% power and 5% (two-sided) significance level.

Statistical Analysis

Data were summarized with the median [minimum-maximum (min-max)]. The normality of data distribution was determined by the Kolmogorov-Smirnov test. Mann-Whitney U, Pearson chi-square test, Yates Corrected chi-square test, and Fisher's Exact chi-square test were used where appropriate for statistical analysis. Receiver operating characteristics (ROC) analysis was performed to determine the most appropriate cut-off points of the relevant variables for predicting preeclampsia and severe preeclampsia. DTROC web-based application developed by Inonu University Faculty of Medicine, Department of Biostatistics and Medical Informatics was used in ROC analysis [Yasar S, Arslan AK, Yologlu S, Colak C. DTROC: Diagnostic tests and ROC Analysis Software (Web-based software), accessed on 2019-10-20 from http://biostatapps.inonu.edu.tr/DTROC/]. For other analyses, IBM Statistical Package for the Social Sciences Statistics 22.0 program was used. Logistic regression analysis was performed for odds ratio estimations. A value of $p<0.05$ was considered statistically significant.

Results

A total of 253 pregnant women were included in the study period. While 61 of these patients were diagnosed with severe preeclampsia, preeclampsia (non-severe) was diagnosed in 96 patients. The control group consisted of age and gestational age-matched 96 normotensive pregnant women administered to our clinic in the same period. When the study groups were compared in terms of hematological and lipid profile; serum lymphocyte, triglyceride, and TC levels were significantly higher ($p<0.001$, $p<0.001$, $p=0.013$, respectively), and HDL-cholesterol levels were found to be considerably lower ($p=0.017$) in the preeclampsia group compared with the control group. There was no significant difference between the two groups regarding leukocytes, neutrophils, monocytes, and LDL-cholesterol ($p=0.589$, $p=0.074$, $p=0.222$, and $p=0.171$, respectively). When pregnant women complicated with severe preeclampsia were compared with the control group, serum leukocytes, neutrophils, monocytes, triglycerides, total cholesterol, LDL-cholesterol were found to be significantly higher ($p<0.001$, $p<0.001$, $p<0.001$, $p<0.001$ and $p=0.022$, respectively) while serum HDL-cholesterol levels were found to be similar ($p=0.564$). When the ratios of hematological and lipid parameters were evaluated, it was found that monocyte/HDL ratio and monocyte/lymphocyte ratios were found to be significantly higher in both preeclampsia and severe preeclampsia groups compared to the control group ($p=0.007$, $p<0.001$, and $p=0.021$, $p<0.001$, respectively). A comparison of monocyte/HDL cholesterol and monocyte/lymphocyte ratios of the study groups compared to the control group is shown in Figure 1. The clinical characteristics and laboratory data of the study and control groups are summarized in Table 1.

ROC analysis was performed to determine the sensitivity, specificity and recommended cut-off values of monocyte/HDL, neutrophil/HDL, neutrophil/lymphocyte, monocyte/lymphocyte, thrombocyte/lymphocyte, and LDL/HDL ratios in terms of predicting the development of preeclampsia and severe preeclampsia. In the prediction of preeclampsia, cut-off value for Monocyte/HDL ratio was 19.34 [area under the receiver operating characteristics (AUROC) 0.613, 95% confidence interval (CI) 0.541-0.683, $p=0.006$] with 78.1% sensitivity and 93.33% specificity; cut-off value for neutrophil/lymphocyte ratio was 4.37 (AUROC 0.612 95% CI 0.532-0.612, $p=0.006$) with 88.5% sensitivity and 37.5% specificity; and cut-off value for monocyte/lymphocyte ratio was detected as 0.314 (AUROC 0.596, 95% CI 0.523-0.666, $p=0.021$) with 57.30% sensitivity, 68.80% specificity. In the prediction of severe preeclampsia, cut-off value for Monocyte/HDL ratio was 16.65 (AUROC 0.756, 95% CI 0.681-0.821, $p<0.001$) with 59.0% sensitivity and 85.4% specificity, cut-off value for neutrophil/HDL ratio was 137.5 (AUROC 0.612 95% CI 0.531-0.688, $p=0.016$) with 59.0% sensitivity and 62.5% specificity, and cut-off value for monocyte/lymphocyte ratio was 0.452 (AUROC 0.710, 95% CI 0.633-0.780, $p<0.001$) with 60.7% sensitivity and 79.2% specificity. The sensitivity, specificity and recommended cut-off values determined after ROC analysis of hematological and lipid parameters to predict the development of preeclampsia and severe preeclampsia are summarized in Table 2 and Table 3. ROC curves are shown in Figure 2.

When the correlations between the parameters in the preeclampsia group were examined; a significant negative correlation was detected between monocyte/HDL ratio and TC ($r=-0.324$; $p=0.001$), LDL-cholesterol ($r=-0.376$; $p<0.001$) and HDL-cholesterol ($r=-0.580$; $p<0.001$). Also, a significant positive correlation was found between the monocyte/HDL ratio and the leukocyte count ($r=0.229$; $p=0.025$) and the monocyte/lymphocyte ratio ($r=0.581$; $p<0.001$). When the correlations between the parameters in the severe preeclampsia group were analyzed; there was a significant negative correlation between monocyte/HDL ratio and TC ($r=-0.395$; $p=0.002$), LDL-cholesterol ($r=-0.316$; $p=0.016$) and HDL-cholesterol ($r=-0.632$; $p<0.001$) and a significant positive correlation was found between body mass index and monocyte/HDL ratio ($r=0.284$; $p=0.027$). The correlations between parameters in the control group, preeclampsia, and severe preeclampsia groups are summarized in Table 4.

Multivariate analysis showed that the monocyte/HDL ratio was independently associated with both preeclampsia and severe preeclampsia [odds ratio (OR): 1.094; 95% CI, 1.009-1.185 and OR: 1.731; 95% CI, 1.218-2.459, respectively] (Table 5 and Table 6).

Discussion

This study demonstrated that serum triglyceride and TC levels were significantly higher, and serum HDL-cholesterol levels were significantly lower in pregnant women with late-onset preeclampsia compared to normotensive pregnant women. Furthermore, the findings imply that the monocyte/HDL and monocyte/lymphocyte ratios are higher in pregnant women with preeclampsia, particularly in severe preeclampsia. These ratios might be valuable laboratory markers for predicting pre-eclampsia and assessing disease severity.

Several studies examining lipid levels during pregnancy and preeclampsia have been reported conflicting results[14]. Preeclampsia is characterized by maternal endothelial dysfunction. Numerous endothelial dysfunctional markers have been identified in preeclamptic women, including an imbalance of anticoagulant and procoagulant factors and increased levels of fibronectin, endothelial cell adhesion molecules, and other coagulation cascade factors[15]. High lipid levels in the bloodstream cause their accumulation within endothelial cells. This accumulation reduces prostacyclin release, resulting in oxidative stress via endothelial dysfunction, a critical mechanism in the preeclampsia pathophysiology[16]. This study found significant elevations in serum triglyceride and TC levels and a substantial reduction in serum HDL-cholesterol levels in patients with late-onset preeclampsia. Consistently, a meta-analysis of studies examining the association between maternal hyperlipidemia and preeclampsia was recently suggested that women with pre-eclampsia had significantly higher triglyceride, total cholesterol, and non-HDL cholesterol levels and lower HDL-cholesterol level than normotensive women[17]. Significantly elevated total cholesterol, triglyceride, and LDL-cholesterol levels in pregnant women with preeclampsia suggested that these lipid measurements obtained in pregnancy follow-up may help identify women at increased risk of developing preeclampsia.

Preeclampsia is a hypertensive disorder associated with severe maternal and neonatal morbidity and mortality. Therefore, pregnant women at high risk of developing preeclampsia or severe preeclampsia should be identified as soon as possible to avoid adverse pregnancy outcomes. However, efforts for

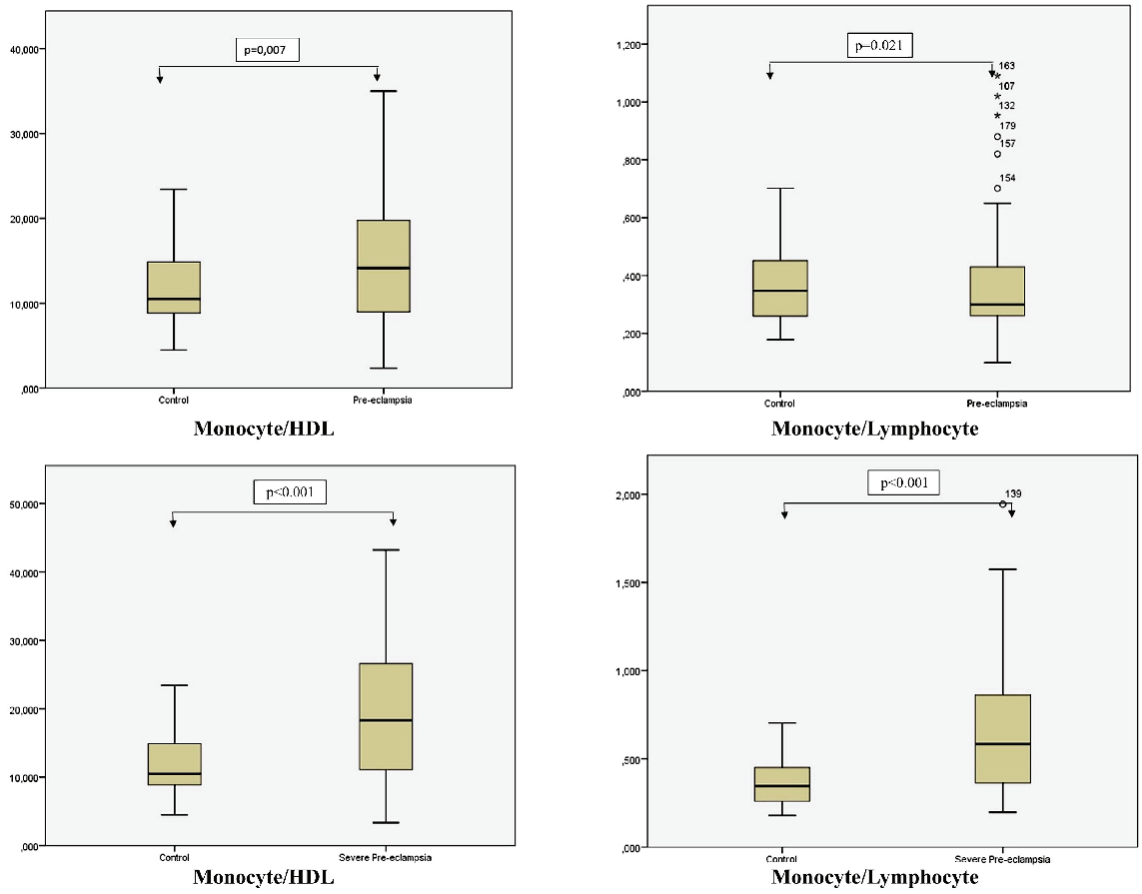

Figure 1. Comparison of monocyte/HDL cholesterol and monocyte/lymphocyte ratios of the study groups compared to the control group

HDL: High-density lipoprotein

Table 1. Clinical characteristics and laboratory data of the study and control groups

Characteristics	Control (n=96)	Preeclampsia (n=96)	Severe preeclampsia (n=61)	p-value[a]	p-value[b]	p-value[c]
Age (years)*	31 (20-42)	32 (18-44)	32 (20-44)	0.179	0.342	0.687
BMI (kg/m^2)*	29.65 (19.38-37.10)	32.8 (20.94-45.70)	29.14 (20.2-47.26)	**0.004**	**0.045**	**<0.001**
Systolic blood pressure (mmHg)*	110 (94-130)	140 (130-155)	170 (150-240)	**<0.001**	**<0.001**	**<0.001**
Diastolic blood pressure (mmHg)*	70 (60-93)	90 (80-102)	110 (90-133)	**<0.001**	**<0.001**	**<0.001**
Hemoglobin (g/L)*	12 (7.5-14.4)	11.5 (6.2-15)	11.7 (9.2-15.5)	0.704	0.414	0.697
Hematocrit (%)*	35 (25.7-40.9)	35.85 (22.4-44.4)	36.3 (26.4-44)	**0.005**	0.134	0.258
Platelet (10^3/mL)*	214 (97-307)	244 (71-431)	232 (84-475)	**0.012**	0.776	0.068
WBC (x10^3/µL)*	10.38 (5.60-14.09)	10.18 (6.14-18.9)	12.40 (6.72-23.70)	0.589	**<0.01**	**<0.001**
Neutrophil (x10^3/µL)*	7.53 (3.73-11.87)	6.29 (4.02-14.53)	8.75 (4.52-22.16)	**0.04**	**0.001**	**<0.001**
Lymphocyte (x10^3/µL)*	1.87 (1.21-2.85)	2.18 (1.11-4.62)	1.88 (0.71-4.56)	**<0.001**	0.860	0.022
Monocytes (x10^3/µL)*	0.67 (0.36-1.19)	0.66 (0.15-1.53)	1.16 (0.24-1.98)	0.222	**<0.001**	**<0.001**
BUN (mg/dL)*	6.05 (2.93-13.60)	8.37 (4.26-14.77)	10.6 (6.05-24.39)	**<0.001**	**<0.001**	**<0.001**
Creatinine (mg/dL)*	0.56 (0.40-0.74)	0.62 (0.51-0.86)	0.64 (0.43-1.07)	**<0.001**	**<0.001**	**0.181**
AST (U/L)*	16 (8-36)	18 (11-35)	20 (12-120)	**0.033**	**<0.001**	**0.003**
ALT (U/L)*	15 (6-53)	12 (6-27)	14 (6-172)	**0.018**	0.461	**0.006**
LDH (U/L)*	200 (152-309)	228 (135-411)	301 (172-701)	**0.002**	**<0.001**	**<0.001**
Uric acid (mg/dL)*	3.94 (2.76-5.9)	5.09 (2.24-6.9)	4.9 (2.57-8.03)	**<0.001**	**<0.001**	0.239
INR*	1 (0.88-1.27)	0.93 (0.83-1.17)	0.88 (0.76-1.15)	**<0.001**	**<0.001**	**<0.001**
APTT (sec)*	25.7 (16.9-68.6)	23.95 (14.8-51.1)	23.4 (18.5-36.7)	0.321	0.288	0.453
Fibrinogen (mg/dL)*	410.6 (306.9-633.7)	474.9 (298.9-759.0)	439.2 (134.8-726.6)	**<0.001**	0.166	**0.024**
CRP (mg/dL)*	0.46 (0.30-2.28)	0.68 (0.30-5.38)	1.4 (0.30-7.61)	0.258	**<0.001**	**<0.001**
Glucose (mg/dL)*	87 (65-161)	86 (58-197)	81 (53-197)	0.072	0.295	0.571
Triglyceride (mg/dL)*	208 (101-295)	242 (109-495)	255 (104-499)	**<0.001**	**<0.001**	0.419
Total cholesterol (mg/dL)*	186 (132-279)	194 (128-378)	245 (115-427)	**0.013**	**<0.001**	**0.005**
LDL-cholesterol (mg/dL)*	114.3 (61.3-163.4)	98.6 (21.5-210)	128.7 (42.5-272.7)	0.171	**0.022**	**0.005**
HDL-cholesterol (mg/dL)*	57.5 (34.4-107.0)	53.6 (31.8-79.5)	57.6 (36.5-126.3)	**0.017**	0.564	**0.008**
Neutrophil/Lymphocyte*	3.74 (2.06-8.72)	3.40 (1.10-5.90)	4.28 (1.89-20.14)	**0.007**	0.107	**<0.001**
Monocyte/Lymphocyte*	0.34 (0.17-0.70)	0.39 (0.09-1.09)	0.58 (0.19-1.94)	**0.021**	**<0.001**	**<0.001**
Platelet/Lymphocyte*	120.53 (41.28-200)	127.01 (29.06-208.48)	106.35 (38.99-388.7)	0.940	0.122	0.381
Monocyte/HDL*	10.51 (4.49-23.43)	14.15 (2.34-34.98)	18.30 (3.36-43.22)	**0.007**	**<0.001**	**0.003**
Neutrophil/HDL*	120.82 (47.04-256.37)	122.57 (58.24-303.98)	140.61 (44.42-449.08)	0.371	**0.018**	0.098
LDL/HDL*	1.82 (0.61-3.21)	1.98 (0.46-3.29)	2.04 (0.89-4.49)	0.846	0.149	0.275
Gravidity*	3.0 (1.0-7.0)	3.0 (1.0-6.0)	3.0 (1.0-7.0)	0.146	0.962	0.322
Parity*	1.0 (0.0-5.0)	1.0 (0.0-5.0)	0.0 (0.0-5.0)	0.388	0.084	0.292
Gestational age at birth (weeks)*	37 (34-39)	34 (39-37)	39 (37-34)	**0.006**	**<0.001**	**<0.001**
Birthweight (g)*	2925 (2230-3550)	2230 (3550-2740)	3550 (2740-1680)	**<0.001**	**<0.001**	**<0.001**

Table 1. Continued

Characteristics		Control (n=96)	Preeclampsia (n=96)	Severe preeclampsia (n=61)	p-value[a]	p-value[b]	p-value[c]
1st minute Apgar score*		8 (5-9)	5 (9-8)	9 (8-6)	**0.023**	**<0.001**	**<0.001**
5th minute Apgar score*		9 (6-10)	6 (10-9)	10 (9-7)	**0.040**	**<0.001**	**<0.001**
Umbilical cord pH*		7.37 (7.27-7.44)	7.27 (7.44-7.33)	7.44 (7.33-7.04)	**<0.001**	**<0.001**	**0.003**
Umbilical cord base excess*		-4.9 (-9.6-1.7)	-9.6 (-1.7-4.8)	-1.7 (-4.8-12.1)	**0.021**	0.106	0.863
Preeclampsia in obstetric history**		0 (0)	4 (4.1)	12 (19.6)	0.061	**<0.001**	**<0.001**
FGR in obstetric history**		4 (4.2)	20 (20.8)	24 (39.3)	**0.001**	**<0.001**	**0.020**
Mode of delivery**	Vaginal	22 (22.9)	16 (16.6)	5 (8.2)	0.365	**0.030**	0.201
	Cesarean section	74 (77.1)	80 (83.3)	56 (91.8)			
Gender**	Female	52 (54.2)	49 (51.0)	33 (54.1)	0.773	0.993	0.709
	Male	44 (45.8)	47 (49.0)	28 (45.9)			
NICU requirement**		11 (11.5)	27 (28.1)	42 (68.8)	**0.007**	**<0.001**	**<0.001**

*Median (min-Max) **n (%), BMI: Body mass index, WBC: White blood count, BUN: Blood urea nitrogen, AST: Aspartate Aminotransferase, ALT: Alanine aminotransferase, LDH: Lactate dehydrogenase, INR: International normalized ratio, APTT: Activated partial thromboplastin time, CRP: C-reactive protein test, LDL: Low-density lipoprotein, HDL: High-density lipoprotein, FGR: Fetal growth restriction, NICU: Neonatal intensive care unit.
[a]Shows statistical significance between preeclampsia and control groups.
[b]Shows statistical significance between severe preeclampsia and control groups.
[c]Shows statistical significance between preeclampsia and severe preeclampsia groups.
Significant p values are shown in bold

Table 2. ROC analysis showing the predictive value of inflammatory markers for preeclampsia

Variables	Cut-off	Sensitivity	Specificity	LR+	LR-	PPV	NPV	AUC (95% CI)	p-value
Neutrophil/ Lymphocyte	4.378	88.5 (80.4-94.1)	37.5 (27.8-48.0)	1.42	0.31	58.61	76.53	0.612 (0.532-0.612)	**0.006**
Monocyte/ Lymphocyte	0.314	57.3 (46.8-67.3)	68.8 (58.5-77.8)	1.83	0.62	64.75	61.71	0.596 (0.523-0.666)	**0.021**
Platelet/Lymphocyte	136.204	40.6 (30.7-51.1)	74.0 (64.0-82.4)	1.56	0.80	60.96	55.47	0.503 (0.430-0.576)	0.941
Monocyte/HDL	19.346	78.1 (69.4-88.2)	96.9 (91.1-99.4)	9.0	0.74	90.06	57.41	0.613 (0.541-0.683)	**0.006**
Neutrophil/HDL	86.376	91.7 (84.2-96.3)	24.0 (15.8-33.7)	1.21	0.35	54.7	74.2	0.537 (0.464-0.609)	0.375
LDL/HDL	2.663	16.7 (9.8-25.6)	95.8 (89.7-98.9)	4.00	0.87	80.0	53.5	0.508 (0.435-0.581)	0.848

LDL: Low-density lipoprotein, HDL: High-density lipoprotein, LR: Likelihood ratio, PPV: Positive predictive value, NPV: Negative predictive value, AUC: Area under the curve, ROC: Receiver operating characteristic, CI: Confidence interval. Significant p values are shown in bold

predicting pre-eclampsia remain elusive up to now. Given that inflammation is thought to be a critical step in preeclampsia development, several studies have been conducted to reveal alterations in hematological inflammatory markers in preeclampsia[18,19]. Systemic inflammatory indices formed from peripheral blood cells have recently gained much importance due to both simplicity of measurement and availability. These combined parameters are derived from basic measures such as the neutrophil-lymphocyte ratio and monocyte-lymphocyte ratio. They have been extensively used to make a diagnosis or predict the severity of septicemia, spondyloarthritis, and hepatocellular cancer[20-22]. Monocyte-lymphocyte ratio and neutrophil-lymphocyte ratio are hematological inflammatory indices determined by activators of inflammation (neutrophils/monocytes) and regulators of inflammation (lymphocytes) that are assumed to be effective predictors of systemic inflammation and immune balance. Although abnormal white blood cell counts have been documented in preeclampsia, their relevance in clinical evaluation, differential diagnosis, and prognostic assessment remains unknown. Recently, Kang et al.[23] carried out a meta-analysis including 3.982 patients who evaluated the predictive role of neutrophil-to-lymphocyte ratio in preeclampsia. They suggested that the neutrophil-to-lymphocyte ratio is a potential predictive biomarker because of its

considerable elevation in preeclamptic pregnancies, particularly in pregnant women with severe preeclampsia. Besides, Wang et al.[24] investigated the contribution of systemic hematological inflammation indices (including neutrophil-lymphocyte ratio and monocyte-lymphocyte ratio) to the pathogenesis of preeclampsia. In pregnant women with preeclampsia, they found higher monocyte-lymphocyte and monocyte-lymphocyte ratios. These results are in accordance with our findings. Therefore, the predictive value of the monocyte-lymphocyte ratio in preeclampsia and severe preeclampsia may arise due to its indicator properties for systemic inflammatory/immune response.

Preeclampsia is a multisystem condition with unknown pathogenetic mechanisms. Given that the only treatment option is delivery, early detection and prevention are critical for avoiding adverse perinatal outcomes. As a result, interest in the role of novel biomarkers that could aid in identifying high-risk pregnant women and give light on the disorder's etiology is developing. Our study data indicate that the monocyte/HDL ratio is higher in preeclamptic pregnant

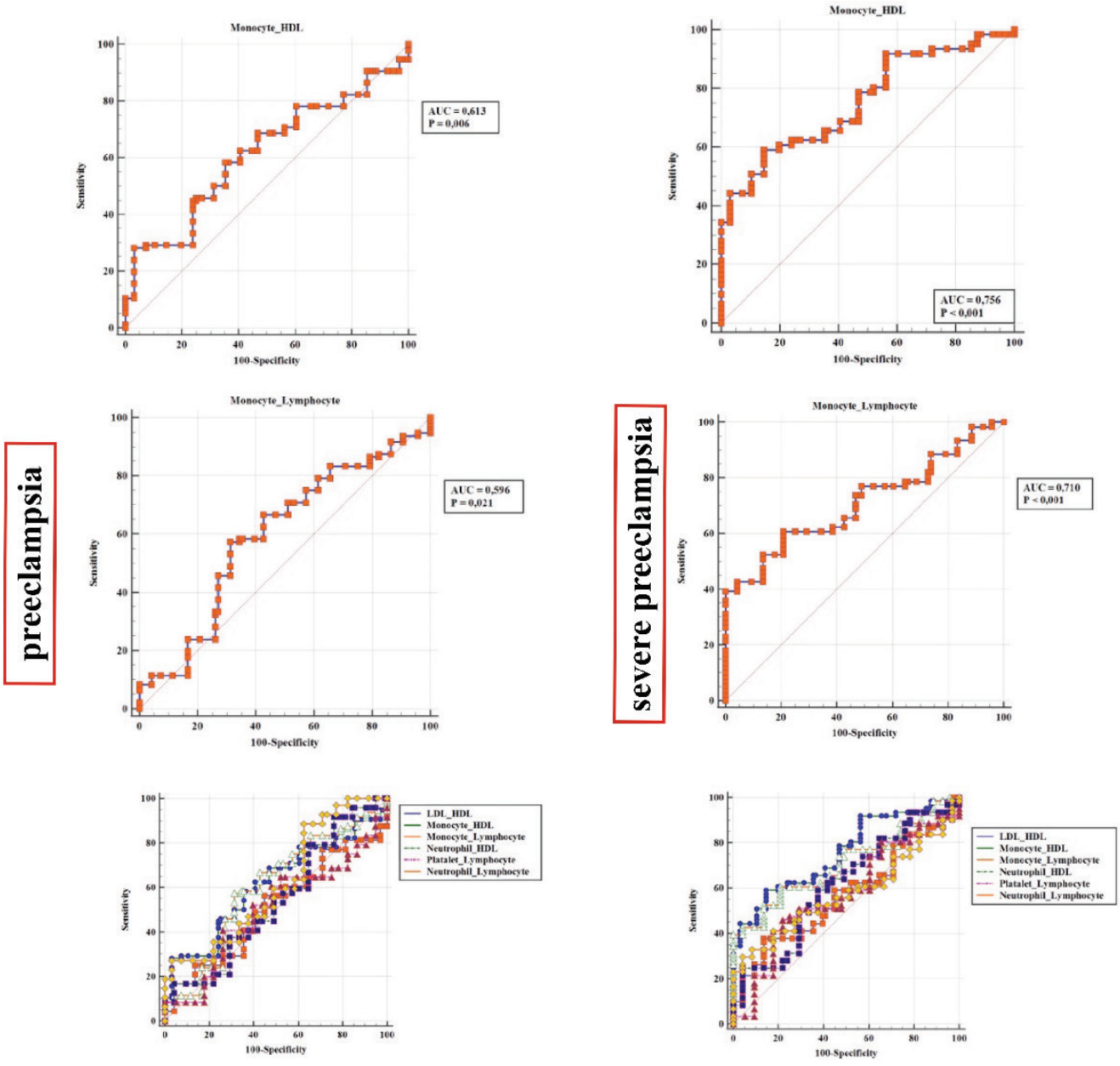

Figure 2. Receiver operating characteristic analysis showing the utility of monocyte/HDL and monocyte/lymphocyte ratios in patients with preeclampsia and severe preeclampsia

HDL: High-density lipoprotein, LDL: Low-density lipoprotein, AUC: Area under the curve

Table 3. ROC analysis showing the predictive value of inflammatory markers for severe preeclampsia

Variables	Cut-off	Sensitivity	Specificity	LR+	LR-	PPV	NPV	AUC (95% CI)	p-value
Neutrophil/ Lymphocyte	7.628	29.5 (18.5-42.6)	95.8 (89.7-98.9)	7.08	0.74	87.6	57.6	0.576 (0.495-0.655)	0.136
Monocyte/ Lymphocyte	0.452	60.7 (47.3-72.9)	79.2 (69.7-86.8)	2.91	0.50	74.4	66.8	0.710 (0.633-0.780)	**<0.001**
Platelet/Lymphocyte	94.889	45.9 (33.1-59.2)	78.1 (68.5-85.9)	2.10	0.69	67.7	59.1	0.573 (0.492-0.652)	0.131
Monocyte/HDL	16.652	59.0 (45.7-71.4)	85.4 (76.7-91.8)	4.05	0.48	80.2	67.6	0.756 (0.681-0.821)	**<0.001**
Neutrophil/HDL	137.575	59.0 (45.7-71.4)	62.5 (52.0-72.2)	1.57	0.66	61.1	60.4	0.612 (0.531-0.688)	**0.016**
LDL/HDL	2.353	37.7 (25.6-51.0)	86.5 (78.0-92.6)	2.78	0.72	73.6	58.1	0.568 (0.487-0.647)	0.166

LDL: Low-density lipoprotein, HDL: High-density lipoprotein, LR: Likelihood ratio, PPV: Positive predictive value, NPV: Negative predictive value, AUC: Area under the curve, CI: Confidence interval, ROC: Receiver operating characteristic

women, and it is a useful biochemical marker for clinical prognosis and disease severity assessment in patients with preeclampsia. Also, the results of this study revealed that the monocyte/HDL ratio is independently associated with both preeclampsia and severe preeclampsia. To the best of our knowledge, this is the first report evaluating maternal serum monocyte/HDL ratio in pregnant women with preeclampsia and severe preeclampsia. Monocytes account for approximately 3-8% of all circulating leukocytes and, along with other granular and agranular cell lines such as basophils, eosinophils, lymphocytes, and neutrophils, are critical elements of innate immunity[25]. Monocytes play an essential role in the onset and regression of inflammation in tissues; this is accomplished primarily through phagocytosis, the release of pro-inflammatory mediators, the presence of reactive oxygen species, and activation of the acquired immune system. By contrast, HDL cholesterol counteracts monocytes' pro-inflammatory and pro-oxidant effects by inhibiting the oxidation of LDL molecules and macrophage migration, as well as promoting cholesterol efflux from these cells[26]. Along with the well-established anti-inflammatory and antioxidant properties of HDL-cholesterol, it has been recently claimed that these molecules act as a suppressive factor in regulating monocyte activation, proliferation, and differentiation of monocyte progenitor cells. The monocyte count to HDL-cholesterol ratio has been reported to be a novel predictor and prognostic indicator of mortality and morbidity in various chronic inflammatory diseases, including cardiovascular disease, chronic kidney disease, abdominal aortic aneurysm, intracerebral hemorrhage, hypertension, and metabolic syndrome[27]. In accordance, recently, Dincgez Cakmak et al.[28] found higher serum monocyte/HDL ratio levels in women with polycystic ovary syndrome (PCOS) that underlie chronic low-grade inflammation in the molecular mechanisms of this syndrome. They showed that monocyte/HDL ratio was an independent predictor of metabolic syndrome in patients with PCOS. Previous studies showed a significant association between monocyte/HDL ratio and inflammation and oxidative stress. Our findings are parallel to the previous studies' observations that confirm our results.

Study Limitations

The current study has a few limitations, such as a cross-sectional study design and the single-center population. However, the number of participants in the study was sufficient to assess the predictive value of inflammatory markers. The major limitation of the study was that only pregnant women with late-onset preeclampsia were included in the study, and cases that presented before 34 weeks of gestation were not included. Additionally, we could only analyze hemato-lipid parameters from the third trimester. Serial maternal serum measurements, including measurements in the first and second trimesters, were not performed during pregnancy. The study's main strength was the first assessment of monocyte/HDL ratio in pregnant women complicated with preeclampsia and severe preeclampsia. The prospective cohort design was additional strength.

Conclusion

This study found that serum triglyceride and TC levels were significantly higher in pregnant women with late-onset preeclampsia, while serum HDL-cholesterol levels were significantly lower. Furthermore, when hematological and lipid parameters were compared, it was shown that preeclamptic and severe preeclamptic pregnant women had considerably greater monocyte/HDL and monocyte/lymphocyte ratios, whereas the monocyte/HDL ratio was independently related to preeclampsia and severe preeclampsia. The results of this study also revealed that the measurement of monocyte/HDL ratio in the pregnant population could be a useful clinical tool for predicting the development of preeclampsia, and further studies must reveal the role of dyslipidemia in elucidating the pathophysiology of complications associated with preeclampsia.

Table 4. Correlations between hemato-lipid profile and inflammatory markers in the control and study groups

Correlations															
Groups			BMI	Hemoglobin	PLT	WBC	Total cholesterol	LDL-cholesterol	HDL-cholesterol	Neutrophil/ Lymphocyte	Monocyte/ Lymphocyte	Platelet/ Lymphocyte	Monocyte/ HDL	Neutrophil/ HDL	LDL/ HDL
Control	Age	Spear rho	-0.030	-0.066	-0.076	0.011	0.042	-0.046	0.010	-0.004	-0.120	-0.004	-0.119	-0.050	-0.078
		p-value	0.770	0.523	0.463	0.913	0.681	0.655	0.919	0.968	0.245	0.971	0.248	0.626	0.452
	BMI	Spearman rho		0.303	-0.047	-0.140	0.093	-0.147	0.101	0.110	-0.076	0.085	-0.170	0.039	-0.046
		p-value		**0.003**	0.647	0.175	0.369	0.154	0.329	0.284	0.460	0.409	0.097	0.708	0.657
	Hemoglobin	Spearman rho			-0.150	0.020	0.236	0.238	0.232	0.122	0.064	-0.048	-0.052	-0.049	0.114
		p-value			0.143	0.847	**0.021**	**0.019**	**0.023**	0.235	0.535	0.644	0.616	0.635	0.267
	PLT	Spearman rho				0.060	0.032	0.057	-0.022	0.257	-0.126	0.750	-0.189	0.195	0.124
		p-value				0.558	0.754	0.582	0.833	**0.012**	0.223	**<0.001**	0.065	0.057	0.228
	WBC	Spearman rho					-0.357	-0.426	-0.206	0.665	0.257	0.008	0.349	0.739	-0.367
		p-value					**<0.001**	**<0.001**	**0.045**	**<0.001**	**0.011**	0.939	**<0.001**	**<0.001**	**<0.001**
	Total cholesterol	Spearman rho						0.508	0.074	-0.014	0.053	0.233	-0.165	-0.274	0.477
		p-value						**<0.001**	0.471	0.889	0.608	**0.022**	0.107	**0.007**	**<0.001**
	LDL-cholesterol	Spearman rho							0.297	-0.040	0.246	0.281	-0.149	-0.488	0.627
		p-value							**0.003**	0.698	**0.016**	**0.005**	0.147	**<0.001**	**<0.001**
	HDL-cholesterol	Spearman rho								-0.266	-0.075	-0.142	-0.550	-0.710	-0.451
		p-value								**0.009**	0.469	0.168	**<0.001**	**<0.001**	**<0.001**
	Neutrophil/ lymphocyte	Spearman rho									0.430	0.580	0.223	0.710	0.061
		p-value									**<0.001**	**<0.001**	**0.029**	**<0.001**	0.552
	Monocyte/ lymphocyte	Spearman rho										0.299	0.698	0.182	0.179
		p-value										**0.003**	**<0.001**	0.075	0.081
	Platelet/ lymphocyte	Spearman rho											0.012	0.272	0.386
		p-value											0.905	**0.007**	**<0.001**
	Monocyte/HDL	Spearman rho												0.502	0.236
		p-value												**<0.001**	**0.021**
	Neutrophil/HDL	Spearman rho													0.024
		p-value													0.816

Table 4. Continued

Correlations															
Groups			BMI	Hemoglobin	PLT	WBC	Total cholesterol	LDL-cholesterol	HDL-cholesterol	Neutrophil/ Lymphocyte	Monocyte/ Lymphocyte	Platelet/ Lymphocyte	Monocyte/ HDL	Neutrophil/ HDL	LDL/ HDL
Preeclampsia	Age	Spearman rho	0.641	0.021	-0.144	-0.083	-0.184	-0.368	-0.145	-0.224	0.150	-0.085	0.157	-0.057	-0.331
		p-value	**<0.001**	0.837	0.163	0.422	0.072	**<0.001**	0.160	**0.029**	0.144	0.409	0.127	0.582	**0.001**
	BMI	Spearman rho		0.022	-0.110	0.021	-0.228	-0.428	-0.017	-0.037	0.009	-0.025	0.064	0.020	-0.403
		p-value		0.833	0.285	0.841	**0.025**	**<0.001**	0.869	0.722	0.931	0.806	0.535	0.844	**<0.001**
	Hemoglobin	Spearman rho			-0.581	0.321	0.089	0.116	0.348	0.084	0.055	-0.519	-0.048	-0.054	0.063
		p-value			**<0.001**	**0.001**	0.388	0.259	**0.001**	0.414	0.597	**<0.001**	0.640	0.602	0.543
	PLT	Spearman rho				0.121	0.246	0.133	-0.045	0.130	0.006	0.646	-0.085	0.151	0.011
		p-value				0.241	**0.016**	0.196	0.662	0.208	0.953	**<0.001**	0.412	0.143	0.912
	WBC	Spearman rho					0.018	-0.031	0.182	0.244	0.016	-0.213	0.229	0.478	-0.158
		p-value					0.862	0.764	0.075	**0.016**	0.874	**0.037**	**0.025**	**<0.001**	0.124
	Total cholesterol	Spearman rho						0.857	0.451	0.315	0.127	0.287	-0.324	-0.202	0.574
		p-value						**<0.001**	**<0.001**	**0.002**	0.219	**0.005**	**0.001**	**0.048**	**<0.001**
	LDL-cholesterol	Spearman rho							0.406	0.375	0.103	0.249	-0.376	-0.186	0.753
		p-value							**<0.001**	**<0.001**	0.317	**0.014**	**<0.001**	0.069	**<0.001**
	HDL-cholesterol	Spearman rho								0.038	-0.062	-0.017	-0.580	-0.636	-0.202
		p-value								0.711	0.549	0.871	**<0.001**	**<0.001**	**0.048**
	Neutrophil/ lymphocyte	Spearman rho									0.524	0.561	0.088	0.506	0.354
		p-value									**<0.001**	**<0.001**	0.392	**<0.001**	**<0.001**
	Monocyte/ lymphocyte	Spearman rho										0.282	0.581	0.234	0.167
		p-value										0.005	**<0.001**	**0.022**	0.104
	Platelet/ lymphocyte	Spearman rho											-0.144	0.139	0.169
		p-value											0.163	0.178	0.099
	Monocyte/ HDL	Spearman rho												0.553	0.001
		p-value												**<0.001**	0.990
	Neutrophil/ HDL	Spearman rho													0.202
		p-value													**0.049**

Table 4. Continued

Correlations															
Groups			BMI	Hemoglobin	PLT	WBC	Total cholesterol	LDL-cholesterol	HDL-cholesterol	Neutrophil/ Lymphocyte	Monocyte/ Lymphocyte	Platelet/ Lymphocyte	Monocyte/ HDL	Neutrophil/ HDL	LDL/ HDL
Severe Preeclampsia	Age	Spearman rho	-0.037	-0.099	0.041	0.012	-0.294	-0.353	-0.112	0.159	0.074	0.087	0.079	0.076	-0.309
		p-value	0.776	0.446	0.753	0.930	**0.022**	**0.005**	0.389	0.221	0.570	0.503	0.543	0.559	**0.015**
	BMI	Spearman rho		-0.096	-0.106	0.178	-0.110	-0.156	-0.189	0.069	0.118	-0.221	0.284	0.233	0.015
		p-value		0.464	0.417	0.170	0.397	0.230	0.144	0.597	0.366	0.087	**0.027**	0.071	0.911
	Hemoglobin	Spearman rho			-0.040	-0.011	0.121	0.061	0.330	-0.184	-0.095	-0.264	-0.153	-0.128	-0.126
		p-value			0.759	0.931	0.354	0.639	**0.009**	0.155	0.468	**0.040**	0.239	0.326	0.332
	PLT	Spearman rho				0.195	0.290	0.304	0.080	-0.263	-0.268	0.517	-0.077	-0.055	0.272
		p-value				0.131	**0.023**	0.017	0.538	**0.041**	**0.037**	**<0.001**	0.556	0.676	**0.034**
	WBC	Spearman rho					0.008	0.029	-0.086	0.471	0.090	0.017	0.245	0.797	0.050
		p-value					0.954	0.823	0.512	**<0.001**	0.489	0.895	0.057	**<0.001**	0.700
	Total cholesterol	Spearman rho						0.934	0.514	-0.288	-0.200	0.065	-0.395	-0.346	0.614
		p-value						**<0.001**	**<0.001**	**0.024**	0.122	0.616	**0.002**	**0.006**	**<0.001**
	LDL-cholesterol	Spearman rho							0.356	-0.316	-0.209	0.052	-0.306	-0.276	0.789
		p-value							**0.005**	**0.013**	0.107	0.693	**0.016**	**0.032**	**<0.001**
	HDL-cholesterol	Spearman rho								-0.039	-0.043	0.127	-0.632	-0.578	-0.223
		p-value								0.765	0.740	0.329	**<0.001**	**<0.001**	0.084
	Neutrophil/ lymphocyte	Spearman rho									0.653	0.437	0.189	0.577	-0.386
		p-value									**<0.001**	**<0.001**	0.145	**<0.001**	**0.002**
	Monocyte/ lymphocyte	Spearman rho										0.297	0.598	0.243	-0.257
		p-value										**0.020**	**<0.001**	0.059	**0.046**
	Platelet/ lymphocyte	Spearman rho											-0.135	0.001	-0.084
		p-value											0.298	0.995	0.521
	Monocyte/HDL	Spearman rho												0.518	0.053
		p-value												**<0.001**	0.683
	Neutrophil/HDL	Spearman rho													0.017
		p-value													0.899

BMI: Body mass index, WBC: White blood count, LDL: Low-density lipoprotein, HDL: High-density lipoprotein. Significant p values are shown in bold

Table 5. Odds ratios for preeclampsia risk calculated by multivariate regression analysis

Variables	Odds ratio	95% CI for odds ratio		p-value
		Lower	Upper	
Age	0.916	0.861	0.975	**0.006**
Monocyte/HDL ratio	1.094	1.009	1.185	**0.029**
Triglyceride	1.008	1.000	1.016	0.061
Total cholesterol	1.040	1.015	1.065	**0.001**
LDL cholesterol	0.953	0.919	0.989	**0.011**
LDL/HDL	1.564	0.507	4.823	0.437

LDL: Low-density lipoprotein, HDL: High-density lipoprotein, CI: Confidence interval. Significant p values are shown in bold

Table 6. Odds ratios for severe preeclampsia risk calculated by multivariate regression

Variables in the equation	Odds ratio	95% CI for odds ratio		p-value
		Lower	Upper	
Age	0.906	0.801	1.024	0.114
BMI	0.953	0.850	1.070	0.417
Neutrophil/lymphocyte ratio	2.198	0.893	5.410	0.086
Monocyte/lymphocyte ratio	0.020	0.000	123.594	0.380
Platelet/lymphocyte ratio	0.996	0.976	1.017	0.734
Monocyte/HDL ratio	1.731	1.218	2.459	**0.002**
Neutrophil/HDL ratio	0.978	0.948	1.009	0.162
LDL/HDL	0.247	0.021	2.946	0.269
Triglyceride	1.022	1.007	1.038	**0.005**
Total cholesterol	1.063	1.015	1.113	**0.010**
LDL cholesterol	0.992	0.925	1.064	0.818

BMI: Body mass index, LDL: Low-density lipoprotein, HDL: High-density lipoprotein, CI: Confidence interval. Significant p values are shown in bold

Ethics

Ethics Committee Approval: Ethical Committee approval was obtained from the Inonu University School of Medicine Clinical Research Ethics Committee for the study, and the researchers committed to comply with the World Medical Association Declaration of Helsinki (including improvements added in 2013) for the conduct of medical research on human subjects throughout the study (approval number: 2019/56).

Informed Consent: All participants gave their written informed consent prior to their inclusion in the study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: R.M., Design: R.M., Data Collection or Processing: Ş.Y., Analysis or Interpretation: Ş.Y., Literature Search: N.Z.Ç., H.Ö., Writing: R.M.

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An evaluation of maternal serum dynamic thiol-disulfide homeostasis and ischemia modified albumin changes in pregnant women with COVID-19

COVID-19 olan gebe kadınlarda maternal serum dinamik tiyol-disülfit dengesinin ve iskemi modifiye albümin değişikliklerinin değerlendirilmesi

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Abstract

Objective: It is thought that oxidative stress, free radicals, reactive oxygen species and reactive nitrogen species affect the pathophysiology of coronavirus disease-2019 (COVID-19). This study aimed to evaluate the oxidative status in pregnant patients with COVID-19 infection according to the changes seen in the levels of maternal serum thiol-disulfide and ischemia-modified albumin (IMA).

Materials and Methods: A study group was formed of 40 pregnant women with confirmed COVID-19 infection (study group) and a control group of 40 healthy pregnant women with no risk factors determined. In this prospective, case-controlled study, analyses were made of the maternal serum native thiol, total thiol, disulfide, IMA, and disulfide/native thiol concentrations.

Results: The maternal serum native thiol and total thiol concentrations in the study group were determined to be statistically significantly lower (p=0.007 and p=0.006, respectively), and the disulfide/native thiol ratio was higher but not to a level of statistical significance (p=0.473). There was no difference between the two groups regarding IMA levels (p=0.731).

Conclusion: The thiol-disulfide balance was seen to shift in the oxidant direction in pregnancies with COVID-19, which might support the view that ischemic processes play a role in the etiopathogenesis of this novel disease.

Keywords: COVID-19, ischemia-modified albumin, pregnancy outcomes, thiol-disulfide homeostasis

Öz

Amaç: Koronavirüs hastalığı-2019 (COVID-19) patofizyolojisinde oksidatif stres, serbest radikaller, reaktif oksijen türleri ve reaktif nitrojen türlerinin rol oynadığı düşünülmektedir. Bu çalışmanın amacı, maternal serum tiyol-disülfit ve iskemi modifiye albümin (İMA) düzeylerinde görülen değişikliklere göre COVID-19 enfeksiyonu olan gebe hastalarda oksidatif durumu değerlendirmektir.

Gereç ve Yöntemler: COVID-19 enfeksiyonu tanısı konulan 40 gebe kadın (çalışma grubu) ve risk faktörü olmayan 40 sağlıklı gebe kadından oluşan kontrol grubu şeklinde gruplar belirlendi. Bu prospektif, olgu-kontrol çalışmasında, maternal serum native tiyol, total tiyol, disülfit, İMA ve disülfit/native tiyol konsantrasyonlarının analizleri yapıldı.

Bulgular: Çalışma grubunda maternal serum native tiyol ve total tiyol konsantrasyonlarının istatistiksel olarak anlamlı daha düşük (p=0,007 ve p=0,006, sırasıyla), disülfit/native tiyol oranının ise daha yüksek olduğu ancak istatistiksel olarak anlamlı düzeyde olmadığı belirlendi (p=0,473). IMA düzeyleri açısından iki grup arasında fark izlenmedi (p=0,731).

PRECIS: The thiol-disulfide balance was seen to shift in the oxidant direction in pregnancies with COVID-19.

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Sonuç: Gebelikte COVID-19 varlığında tiyol-disülfit dengesinin oksidan yöne kaydığı görülmüştür. Bu durum, bu yeni hastalığın etiyopatogenezinde iskemik süreçlerin varlığını desteklemektedir.

Anahtar Kelimeler: COVID-19, iskemi modifiye albümin, gebelik sonuçları, tiyol-disülfit dengesi

Introduction

The emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in China in December 2019, as the agent of coronavirus disease-2019 (COVID-19), affected the whole world. This highly contagious infectious disease was declared a pandemic on 11.02.2020 by the World Health Organization (WHO)[1]. Researchers worldwide have been studying to reveal the complex pathophysiological mechanisms behind this deadly disease at the beginning of the pandemic. However, knowledge of this disease is still very limited and there is no efficient treatment at present. SARS-CoV-2 infects the host respiratory epithelium by cleaving to angiotensin-converting enzyme 2 (ACE-2) receptors. ACE-2 is preponderantly expressed in type II alveolar cells in the lungs[2]. The invasion of the respiratory epithelium usually results in acute respiratory distress syndrome (ARDS). As there is an incremental effect on the permeability of the alveolar-capillary membrane, microthrombus, refractory hypoxemia, and bilateral pulmonary infiltrates can be seen in ARDS[3]. Hypoxia and impaired perfusion seem to be the main factors responsible for most systemic complications of COVID-19. Therefore, it has been suggested that oxidative stress (OS), free radicals, reactive oxygen species (ROS), and reactive nitrogen species (RNS) affect the pathophysiology of COVID-19. Moreover, as it has been shown that viral infections could activate the free radical production and could deplete antioxidants, SARS-CoV-2 may trigger OS, in the same way as other RNA viruses[4,5].

During pregnancy, there is a physiological increase in the oxygen consumption of tissues due to the needs of both mother and fetus. Physiological immune tolerance is maintained, thereby allowing the growth of the semi-allograft fetus[6]. Although concerns have been raised by physicians about the potential effects of COVID-19 on pregnancy, the above-mentioned adaptive changes may be of benefit to pregnant women as an increased pro-inflammatory cytokine response (cytokine storm) in the host has been reported to be the main pathological event in severe and critical COVID-19 cases[7].

Thiols are organic compounds, which contain a sulfhydryl group that can oxidate to covalent disulfide bonds in OS states. This dynamic balance of thiol-disulfide is essential for the antioxidant defense system, critical cell functions such as cellular transcription, cellular signal transmission, detoxification, enzymatic and apoptotic pathways[8-10]. Furthermore, the thiol-disulfide homeostasis is a good reflection of the cellular redox system[11]. ACE-2 and SARS-CoV-2 spike proteins have been shown to be highly disrupted when all disulfide molecules are reduced to thiol groups. Hence, it has been concluded that this computational result may provide molecular principle for discriminative COVID-19 cellular signaling through OS[12]. A recent study showed that COVID-19 patients had depleted thiol status, and therefore concluded that this could be an effective biomarker in the prediction of the severity of COVID-19[11].

Ischemic conditions activate substantial modifications in the metal-binding capability of albumin, resulting in the altered oxidized form known as ischemia-modified albumin (IMA)[13]. Although OS has generally been examined in ischemic cardiac pathologies, ischemia and reperfusion in different tissues may result in elevated IMA levels. Normal trophoblast evolution is related to ischemic conditions and increased IMA concentrations. Higher IMA levels have also been reported in pregnancies with perinatal hypoxia, fetal growth restriction and fetal distress[9,14].

Although the course of pregnancy in women with COVID-19 infection is similar to that of female adult patients of the same age group, there have been reports in literature of increased rates of obstetric complications such as fetal distress, preterm labor and higher cesarean section rates[7]. These complications could be explained by OS in COVID-19 infection. No previous study could be found in the literature that has evaluated the oxidative status of pregnant women with COVID-19 infection, through the examination of the maternal serum thiol-disulfide balance and IMA levels.

Therefore, this study aimed to evaluate the oxidative status of pregnant women with COVID-19 by analyzing the changes in maternal serum dynamic thiol-disulfide homeostasis and IMA levels.

Materials and Methods

This prospective, case-control study was approved by the Ethics Committee of The Republic of Turkey Ministry of Health Ankara City Hospital (E1-20-954). This hospital is a tertiary-level reference hospital, which has played a leading role in COVID-19 management throughout the pandemic[15]. All the pregnant women included in the study provided informed consent for participation. The study included pregnant women who presented at the Department of Obstetrics and Gynecology between June 19-July 16, 2020. The required data were collected from patient records.

For the calculation of the sample size required by the study, power analysis was performed using G*Power 3.1.9.4 statistical software on the basis of previous study results. Target alpha (α) and 1-beta (β) error levels were taken as 0.05 and 0.95, respectively, and to obtain 95% power, a minimum of 34 patients were required in each group (total 68)[16,17].

The study group was formed of 40 pregnant women with a confirmed diagnosis of COVID-19 infection during clinical follow-up and a control group was formed of 40 healthy pregnant women, selected to be similar in terms of demographic

characteristics. The inclusion criteria were defined as pregnant women with confirmed COVID-19 infection positivity, who were hospitalized in the relevant period. Patients were excluded from the study if they had any systemic comorbidities or were smokers. The COVID-19 diagnosis was made according to the results of real-time polymerase chain reaction (RT-PCR) test applied to nasopharyngeal and oropharyngeal smears[18]. Gestational age was calculated according to the last date of menstruation or ultrasonography in the first trimester. Patients with ongoing pregnancy were followed up, and their neonatal results were recorded.

Study Parameters

Comparisons were made between the groups of maternal age, body mass index (BMI), gravida, parity, previous miscarriage, gestational age at diagnosis, pregnancy status, initial laboratory tests, gestational age at birth, the type of delivery, labor anesthesia, birth weight, Apgar scores at 1 and 5 mins, neonatal outcomes, admission to neonatal intensive care unit (NICU), clinical characteristics and obstetric outcomes.

A record was made of the laboratory parameters on hospital admission, including hemoglobin, hematocrit, leukocyte, neutrophil, lymphocyte, neutrophil- lymphocyte ratio, platelets, erythrocyte sedimentation rate, C-reactive protein, procalcitonin interleukin 6 (IL-6), blood urea nitrogen, creatinine, alanine aminotransferase, and aspartate aminotransferase.

At the time of hospital admission, blood samples were taken from all patients. For the maternal blood serum sample, 5-10 cm^3 of blood was taken into a biochemistry tube. Following centrifugation at 3,500 rpm for 10 min, sera were obtained, and the samples were transferred into Eppendorf tubes and stored at -80 °C until assay. When the number of patients required for the study was reached, analysis was made of the specimens in the biochemistry laboratory of our institution.

The thiol-disulfide levels were determined using the spectrophotometric procedure defined by Erel and Neselioglu[8]. The albumin-cobalt binding test was used to determine the presence of IMA[19].

Statistical Analysis

Statistical analysis of the study data was performed using IBM SPSS Statistics 22.0 software (IBM Corp., Armonk, NY, USA). Whether or not the data conformed to the normal distribution was assessed with the Kolmogorov-Smirnov test and histograms. As the data were seen to be normally distributed, descriptive numerical statistics were stated as mean and standard deviation values, and categorical variables as number (n) and percentage (%). The Student's t-test was applied to comparisons between groups of numerical data, and the chi-square test was used to compare categorical variables. The level of statistical significance was set at $p<0.05$.

Results

The neonatal results and demographic data of maternal age, BMI, gestational age at diagnosis, gravida, parity, gestational status, delivery mode, birthweight, Apgar scores, and NICU admission rates are presented in Table 1. The results of the analyses of IMA, native thiol, total thiol, disulfide, and disulfide/native thiol ratio are shown in Table 2. Inflammatory and other laboratory parameters are presented in Table 3.

The clinical course in the study group of pregnant women diagnosed with COVID-19 was seen to be similar to that of the general population [mild n=31 (77.5%), moderate n=5 (12.5%), severe n=4 (10%)[20,21]. There was seen to be no difference between the two groups in terms of demographic features and neonatal outcomes ($p>0.05$). The rate of admission to NICU was seen to be higher (23.1%) in the study group of pregnant patients with COVID-19, but not to a statistically significant level ($p=0.142$).

There was determined to be a statistically significant difference between the groups in terms of the thiol-disulfide homeostasis parameters and IMA levels. Significantly lower native thiol and total thiol values were observed in the COVID-19 group than in the control group ($p=0.007$ and $p=0.006$, respectively). A higher but not statistically significant disulfide/native thiol ratio was determined in the COVID-19 group ($p=0.473$). IMA levels were seen to be similar in the two groups ($p=0.731$) (Table 2). Inflammation parameters were determined to be statistically significantly higher in the COVID-19 group than in the control group ($p<0.001$, $p=0.047$ and $p=0.020$, respectively) (Table 3).

Discussion

This study aimed to evaluate the role of OS in pregnant women with COVID-19 infection. The study results demonstrated a significant decrease in native thiol and total thiol concentrations in the study group supporting the tendency of thiol-disulfide homeostasis shifting to oxidant status. OS is associated with various conditions such as diabetes mellitus, hypertensive disorders, ischemic coronary artery diseases, premature aging, and different types of cancers[9,22]. Increased free radical production and antioxidant consumption can be observed in viral infections, and thus infections caused by RNA viruses, including Herpes, HIV 1, Hepatitis B, C, D, respiratory viruses and coronaviruses could trigger OS[23,24].

Cytokine storm related to the release of several cytokines such as interleukin 2 (IL-2), interleukin 6 (IL-6), interleukin 7 (IL-7) and tumor necrosis factor-alpha has been defined in the etiopathogenesis of COVID-19[25]. However, ROS and RNS can also cause an oxidative storm that may cause lipid peroxidation, hyalinization, and alterations in the pulmonary alveolar membranes leading to fatal respiratory consequences[4]. In elderly patients and those with comorbid conditions, a poor prognosis has also been reported to be associated with exacerbation of pre-existing OS by viral infections[26].

Because of physiological adaptations in the cardiorespiratory system and the immune system during pregnancy, pregnant women may become more vulnerable to infections in general. Resistance to hypoxia is lower during pregnancy because of an increase in the transverse diameter of the thorax and elevation of the diaphragm. Because of lung volume changes and vasodilation, mucosal edema may develop with increased secretions in the respiratory tract. Moreover, susceptibility to infection caused by intracellular organisms such as viruses is increased with changes in cellular immunity. Although there is a

Table 1. Demographic features, clinical characteristics and obstetric outcomes of the study and control groups[a]

Parameters	Group 1 COVID-19 (n=40)	Group 2 Control (n=40)	p-value[b,c]
Maternal age (years)	28.6±6.7 (15-45)	27.3±5.1 (17-41)	0.353[b]
BMI (kg/m^2)	27.8±5.7 (21.4-43.9)	27.5±4.7 (16.6-39.2)	0.764[b]
Gravidity	2.7±1.5 (1-7)	2.52±1.4 (1-6)	0.594[b]
Parity	1.3±1 (0-4)	1±1.14 (0-5)	0.261[b]
Previous miscarriage	0.2±0.5 (0-2)	0.3±0.5 (0-2)	0.692[b]
Gestational age at diagnosis (weeks)	27.9±11.1 (5-40)	27.3±10.7 (6-40)	0.870[b]
Pregnancy status Ongoing pregnancy (n=57) (72.2%) Delivered (n=21) (26.6%) Miscarriage (n=1) (1.3%)	 (n=26) (65%) (n=13) (32.5%) (n=1) (2.5%)	 (n=31) (79.5%) (n=8) (20.5%) (n=0) (0%)	0.221[c]
Gestational age at birth (weeks)	37.0±3.6(28-41)	38.2±1.1 (36-40)	0.263[b]
Route of delivery (n=21) Vaginal (n=7) (33.3%) Cesarean Section (n=14) (66.7%)	 (n=5) (38.5%) (n=8) (61.5%)	 (n=2) (25%) (n=6) (75%)	0.525[c]
Labor anesthesia (n=14) Regional (n=12) (57.1%) General (n=2) (9.5%)	 (n=7) (53.8%) (n=1) (7.7%)	 (n=5) (62.5%) (n=1) (12.5%)	0.797[c]
Birth weight (g)	2923±798 (1200-3780)	3249±275 (2900-3700)	0.197[b]
1st minute Apgar score	8.0±1.15 (6-9)	7.75±0.7 (7-9)	0.546[b]
5th minute Apgar score	9.4±0.8 (8-10)	9.3±0.5 (9-10)	0.779[b]
Neonatal intensive care unit (NICU) admission rate (n, %)	3/13 (23.1%)	0/8 (0%)	0.142[c]

BMI: Body mass index, NICU: Neonatal intensive care unit, COVID-19: Coronavirus disease-2019
[a]Values are given as number (percentage) or mean ± standard deviation (range)
[b]Statistical analysis was performed using the Independent sample test (t-test)
[c]Statistical analysis was performed using the chi-square test

Table 2. Comparisons of thiol-disulfide homeostasis parameters and IMA levels between the groups[a]

Parameters	Group 1 COVID-19 (n=40)	Group 2 Control (n=40)	p-value[b]
IMA (U/mL)	0.67±0.02 (0.63-0.74)	0.68±0.01 (0.66-0.71)	0.731
Native thiol (µmol/L)	356.6±42.87 (260-452)	381.45±37.45 (309-461)	**0.007**
Total thiol (µmol/L)	396.15±43.13 (293-487)	421.87±38.31 (333-511)	**0.006**
Disulfide (µmol/L)	19.77±5.6 (7.5-31)	20.21±6.01 (2.5-30)	0.737
Disulfide/native thiol (%)	5.63±1.78 (0.60-8.10)	5.35±1.66 (2.51-9.54)	0.473

IMA: Ischemia-modified albumin, COVID-19: Coronavirus disease-2019
[a]Values are given as number (percentage) or mean ± standard deviation (range)
[b]Statistical analysis was performed using the Independent sample test (t-test)

lack of data, current literature supports the view that the course of COVID-19 in pregnant women is not different to that of non-pregnant women[27]. The most common symptoms in pregnant women are mild or moderate cold/flu-like symptoms[7,28]. However, it has also been reported that poor prognosis can also be observed in pregnant women, especially in those with comorbid diseases[29]. There have also been reported to be higher rates of obstetric complications such as preterm birth, pre-labour rupture of membranes, pre-eclampsia and cesarean delivery due to fetal distress[30].

Thiols are organic molecules including sulfhydryl groups, which play a critical role in oxidation-reduction reactions and redox balance. Thiols can be oxidized and transformed into disulfides, which may reduce to thiols, thereby maintaining dynamic thiol-disulfide homeostasis. The new automated process improved by Erel and Neselioglu[8], has enabled the measurement of native thiol, total thiol, and disulfide levels. It has been previously reported that disulfide levels are increased in inflammatory diseases and are decreased in malignant diseases[31]. Therefore, it has been stated that the increase in disulfide concentrations is related to OS and the increase in native thiol levels could be a marker of a reaction to the oxidative environment[9,32].

Any disturbance in the thiol-disulfide balance dissuades viruses from entering target cells. Alterations in pH and the reduction of the disulfide viral spike protein to thiol molecules restore these conformational modifications. Under severe OS, the cell surface receptor ACE-2 and receptor-binding domain of the penetrating viral spike protein can be present in the oxidant model with mostly disulfide bonds. In a computational analysis, the absence of a reducing medium under OS caused the viral protein to bind significantly to the cell surface ACE-2, and the reduction of all disulfides to sulfhydryl groups entirely disrupted the process of the SARS-CoV-2 spike protein binding to ACE-2[12]. In a recent study evaluating thiol status in patients with COVID-19 infection, thiol levels were found to be significantly lower in 517 COVID-19-positive patients compared to the control group (n=70). It has also been reported that these low thiol levels were correlated with the severity of COVID-19 (area under the curve: 0.949, sensitivity 98.6%, specificity 80.4%). Therefore, it was concluded that thiol status could be a potential biomarker for prediction of the severity of COVID-19[11]. In this study, a similar decrease in native thiol and total thiol concentrations was observed in the COVID-19 group, consistent with findings in the literature.

Table 3. Comparisons of laboratory parameters between the groups[a]

Parameters	Group 1 COVID-19 (n=40)	Group 2 Control (n=40)	p-value[b]
Hb (g/dL)	11.44±1.33 (8.3-14.1)	11.96±1.21 (9.7-14.7)	0.461
Hct (%)	35.03±3.56 (26.9-43.8)	36.65±3.65 (28.6-44.0)	**0.048**
Leukocyte (10^3/mm^3)	11146.75±1423.15 (4900-21600)	8562.00±1927.65 (3630-12420)	**<0.001**
Neutrophil (10^3/mm^3)	6151.00±1504.32 (2610-9940)	4686.25±1953.22 (1810-10580)	**<0.001**
Lymphocyte (10^3/mm^3)	1146.75±423.15 (490-2160)	1710.50±475.79 (770-3230)	**<0.001**
Neutrophil to lymphocyte ratio	4.66±2.41 (0.98-10.57)	3.79±1.24 (1.93-6.79)	**0.047**
Platelet (10^3/mm^3)	208.0±720.48 (82-354)	241.25±65.66 (147-373)	**0.034**
ESR (mm/h)	45.25±12.01 (23-73)	29.52±16.58 (3-66)	**<0.001**
CRP (mg/dL)	14.3±16.6 (3.1-95)	7.0±9.9 (0.4-64)	**0.020**
Procalcitonin (ng/mL)	0.25±0.17 (0.03-0.73)	0.02±0.01 (0.01-0.03)	**<0.001**
IL-6 (pg/mL)	8.71±5.42 (1-19.4)	3.71±1.05 (1.7-6.1)	**<0.001**
Ferritin (ng/mL)	28.47±33.44 (5-204)	14.67±11.05 (2-43)	**0.017**
BUN (mmol/mL)	14.80±3.79 (9-26)	17.72±6.71 (9-41)	**0.019**
Creatinine (mg/dL)	0.50±0.10 (0.29-0.81)	0.48±0.08 (0.30-0.65)	0.347
ALT (IU/L)	16.67±5.77 (9-32)	18.92±15.48 (8-106)	0.393
AST (IU/L)	19.37±9.90 (7-53)	16.60±6.64 (8-43)	0.146

ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, BUN: Blood urea nitrogen, COVID-19: Coronavirus disease-2019, CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate, Hb: Hemoglobin, Hct: Hematocrit, IL-6: Interleukin 6, LDH: Lactate dehydrogenase

[a]Values are given as number (percentage) or mean ± standard deviation (range)

[b]Statistical analysis was performed using the Independent sample test (t-test)

IMA is a sensitive biomarker in the identification of suspected myocardial ischemia. OS is triggered by ischemia, resulting in changes in the N-terminal part of albumin, and thus metal-binding cannot occur. Therefore, IMA is an oxidatively altered albumin form that develops a reaction to ROS due to ischemia. There have been reported to be high IMA levels in several clinical pathological conditions when the oxidative status is affected[9,33]. Within minutes of the onset of ischemia, the IMA level in the blood begins to rise, reaches a peak within 6 h and maintains a high level for up to 12 hours[34]. The fact that no difference was found between maternal serum IMA levels in the current study suggests the presence of underlying non-acute ischemic oxidant processes in COVID-19.

One of the most striking of the COVID-19 laboratory findings is high serum ferritin levels. In this study, high ferritin values were determined in the COVID-19 group. Iron can increase virulence and pro-oxidant responses and contribute to OS in the lungs. Increased molecular iron levels in bronchoalveolar lavage fluid have been demonstrated in patients with ARDS. In viral infections, the presence of extracellular iron in healthy lungs creates a predisposition to oxidative damage and infection[35].

Prevention and treatment strategies for COVID-19 also include supportive antioxidant therapy to reduce OS. Selenium is a co-factor in glutathione peroxidase, and it has been stated that the thiol groups in the virus protein disulfide Isomerase are oxidized by the chemical form, sodium selenite, so that the virus cannot penetrate the healthy cell membrane. Thus, selenite could be used in the fight against the coronavirus pandemic[36].

Study Limitations

The strong aspects of this study were the prospective design and the evaluation of a high number of parameters. However, there were also some limitations, primarily the relatively small number of patients, and that the fetal serum thiol-disulfide balance and IMA levels were not evaluated.

Conclusion

With the limited data available on the etiopathogenesis of COVID-19, the oxidative status in pregnant women with COVID-19 was evaluated together with the maternal serum thiol-disulfide balance and IMA levels, for the first time in the literature. The results of the study showed that the thiol-disulfide balance had shifted in the oxidant direction. In addition to supporting previous evidence that OS is characterized by ROS and RNS production, these findings demonstrate an antioxidant deficiency in patients with COVID-19 and that ischemic processes are present in the etiopathogenesis of the disease.

Ethics

Ethics Committee Approval: This prospective, case-control study was approved by the Ethics Committee of The Republic of Turkey Ministry of Health Ankara City Hospital (E1-20-954).
Informed Consent: All the pregnant women included in the study provided informed consent for participation.
Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Supervision: Ö.E., Ö.M.T., D.Ş., Critical Review: H.L.K., D.Ş., Concept: S.A.E., D.Ş., Design: S.A.E., A.T., H.L.K., S.N., D.Ş., Data Collection or Processing: S.A.E., A.T.A., H.S., Analysis or Interpretation: A.T., S.N., Literature Search: S.A.E., A.T., D.Ş., Writing: S.A.E., A.T.A., A.T., S.N., D.Ş.

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Comparison of natural and artificial cycles in frozen-thawed embryo transfer: A retrospective analysis of 1696 cycles

Dondurulmuş çözülmüş embriyo transferinde doğal siklus ve yapay siklusun karşılaştırılması: 1696 döngünün retrospektif analizi

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Abstract

Objective: This study aimed to compare the pregnancy outcomes of natural cycles (NC) and artificial cycles (AC) in patients undergoing endometrial preparation for frozen-thawed embryo transfer (FET).

Materials and Methods: This retrospective cohort study was conducted in a private infertility clinic between September 2016 and January 2021 and reviewed 1696 FET cycles. Among these FET cycles, endometrial preparation protocols that are performed as the NC (group 1) and AC (group 2) were analyzed. Outcome measures were live birth rates (LBR), clinical pregnancy rates (CPR), implantation rates (IR), and miscarriage rates (MR).

Results: The mean serum estradiol level before progesterone supplementation was significantly higher in group 2, whereas endometrial thickness before progesterone supplementation was higher in group 1 ($p<0.05$). The mean number of transferred embryos and embryo quality score rates regarding cleavage and blastocyst stages were similar in both groups. The IR and MR were similar between groups ($p>0.05$). Additionally, CPR and LBR were similar in groups 1 (39.2% and 32.8%) and 2 (37.3% and 28.5%) ($p=0.517$, $p=0.134$, respectively). Multivariate logistic regression analyses revealed that female age at embryo freezing time and the number of transferred embryos were predictable variables of live birth [odds ratio (OR): 0.970, confidence interval (CI): 0.948-0.991, $p<0.05$, and OR: 1.359, CI: 1.038-1.780, $p<0.05$, respectively].

Conclusion: Suitable endometrial preparation is essential to obtain successful pregnancy rates; however, no superiority was determined in NC or AC protocols in frozen-thawed cycles. One of these protocols may be performed depending on menstrual regularity and clinical experience.

Keywords: Infertility, assisted reproductive techniques, cryopreservation, embryo transfer, pregnancy outcome

Öz

Amaç: Bu çalışma donmuş çözülmüş embriyo transferi için endometriyal hazırlık yapılan hastalarda doğal ve yapay siklusların gebelik sonuçlarını karşılaştırmayı amaçlamaktadır.

Gereç ve Yöntemler: Bu retrospektif kohort çalışması Eylül 2016 ile Aralık 2020 arasında özel bir infertilite kliniğinde yürütülmüştür. Toplamda 1696 siklus gözden geçirildi. Bu sikluslardan, endometriyal hazırlık protokolleri doğal siklus (grup 1) ve yapay siklus (grup 2) olarak gerçekleştirilenler bu çalışmada incelenmiştir. Sonuç ölçütleri canlı doğum oranları, klinik gebelik oranları, implantasyon oranları ve düşük oranlarıydı.

Bulgular: Grup 2'de progesteron takviyesi öncesi ortalama serum estradiol seviyesi anlamlı olarak yüksek iken, progesteron takviyesi öncesi endometriyal kalınlık grup 1'de daha yüksekti ($p<0,05$). Gruplar arasında ortalama transfer edilen embriyo sayısı ve klivaj ve blastosist evreleri ile ilgili embriyo kalite skor oranları benzerdi. İmplantasyon ve düşük oranları gruplar arasında farklı değildi ($p>0,05$). Ayrıca, klinik gebelik ve canlı doğum oranları grup 1

PRECIS: The impact of natural and artificial endometrial preparation protocols is similar regarding pregnancy outcomes in frozen-thawed embryo transfer cycles.

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(%39,2 ve %32,8) ve grup 2 (%37,3 ve %28,5) arasında benzerdi (sırasıyla, p=0,517, p=0,134). Çok değişkenli lojistik regresyon analizleri, embriyo dondurma zamanındaki kadın yaşının ve transfer edilen embriyo sayısının canlı doğumun predikte edilebilir değişkenleri olduğunu ortaya çıkardı [risk oranı (OR): 0,970, güven aralığı (GA): 0,948-0,991, p<0,05 ve OR: 1,359, GA: 1,038-1,780, p<0,05, sırasıyla].

Sonuç: Başarılı gebelik oranlarını elde edebilmek için uygun endometriyumun hazırlanması mutlak gerekli olsa da, donmuş çözülmüş sikluslarda doğal veya yapay siklus protokolleri arasında bir üstünlük görülmemektedir. Bu protokollerden biri adet düzenine ve klinik deneyime bağlı olarak uygulanabilir.

Anahtar Kelimeler: İnfertilite, yardımlı üreme teknikleri, dondurarak saklama, embriyo transferi, gebelik sonucu

Introduction

Frozen-thawed embryo transfer (FET) has become a promising approach in assisted reproductive technique cycles in recent years due to cryopreservation technique improvements. Survival rates, embryo morphology, and pregnancy outcomes have improved in FET cycles after the widespread use of the vitrification method relative to slow freezing for cryopreservation[1,2]. Frozen-thawed cycles are considered as an option for clinicians to use the freeze-all strategy, prevent ovarian stimulation syndrome, and perform preimplantation genetic testing (PGT) in recent years[3,4]. Moreover, some studies revealed higher pregnancy outcomes in FET cycles than fresh ones[5-7]. Favorable results have been attributed to better synchronization between the endometrium and transferred embryo in FET cycles[5,6].

With the increased use of FET cycles and knowledge on the importance of endometrial thickness, maturation, and receptivity in achieving pregnancy, clinicians have more closely focused on optimal endometrial preparation. Numerous protocols are available; however, the most commonly used are natural cycle (NC) [true (tNC) or modified NC (mNC)] and artificial cycle (AC). The tNC occurs by preparing the endometrium for implantation with endogenous hormones in patients with regular menstrual cycles. Luteinizing hormone (LH) surge and ovulation are determined by serial ultrasonographic examination of follicular growth, serum LH level measurement, or urinary LH kits. However, it might be difficult even in the frequent follow-up and monitoring. To overcome this problem, mNC is the preferred protocol in which human chorionic gonadotropin (hCG) is administered to trigger ovulation when the leading follicle is measured at ≥18 mm. Embryo transfer (ET) is performed according to the embryonic stage after ovulation is identified in both protocols. In AC protocol, exogenous estrogen (E2) and progesterone are administered to prepare the endometrium and provide endometrial maturation, respectively. It might be used in all patients with or without regular cycles for controlled cycles and optimal ET timing[3,4].

Despite the growing evidence on the impact of endometrial preparation protocols for the success of FET cycles, any protocol superiority is unclear. Generally, pregnancy outcomes are similar between NC and AC[4,8-11]. However, others have presented that NC has higher pregnancy rates than AC[3,12,13]. Additionally, many modifications are made regarding these protocols, and choosing one of these approaches may be challenging and generally depends on clinicians' preferences.

Therefore, this study aimed to compare the impact of the endometrial preparation protocols on pregnancy outcomes in a large number of cycles in patients undergoing FET with NC and AC.

Materials and Methods

This observational cohort study was retrospectively conducted at a private infertility clinic (Novaart IVF Center) in Ankara from September 2016 to January 2021. Approval was obtained from the local Ethics Committee of Gazi University Faculty of Medicine (approval number: 2021-918, date: 23.03.2021). All FET cycles were reviewed from the medical records of the clinic. All completed frozen-thawed cycles performed in the same clinic were included in the study. A total of 1696 thaw cycles of 1297 patients were evaluated. Some multiple cycles were recruited from the same patients; however, the same endometrial preparation protocol was performed in these patients during each FET cycle. All included patients had normal intrauterine cavities assessed by hysteroscopy or hysterosalpingography, of whom two groups were formed according to endometrial preparation protocol for FET cycles: NC (group 1) and AC (group 2). Patients with previous intrauterine surgery; intrauterine lesions, such as polyps, fibroids, and septum; undergoing PGT cycles; and diagnosed with endometriosis, autoimmune diseases, and antiphospholipid antibody syndrome were excluded from the study.

Endometrial preparation protocols were performed according to clinicians' decisions. NC was performed in patients with regular menstrual cycles, whereas AC in patients who are oligo-ovulatory with a possible unpredictable follicular growth pattern and in some who are normo-ovulatory. In NC, baseline monitoring was started on day 3 of the cycle. Patients were then followed up with 2-3 days intervals by ultrasound and serum E2, LH, and progesterone level measurement to evaluate the endometrial thickness and follicle growth, and determine ovulation timing. LH surge (>15 mIU/mL) was determined with the blood test performed on the morning during follow-up visits. Progesterone supplementation was administered by vaginal gel (Crinone 8% gel, Merck Serono, Turkey) at 90 mg once a day or vaginal micronized progesterone capsules (Progestan, Koçak Farma, Turkey) at 200 mg three times a day for luteal phase support (LPS) 48 h after detecting serum LH surge and 24 h after the leading follicle collapse. Progesterone was used for 3 or 5 full days before cleavage stage or blastocyst stage ET, respectively. Thawing of embryo and transfer was performed one day after 3 or 5 full days of progesterone supplementation. Progesterone was continued until the 10 weeks of gestation.

In AC, oral 2 mg of estradiol (Estrofem, Novo Nordisk, Turkey) two times daily was started on day 3 of the menstrual cycle. Patients were evaluated after 7 days, with 3-4 days intervals by transvaginal ultrasound and serum E2 levels to adjust the estradiol dosage relative to the endometrial thickness and E2 concentration. Both vaginal (Crinone 8% gel, Merck Serono, Turkey) at 90 mg once a day or vaginal micronized progesterone capsules (Progestan, Koçak Farma, Turkey) at 200 mg three times a day for LPS was administered when the endometrial thickness was ≥7 mm in diameter and serum E2 was >150 pg/mL. ET was performed after 4-6 full days of progesterone administration according to the embryonic stage. E2 and progesterone administration were continued until the end of the first trimester of gestation.

The vitrification method was performed for cryopreservation in all FET cycles. All embryos were fertilized by intracytoplasmic sperm injection procedure in the same clinic and obtained from the fresh cycles of the same patients who undergo FET cycles. Thawing of embryos was performed on the planned ET day. The morphology and cell number of embryos were evaluated on the transfer day to determine their quality. Good quality embryos were defined as grades 1 and 2 according to the grading system of Hardarson et al.[14] for day 3 cleavage stage embryos. For day 5 blastocyst stage embryos, good quality embryos were defined as ≥3 BB according to the Gardner and Schoolcraft embryo grading system[15]. One to two ET was performed under the transabdominal ultrasonographic guidance using a flexible catheter (Wallace; Irvine Scientific, Santa Ana, CA).

The primary outcome measure was live birth rates (LBR). Secondary outcome measures were clinical pregnancy rates (CPR), implantation rates (IR), and miscarriage rates (MR).

Clinical pregnancy was proven when a gestational sac or a fetus was monitored by ultrasonography. The biochemical loss was defined as pregnancy loss before the gestational sac was identified on ultrasonography. Miscarriage was determined when a non-viable fetus before 23 weeks of gestation was delivered. Live birth was defined as the delivery of a viable fetus at ≥23 weeks of pregnancy. Implantation rate was determined as the ratio of the total number of the gestational sac to the total number of transferred embryos.

Statistical Analysis

Data were analyzed by Statistical Package for Social Sciences (SPSS, version 21.0, Statistics, 2013, Chicago, IBM, USA). Normality tests, including visual (histograms and probability plots) and analytical methods (Kolmogorov-Smirnov test), were performed to determine the normal distribution of variables before analysis. The Student's t-test was used to compare normally distributed parametric variables between groups. Non-normally distributed metric data were compared by the Mann-Whitney U test if required. The chi-square test or Fischer's exact test was used to compare categorical variables. Data were presented as mean ± standard deviation (SD), percentages, and median (25-75 percentile). Multivariate logistic regression analysis was performed to identify independent variables in predicting live birth. Cycle numbers and fresh cycle pregnancy were used as categorical covariates in the multivariate logistic regression analysis. The model fit was evaluated by Hosmer-Lemeshow goodness of fit statistics. Statistical significance was accepted as $p<0.05$.

Results

This study analyzed 1696 completed FET cycles with two different protocols for endometrial preparation. Group 1 (NC) had 311 cycles and group 2 (AC) had 1385 cycles. Of 1696 cycles, clinical pregnancy was obtained in 638 (37.6%) and live birth was delivered in 497 (29.3%).

Baseline characteristics of groups are presented in Table 1. No significant differences were found except for the causes of infertility ($p<0.001$) between groups. Among the causes of infertility, no ovulatory dysfunction was determined in group 1, as expected. Unexplained infertility was the frequent etiology in groups 1 and 2 (56.6%, and 46.8%, respectively).

The comparison of the cycle and pregnancy outcomes between groups is shown in Table 2. The mean serum estradiol level one day before progesterone supplementation was significantly higher in group 2 (223.2±74.2) than that in group 1 (215.5±56.0) ($p<0.05$). However, endometrial thickness before progesterone supplementation was significantly higher in group 1 (10.3±1.4, respectively) than that in group 2 (10.0±1.5, respectively) ($p<0.05$). The mean number of transferred embryos and embryo quality scores regarding both cleavage and blastocyst stages were similar among the two preparation protocols. LBR were similar between groups 1 (32.8%) and 2 (28.5%) ($p=0.134$). CPR were also similar between the groups (39.2% and 37.3%, respectively) ($p=0.517$). Besides, IR and MR were not different between groups (24.9%, 23%, $p=0.355$, and 6.4%, 8.7%, $p=0.183$, respectively).

Multivariate logistic regression analysis to predict live birth was presented in Table 3. Female age at the time of embryo freezing and the number of transferred embryos were found as independent predictors of live birth [odds ratio (OR): 0.970, confidence interval (CI): 0.948-0.991, $p<0.05$, and OR: 1.359, CI: 1.038-1.780, $p<0.05$, respectively].

Discussion

This study revealed that LBR, CPR, IR, and MR were similar among natural and artificial endometrial preparation protocols performed for FET cycles. Our NC group may be accepted as mNC due to progesterone supplementation for LPS. Despite the well-known importance of endometrial preparation for achieving pregnancy, the optimal protocol in patients who undergo FET cycles is still debatable.

Generally, NC (tNC or mNC) and AC are mostly evaluated endometrial preparation protocols for FET cycles in the literature. However, results are inconsistent regarding any of these preferred protocols. A recent Cochrane review reported

Table 1. Comparison of baseline characteristics of the patient groups who received endometrial preparation with natural cycle and artificial cycle before FET

Variables	Natural cycle (group 1) (311 cycles)	Artificial cycle (group 2) (1385 cycles)	p-value
Age at the time of embryo freezing (years)	33.0±4.8	32.5±5.4	0.092
Age at the time of embryo thawing (years)	33.9±4.6	33.3±5.3	0.060
BMI (kg/m^2)	22.8±2.2	23.1±2.0	0.104
Duration of infertility (years)	5.5±3.6	5.3±4.0	0.278
Duration of cryopreservation (years)	1.1±1.0	1.1±1.2	0.253
Number of prior IVF attempts	1 (1-2)	2 (1-3)	0.242
Number of previous thaw cycle attempts	1 (1-2)	1 (1-2)	0.100
Fresh cycle pregnancy (%)	14.1	15.5	0.542
Infertility etiology (%)			<0.001
Unexplained	56.6	46.8	
Male factor	31.5	25.7	
Tubal factor	9	6.9	
Ovulatory dysfunction	-	18.6	
Mixt	2.9	2.1	

Data were presented as mean ± SD, percentage, and median (25-75 percentile). BMI: Body mass index. A p-value of <0.05 was considered significant, IVF: In vitro fertilization, FET: Frozen-thawed embryo transfer, SD: Standard deviation

Table 2. Comparison of cycles and pregnancy outcomes of the patient groups who received endometrial preparation with natural cycle and artificial cycle before FET

Variables	Natural cycle (group 1) (311 cycles)	Artificial cycle (group 2) (1385 cycles)	p-value
Serum E_2 level, 1 day prior to progesterone supplementation (pg/mL)	215.5±56.0	223.2±74.2	0.041
Serum LH level, 1 day prior to progesterone supplementation (mIU/mL)	18.1±8.6	16.6±7.7	0.062
Serum P level, 1 day prior to progesterone supplementation (ng/mL)	1.7±1.4	1.5±1.3	0.105
Endometrial thickness, 1 day prior to progesterone supplementation (mm)	10.3±1.4	10.0±1.5	0.002
Number of transferred embryos	1.7±0.4	1.8±0.4	0.090
Embryo stage at the day of transfer (%)			0.001
Cleavage	24.8	34.9	
Blastocyst	75.2	65.1	
Quality scores of Cleavage stage embryos (%)			0.665
Grade 1-2	90.9	92.3	
Grade 3	9.1	7.7	
Quality scores of Blastocyst stage embryos (%)			0.412
≥3 BB	92.3	93.8	
<3 BB	7.7	6.2	
Implantation rates (%)	24.9	23	0.355
Biochemical loss rate (%)	5.5	6.9	0.349
Miscarriage rate, n (%)	20 (6.4)	121 (8.7)	0.183
Clinical pregnancy rate, n (%)	122 (39.2)	516 (37.3)	0.517
Live birth rate, n (%)	102 (32.8)	395 (28.5)	0.134

Data were presented as numbers and percentages. E_2: Estradiol; LH: Luteinizing hormone, P: Progesterone. A p-value of <0.05 was considered significant, FET: Frozen-thawed embryo transfer

a low quality of evidence that LBR was comparable between mNC and AC[11]. Similarly, no significant difference was found in another review regarding pregnancy outcomes among these two groups[16]. However, the authors concluded that tNC with either LPS or not has higher CPR than AC without suppression, although LBR was similar between groups. Comparable pregnancy outcomes among mNC and AC without suppression were also reported in a large randomized controlled Antarctica trial[10]. Additionally, two recent studies have found similar IR, CPR, and LBR between NC and AC[4,9]. Our study also showed similar findings in terms of IR, CPR, and LBR among NC and AC groups, which was in line with these previous studies. contrarily, some other studies reported more favorable reproductive outcomes in either NC[3,12,17,18] or AC[19,20] than others in FET. Numerous modifications were presented in these studies, such as NC with or without LPS, AC with or without suppression, and different dosages or types of exogenous E2 in AC. Therefore, controversial results are speculated to be attributed to these different endometrial preparation approaches. Moreover, heterogeneous study populations, including patients with only regular menses or both regular and irregular menses, could also contribute to these inconsistent results. Two reviews have reported comparable outcomes in CPR and LBR among tNC and mNC in which hCG has been used to trigger ovulation during NCs[13,16].

Outcomes of AC and mNC are comparable as in our and other recent studies[21]; however, some advantages or disadvantages are determined in both treatment protocols. The main superiorities of hormonal endometrial preparation include the timing flexibility for an ET in clinics that deal with many cycles and the unnecessary regular menstrual cycles. Additionally, the cycle cancellation due to unexpected follicle development is very rare in artificial endometrial preparation cycles. Likely, no cycle cancellations were determined in our study population. Interestingly, a recent study revealed better outcomes in AC in which spontaneous follicles developed contrary to previous reports[22].

The possible disadvantage of AC is that serum estradiol and progesterone levels are directly correlated with the live birth[23], thus an increased dose of steroid hormones may be required in patients with thin endometrium and having difficulty in reaching the desired serum steroid hormone levels. In these individualized treatment cycles, an increased risk of thromboembolic events related to higher doses of steroid hormones besides other risk factors for thrombosis is possible. This problem can be avoided by using daily low molecular weight heparin injections to reduce the risk of iatrogenic complications. However, this adverse effect is too rare in usual doses of estradiol hemihydrate (2 mg, 3 or 4 times/day) and micronized progesterone (200 mg 3 or 4 times/daily), which is approximately equal to the low dose Ethinyl estradiol-containing oral contraceptive pills. This event has not been previously reported in AC thaw studies, and no case was complicated with thromboembolic events in our study. mNC is a patient-friendly approach in economic burden and unexposed to adverse effects of exogenous E2. However, it is unsuitable for patients with oligo-anovulatory cycles and requires more frequent visits to detect unexpected ovulation leading to cycle cancellations.

The literature has also investigated the impact of serum estradiol levels and endometrial thickness in FET cycles. The increased endometrial thickness (9-14 mm) is considered a positive factor in favorable pregnancy outcomes[24]; however, higher estradiol levels may not guarantee thicker endometrium and higher pregnancy outcomes[9]. Two studies that compared NC and AC for endometrial preparation in FET revealed a consistently greater endometrial thickness and lower serum estradiol levels with higher pregnancy outcomes in NC[17,25], which as referred to the appropriate implantation window in lower estradiol levels combined with thick endometrium. Our study revealed similar findings. Our NC group had relatively higher IR and LBR than the AC group but without significant difference. Lower estradiol levels and the greater endometrial thickness could lead to better synchronization between the endometrial and embryonic stage in the NC group.

Table 3. Multivariate logistic regression analyses of variables to predict live birth

Variables	Live birth		
	Odds ratio	95% Confidence interval	p-value
Age at the time of embryo freezing	0.970	0.948-0.991	0.006
Duration of infertility	0.989	0.959-1.020	0.496
Serum E_2 level, 1 day prior to progesterone supplementation	1.001	1.000-1.003	0.155
Endometrial thickness, 1 day prior to progesterone supplementation	1.034	0.963-1.111	0.356
Fresh cycle pregnancy [(a)]	1.252	0.931-1.684	0.136
Number of transferred embryos	1.359	1.038-1.780	0.026
Number of prior IVF attempts	0.977	0.903-1.057	0.561
Cycle number [(a)]			0.709

Variable with Superscript [(a)] was selected as the categorical covariate, IVF: In vitro fertilization

The need for LPS in the natural ET cycles is also controversial in the literature. Higher LBR[26,27], as well as lower MR[26], was observed in some studies; however, other studies reported similar pregnancy outcomes with or without LPS in the NC of FET[28,29]. We used progesterone supplementation in all NC for LPS due to possible luteal phase defects in some ovulatory patients. Additionally, insufficient corpus luteum (CL) is suggested to be associated with lower implantation and some pregnancy complications in AC[3]. Generally, MR was reported higher in AC than NC[3,8]. Our study revealed a relatively higher MR in the AC group, which is explained by the insufficient uterine milieu in AC compared to NC due to insufficient other hormones released from the CL, although exogenous E2 and progesterone were extensively used in AC until the end of the first trimester of pregnancy. Additionally, using progesterone in our NC group may have supported the effectiveness of the CL and decreased the possible pregnancy losses.

The present study revealed that female age at embryo freezing time and the number of transferred embryos were significant variables in predicting live birth. Maternal age is known to be a major determinant factor on pregnancy success in IVF and FET cycles, and the chance of pregnancy notably decreased after 35 years of age[17]. Considering the increasing frequency of ovulatory dysfunction with advancing age, cryopreserved embryos should be transferred with a higher number in older patients, especially in AC.

Study Limitations

The main strength of the current study is the comparison of the most commonly used endometrial preparation protocols in a large cohort of patients who undergo FET cycles, as well as the similarly higher rates of transferred good quality embryos in both NC and AC by experienced clinicians and the use of vitrification method in all embryos in a single-center, which may affect favorable pregnancy outcomes. The major limitations are the retrospective study design and the bias potential of medical records.

Conclusion

The pregnancy outcome of NC and AC seems to be similar in FET. The NC is more convenient for patients with regular menses as there is no need for excessive exogenous hormonal therapy. However, frequent monitoring might be bothersome for some patients. The AC is reasonable, especially for irregular cycles, to control the cycle with less monitoring. It was in line with some reports in the literature; however, the results of our large cohort may provide the flexibility in choosing one of these protocols for clinicians to prepare endometrium in FET cycles. Therefore, one of these approaches might be preferred according to patients' characteristics and clinicians' experience.

Ethics

Ethics Committee Approval: Approval was obtained from the local Ethics Committee of Gazi University Faculty of Medicine (approval number: 2021-918, date: 23.03.2021).
Informed Consent: Retrospective study.
Peer-review: Internally peer-reviewed.

Authorship Contributions

Concept: A.E., Design: A.E., Data Collection or Processing: E.D., İ.G., M.F.C.A., E.Ş., A.D.T., M.E., Analysis or Interpretation: İ.G., Literature Search: E.D., Writing: E.D.
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Effect of metformin on proliferative markers in women with endometrial carcinoma: Systematic review and meta-analysis

Metforminin endometriyal karsinomlu kadınlarda proliferatif belirteçler üzerine etkisi: Sistematik derleme ve meta-analiz

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Abstract

Objective: Endometrial carcinoma (EC) is the most common gynecologic malignancy in the USA and Western Europe. Surgery is the mainstay of both staging and treatment of EC. Fertility sparing medical therapies are often offered to young women who desire fertility. Metformin has been suggested to be an anti-cancer agent as evidenced by previous studies. It decreases Antigen Ki-67 (Ki-67) proliferation and expression which is associated with proliferative activity of malignant tumors. In this systematic review and meta-analysis, we assessed the efficacy of metformin on patients with EC.

Materials and Methods: We searched PubMed, Cochrane CENTRAL, Web of Science, and SCOPUS for relevant clinical trials and excluded observational studies. The quality appraisal was evaluated according to GRADE, and we assessed the risk of bias using Cochrane's risk of bias tool. We conducted the analysis of continuous data using mean difference (MD). We included the following outcomes: Ki-67 index, glucose, insulin, P-S6, body mass index (BMI), C-peptide, Insulin-like growth factor (IGF-1), leptin, and hemoglobin.

Results: Nine studies were eligible for our meta-analysis. We found that compared to the control group, metformin is highly effective in reducing Ki-67 proliferation and expression [MD=-10.14 (-19.10, -1.17)], (p=0.03), P-S6 [MD=-1.82 (-3.17, -0.46)], (p=0.009), plasma glucose level [MD=-1.76 (-4.88, 1.37), p=0.27], and BMI [MD=-1.07 (-1.49, -0.65)], (p<0.001).

Conclusion: We conclude that metformin administration is effective in patients with EC. It decreases Ki-67 proliferation and expression, serum glucose, and p-S6 significantly.

Keywords: Metformin, glucophage, dimethylbiguanide, endometrial carcinoma, meta-analysis

Öz

Amaç: Endometriyal karsinom (EK), ABD ve Batı Avrupa'da en sık görülen jinekolojik malignitedir. EK'nin hem evrelemesinin hem de tedavisinin temeli cerrahidir. Doğurganlığı koruyucu tıbbi tedaviler genellikle doğurganlık isteyen genç kadınlara sunulur. Metforminin, önceki çalışmalardan elde edilen kanıtlara göre bir anti-kanser ajanı olduğu öne sürülmektedir. Metformin malign tümörlerin proliferatif aktivitesi ile ilişkili Antijen Ki-67 (Ki-67) proliferasyonunu ve ekspresyonunu azaltır. Bu sistematik derleme ve meta-analizde, metforminin EK'li hastalardaki etkinliğini değerlendirmeyi amaçladık.

Gereç ve Yöntemler: İlgili klinik araştırmalar için PubMed, Cochrane CENTRAL, Web of Science ve SCOPUS'yi taradık ve gözlemsel çalışmaları hariç tuttuk. Kalite değerlendirmesi GRADE'ye göre değerlendirildi ve biz de Cochrane'nin yanlılık riski aracını kullanarak yanlılık riskini değerlendirdik. Ortalama farkı (MD) kullanarak sürekli verilerin analizini gerçekleştirdik. Şu sonuçları dahil ettik: Ki-67 indeksi, glukoz, insülin, P-S6, vücut kitle indeksi (VKİ), C-peptid, insülin benzeri büyüme faktörü (IGF-1), leptin ve hemoglobin.

PRECIS: Metformin is effective in patients with endometrial carcinoma. It significantly decreases Ki-67 proliferation and expression, serum glucose, and p-S6.

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Bulgular: Dokuz çalışma meta-analizimiz için uygun bulundu. Kontrol grubu ile karşılaştırıldığında metforminin Ki-67 proliferasyonunu ve ekspresyonunu [MD=-10,14 (-19,10, -1,17)], (p=0,03), P-S6 [MD=-1,82 (-3,17, -0,46), (p=0,009), plazma glukoz düzeyini [MD=-1,76 (-4,88, 1,37), p=0,27] ve VKİ'yi [MD=-1,07 (-1,49, -0,65)], (p<0,001) azaltmada oldukça etkili olduğunu bulduk.

Sonuç: EK'li hastalarda metformin uygulamasının etkili olduğu sonucuna varılmıştır. Metformin Ki-67 proliferasyonunu ve ekspresyonunu, serum glukozunu ve p-S6'yı önemli ölçüde azaltmaktadır.

Anahtar Kelimeler: Metformin, glukofaj, dimetilbiguanid, endometriyal karsinom, meta-analiz

Introduction

Endometrial carcinoma (EC) is the most common gynecologic malignancy in the USA and Western Europe[1]. The main symptoms of EC are dysfunctional uterine bleeding and infertility[2]. EC is divided into two major types. Type I, known as estrogen-dependent or endometrioid, is the more common type. It is associated with unopposed hyperestrogenemia and is often preceded by endometrial hyperplasia. Moreover, type II, known as estrogen-independent or non-endometroid, has a poorer prognosis and less differentiation than type I[3]. Many factors increase the risk for developing both low-grade and high-grade EC, including obesity, diabetes especially type II (which is associated with insulin resistance), menstrual irregularity, anovulation, and infertility[4]. Fortunately, most women are usually diagnosed at an early stage in which the disease is limited to the uterine corpus. Therefore, about 75% of women survive for 5 years[5,6].

Treatment options for EC vary depending on the stage and grade of the disease. Surgery is the mainstay of both staging and treatment of EC. Surgery includes hysterectomy, bilateral salpingo-oophorectomy, and lymph node assessment[7]. Fertility sparing medical therapies are often offered to young women who desire fertility. The standard conservative medical treatment of EC is high-dose oral progestin such as megestrol acetate or medroxyprogesterone acetate[8]. However, women experience many side effects, including liver damage, weight gain, thrombosis, and progesterone resistance, which limits the usage of this drug[9].

Metformin is the first-line medication for treating type 2 diabetes mellitus[10]. It has been suggested to be an anticancer agent[11]. Previous studies reported the anti-carcinogenic properties of metformin on gastric cancer, medullary thyroid carcinoma, pancreatic cancer, and EC[12,13]. A recent study revealed that metformin and progestins have a synergistic effect on the inhibition of proliferation of EC cells[14]. Metformin also affects Adenosine monophosphate-activated protein kinase (AMPK)-independent pathways responsible for tumor growth and cell proliferation. Therefore, it decreases Antigen Ki-67 (Ki-67) proliferation and expression[15,16]. Expression of Ki-67 is associated with proliferative activity of malignant tumors, so it has been used as a marker for tumor aggressiveness[17,18].

There are no sufficient data from previous trials regarding the effect of metformin on endometrial neoplasms. Therefore, we performed this systematic review and meta-analysis to estimate the effect of metformin on the proliferation and expression of tumor cells and the change of tumor markers in cases of EC.

Materials and Methods

In this meta-analysis, We followed the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA)[19] guidelines and conducted every step in this study according to the Cochrane Handbook for Systematic Reviews of Interventions[20]. The ethics statement is not applicable because this study is based exclusively on published literature.

Literature Search

We searched four databases: Web of Science, SCOPUS, Cochrane CENTRAL, and PubMed, from inception until October 2020. We followed this search strategy with no restriction on time or languages: (metformin OR glucophage OR dimethylbiguanide OR dimethylguanylguanidine) AND (endometrial cancer OR EC OR endometrial hyperplasia OR endometrial proliferation OR endometrial thickness).

Eligibility Criteria

We included studies according to these eligibility criteria: (I) **Population:** Patients with EC or endometrial hyperplasia with atypia, (ii) **Intervention:** Metformin regardless of the dose and mode of administration, (iii) **Comparator:** Placebo or no treatment, (IV) **Outcomes:** Ki-67 proliferation and expression index as a primary outcome. The secondary outcomes were plasma glucose level, body mass index (BMI), p-S6, insulin, C-peptide, insulin growth factor (IGF-1), Leptin, p-AKT, p-4EBP1, hemoglobin. (v) **Study design:** We included only randomized clinical trials (RCTs). Our exclusion criteria were (1) non-randomized controlled clinical trials, (2) studies that did not report data or measures for our selected outcomes (3) single-armed trials, or (4) that with no available full-text.

Screening of Results

After retrieving the search results, we exported the data into EndNote X8.0.1 (Build 1044), with the automatic removal of any duplicates. We screened the included articles through two steps, the first step was the title and abstract screening, and the second was full-text screening. Two independent authors performed the screening steps and obtained the full-text files for all included studies based on our criteria for eligibility criteria. A third author solved any deflection.

Data Extraction and Analysis

After the screening process, we performed the data extraction step. We extracted the data into three main categories: 1) baseline and demographic data of patients in each study, including age, BMI, myometrium invasion, and menopausal

state. 2) Data about Fédération Internationale de Gynécologie et d'Obstétrique (FIGO) staging and tumor grades, and 3) Data for analysis including outcome values of Ki-67 proliferation and expression index, glucose level, BMI, p-S6, insulin, c-peptide, IGF-1, Leptin, p-AKT, p-4EBP1, hemoglobin. In addition to the previous three categories, we extracted the data about the seven domains assessing the risk of bias according to Cochrane's risk of bias.

Statistical Analysis

We performed our analysis using Review Manager Software (RevMan 5.4.1) under the Inverse variance method. Continuous data were expressed using mean difference (MD) and standard error, relative to 95% confidence interval (CI), while dichotomous outcomes were expressed using percentage and total. Two main tests indicate inconsistency among studies[21], the I-square test (I^2) and the p-value of the chi-square test. The outcomes with I^2>50%, p<0.1 were considered heterogeneous, while outcomes with I^2<50%, p>0.1 were considered homogeneous, according to the Cochrane Handbook. Homogenous data were analyzed using a fixed-effects model, while heterogeneous outcomes were analyzed using the random-effects model.

Quality Assessment

Quality assessment of this meta-analysis was performed using the guidelines of the Grading of Recommendations, Assessment, Development, and Evaluations (GRADE). We included only the controlled trials and excluded the observational evidence. We used Cochrane's risk of bias tool to perform the risk of bias assessment for the included studies[22]. The tool depends on the following domains for the assessment of the risk of bias: 1) proper randomization, 2) blinding allocation of the included patients into each group, 3) blinding of patients only (single-blinding), blinding of both personnel and participants (double-blinding), or not blinding at all, 4) attrition bias, 5) selection bias (outcomes reported matches with that of the protocol or not), 6) awareness of the outcome assessor (whether blinded or not), 7) other bias. The total risk of bias for the studies has been assessed as well.

Results

Summary of Included Studies

Figure 1 shows a PRISMA flow diagram of our literature search. In our study, we performed an analysis of 397 patients from nine studies[23-31]. A total of 221 patients were allocated to receive metformin, and 176 patients entered the control group. The mean age of the percipient in the treatment group was 56.4±8.8 years, while that of the control group was 60±7.5. The mean BMI of the patients in the metformin group was 34.14±6.1, while that of the control group was 32.84±9.7. Table 1 shows a detailed summary of the included participants, their demographic data, and the menopausal state. Additionally, Table 2 illustrates the FIGO staging and Tumor grade.

Results of Risk of Bias Assessment

The result of the risk of bias assessments yielded an overall low risk of bias, according to Cochrane's tool[22]; Figure 2 summerizes the quality assessment of included studies. Regarding randomization, all studies were at low risk of randomization, except Sivalingam et al.[24], and Mitsuhashi et al.[25] were non-randomized trials. As for the allocation concealment, three studies[23,27,29] reported adequate allocation concealment; therefore, there were put to a low risk of bias. Five studies[24-26,30,31] did not report enough data about allocation concealment, thus put to an unclear risk of bias. One study reported no allocation concealment. Most included studies[23,24,26,27,29,30] were blinded, and only three studies[25,28,31] did not report enough data about blinding of the participants and personnel, thus put to an unclear risk of bias. Six studies[23,24,26,27,29,30] were at low risk of blinding of outcome assessment. Zhao et al.[31] and Pabona et al.[28] did not report enough data about blinding of outcome assessment. The remaining domains of the Cochrane tool were all at low risk of bias, except two studies: Zhao et al.[31] did not report enough evidence aonthe attrition bias domain, and Tehranian et al.[29] did not report enough evidence on the reporting bias domain.

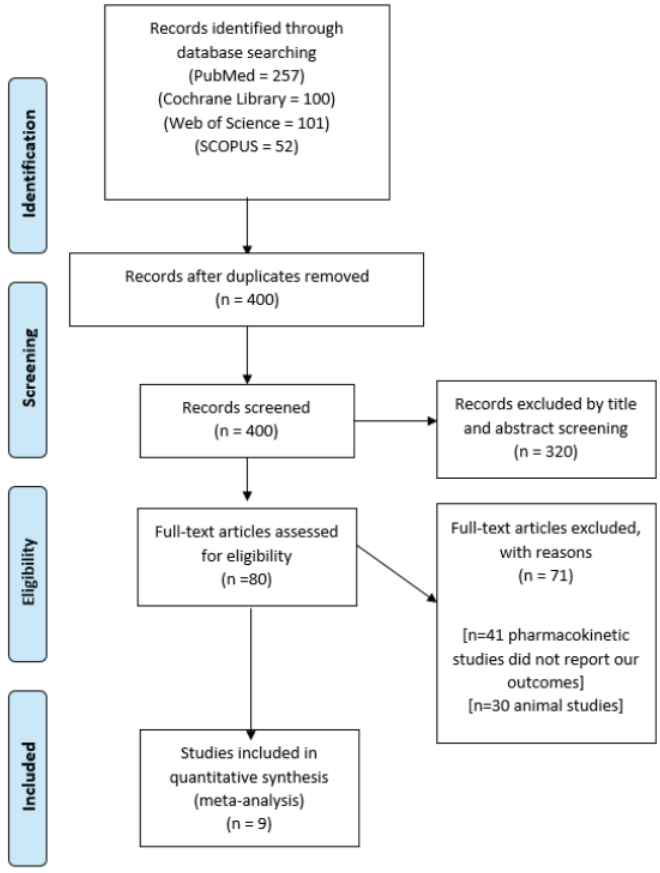

Figure 1. Shows a PRISMA flow diagram of our literature search

PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses

Analysis of Outcomes

1-Ki-67 index:

Ki-67 index was reported by six studies[23-26,28,31].The overall mean difference favored the metformin group over the control group [MD=-10.14 (-19.10, -1.17)], (p=0.03). Pooled analysis was heterogeneous (p<0.001); I^2=89% as shown in Figure 3A.We solved the heterogeneity by the exclusion of Pabona et al.[28] (p=0.53); I^2=0%. The pooled analysis after the exclusion also favored the metformin group significantly [MD=-11.82 (-15.22, -8.42)], (p=0.01). Figure 3B illustrates the analysis after the exclusion of one study.

2-P-AKT:

Two studies[24,31] reported P-AKT. There was no significant difference between both groups [MD=0.40 (-1.32, 2.13)]. Pooled analysis was homogenous (p=0.97); I^2=0% as shown in Figure 4.

3- P-S6

Two studies reported p-S6 outcome[24,26]. P-S6 was significantly decreased in the metformin group [MD=-1.82 (-3.17, -0.46)], (p=0.009). Analysis was homogenous (p=0.15); I^2=52% as shown in Figure 5.

4-P-4EBP1

p-4EBP1 was reported in two studies[24,31]. The overall analysis did not show any variation between both groups [MD=-2.28 (-5.75, 1.20)], (p=0.20). Data were homogeneous (p=0.90); I^2=0% as shown in Figure 6.

5-Hemoglobin (g/dL)

Two studies reported hemoglobin outcome[29,30]. The analysis did not show any significant difference between both groups [MD=-0.03 (-0.33, 0.26)], (p=0.82). Data were homogenous, (p=0.65); I^2=0% as shown in Figure 7.

6-Glucose (mg/dL)

Glucose outcome was reported in five studies[23,24,27,29,30]. The overall mean difference did not reveal any difference between both groups [MD=-1.76 (-4.88, 1.37)], p=0.27. Analysis was heterogeneous (p=0.07); I^2=54% as shown in Figure 8A. To solve heterogeneity we excluded Tehranian et al.[29] (p=0.75); I^2=0%. The total mean difference after solving heterogeneity also favored metformin group [MD=-0.40 (-0.68, -0.11)], (p=0.006) as shown in Figure 8B.

7-Insulin (mUI)

Three studies reported insulin outcome[24,27,30]. The total analysis showed increased insulin level in the metformin group than the control group [MD=1.99 (1.86, 2.12)], (p<0.001), Data were homogeneous (p=0.40); I^2=0% as shown in Figure 9.

8-BMI

Three studies reported BMI[24,29,30]. The total mean difference favored BMI significantly [MD=-1.07 (-1.49, -0.65)], (p<0.001).

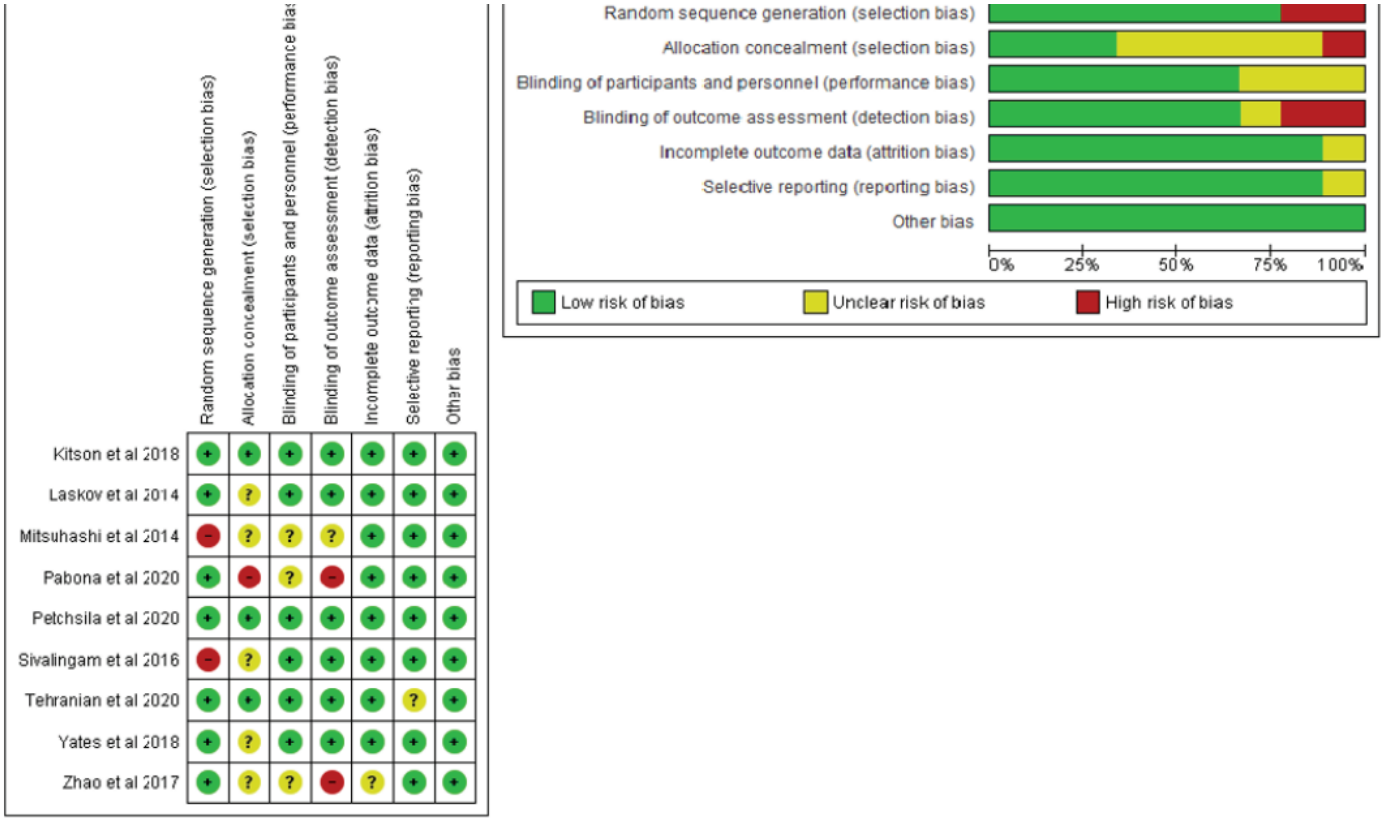

Figure 2. Shows both a summary and a graph of the risk of bias of the included studies

Table 1. Shows a detailed summary of the included participants, their demographic data, and the menopausal state

Study ID	Age, years (mean ± SD)/ median (range)		BMI kg/m² (mean ± SD)/ median (range)		Post-menopausal n (%)		Myometrial invasion, n (%)			
							<50		≥50	
	MFM	C	MFM	C	MFM	C	MFM	C	MFM	C
Kitson et al. 2018[27]	64.375±13.525	64.8±11.35	31.0 (20.2-54.2)	32.0 (17.8-47.6)	36 (80.0)	36 (83.7)	NR	NR	NR	NR
Laskov et al. 2014[26]	61±6.5	68.25±4.75	28.6 (20.5-34.9)	28.8 (25-40)	11 (100)	10 (100)	NR	NR	NR	NR
Mitsuhashi et al. 2014[25]	50.25±11.25		NR	NR	NR	NR	NR	NR	NR	NR
Pabona et al. 2020[28]	55.4±4.7	60.5±1.8	42.5±4.9	38.2±2.8	NR	NR	NR	NR	NR	NR
Petchsila et al. 2020[23]	55.5±10.0	54.9±11.9	NR	NR	17 (68.0)	15 (62.5)	13 (52.0)	18 (75.0)	10 (40.0)	6 (25.0)
Sivalingam et al. 2016[24]	63.6±8.9	67.8±9.2	35.5±11.3	32±5.9	NR	NR	22 (78.6)	7 (58.3)	6 (21.4)	3 (25.0)
Tehranian et al. 2020[29]	44.85±6.80	43.16±6.08	NR	NR	3 (9.4)	4 (16)	NR	NR	NR	NR
Yates et al. 2018[30]	60.0±4.5	55.8±5.2	36.7±5.5	38.3±5.1	NR	NR	NR	NR	NR	NR
Zhao et al. 2017[31]	NR	NR	27.4 (23.7-36.1)	26.9 (24.5-35.6)	24 (72.7)	22 (68.75)	26 (78.7)	24 (75)	7 (21.2)	8 (25)

Data are reported as mean ± SD or n (%) unless otherwise specified. NR: Unreported, MFM: Metformin, C: Control group, BMI: Body mass index, SD: Standard deviation

Table 2. Illustrates the FIGO staging and tumor grade

Study ID	FIGO Stage n (%)				Tumor grade n (%)					
	Early stage (I-II)		Advanced stage (III-IV)		G1		G2		G3	
	MFM	C	MFM	C	MFM	C	MFM	C	MFM	C
Kitson et al. 2018[27]	34 (75)	38 (88.3)	9 (25)	3 (6.9)	26 (57.8)	23 (53.5)	10 (22.2)	12 (27.9)	6 (13.3)	6 (14.0)
Laskov et al. 2014[26]	9 (81)	2(11)	9 (90)	1 (10)	2 (18)	5 (50)	5 (45)	2 (20)	4 (36)	3 (30)
Mitsuhashi et al. 2014[25]	26 (80)		5 (20)		NR	NR	NR	NR	NR	NR
Pabona et al. 2020[28]	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Petchsila et al. 2020[23]	20 (80.0)	21 (87.5)	5 (20.0)	3 (22.5)	15 (60.0)	17 (70.8)	6 (24.0)	5 (20.8)	4 (16.0)	2 (8.3)
Sivalingam et al. 2016[24]	23 (83)	10 (100)	5 (17)	0(0)	14 (50.0)	1 (8.3)	13 (46.4)	6 (50.0)	1 (3.6)	3 (25.0)
Tehranian et al. 2020[29]	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Yates et al. 2018[30]	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Zhao et al. 2017[31]	23 (69.6)	19 (59.3)	10 (30.3)	13 (39.3)	19 (57.5)	18 (56.25)	8 (24.4)	7 (21.8)	6 (18.1)	7 (21.8)

Data are reported as mean ± SD or n (%) unless otherwise specified. NR: Unreported, MFM: Metformin, C: Control group, FIGO: Fédération Internationale de Gynécologie et d'Obstétrique. SD: Standard deviation

Pooled analysis was homogeneous (p=0.17); I^2=43% as shown in Figure 10.

9-C-peptide (pg)

The C-peptide outcome was reported in two studies[24,30]. The combined mean difference did not show any significant difference between both groups [MD=-93.12 (-422.60, 236.36)], (p=0.58) Data were heterogeneous (p=0.01); I^2=84% as shown in Figure 11. We could not solve heterogeneity because only two studies reported this outcome.

Discussion

In our meta-analysis, we investigated the effect of metformin on tumor markers of EC. Six studies[23-26,28,31] evaluated the association of metformin use with Ki-67 proliferation and expression. Out of the six studies that reported the Ki-67 index, five studies[23-26,31] found that metformin significantly decreased the positive rate of Ki-67. Pabona et al.[28] found that metformin did not affect Ki-67 proliferation, which may be due to a short-term metformin administration and/or the non-diabetic status of the patients.

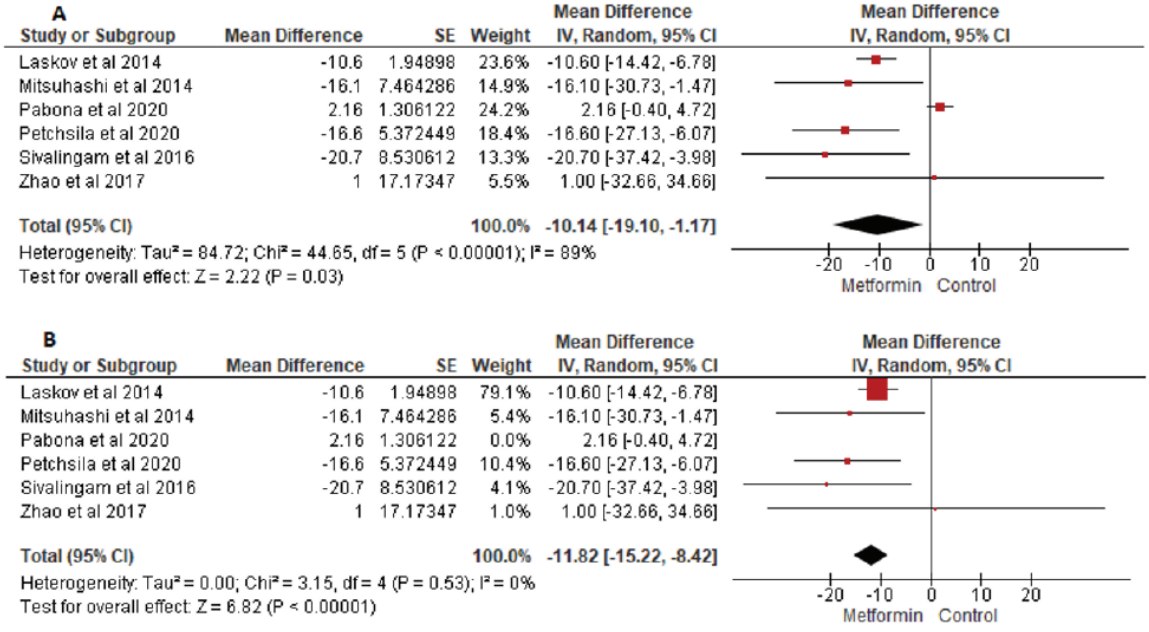

Figure 3. Shows the Ki-67 proliferation index outcome

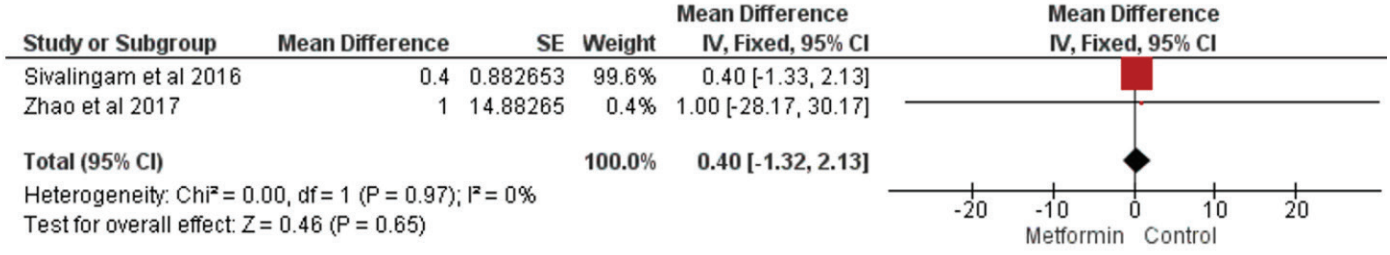

Figure 4. Shows the P-AKT outcome

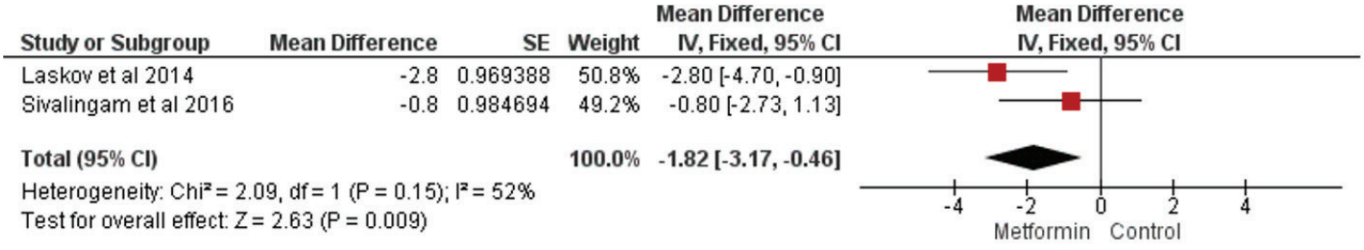

Figure 5. Shows the P-S6 outcome

Ki-67 protein is a proliferation marker for many human tumors for decades. Recently, we have understood the molecular functions of the Ki-67 protein[32]. Ki-67 affects the active phases of the cell cycle. It accumulates only during S, G2, and M phases but is absent from resting cells G0; therefore, it is an excellent marker for cell proliferation[33]. Zhao et al.[31] and Sivalingam et al.[24] reported the effect of metformin on p-AKT expression. The two studies showed that metformin significantly decreased the rate of p-AKT. Akt is a serine kinase that participates in the PI3K signaling pathway. It can be activated by various growth signals. Once activated, Akt modulates the function of many proteins involved in cellular proliferation, survival, metabolism, -and angiogenesis.

Two studies[24,26] showed that reduction of pS6 expression was evident in all patients who received metformin. The expression of ps6 was increased in abnormal epithelial glands compared to the normal endometrium. Five studies that reported glucose level showed a significant decrease in glucose level after metformin administration as expected[23,24,27,29,30]. The main underlying mechanism is that metformin improves insulin sensitivity and prevents gluconeogenesis, lowering plasma glucose[34]. A previous study found impaired glucose

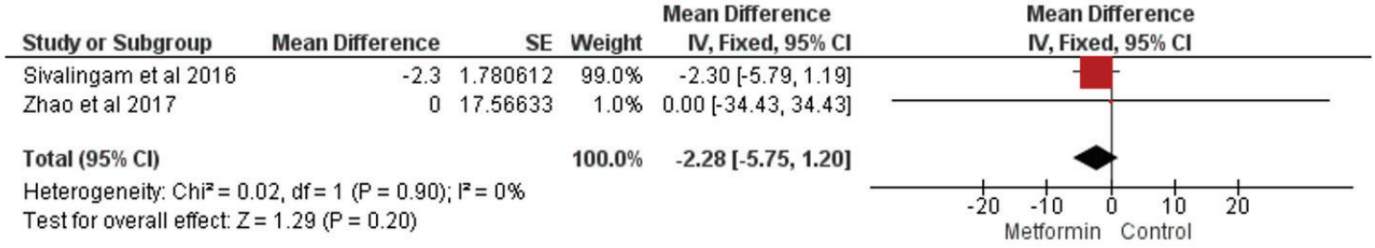

Figure 6. Shows the p-4EBP1 outcome

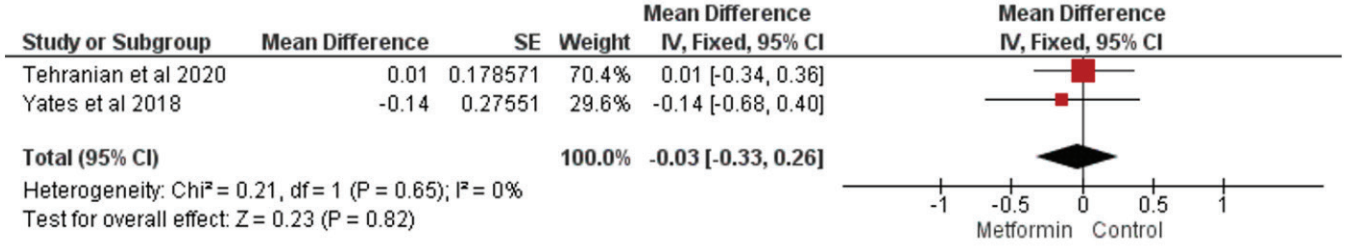

Figure 7. Shows the hemoglobin (gm) outcome

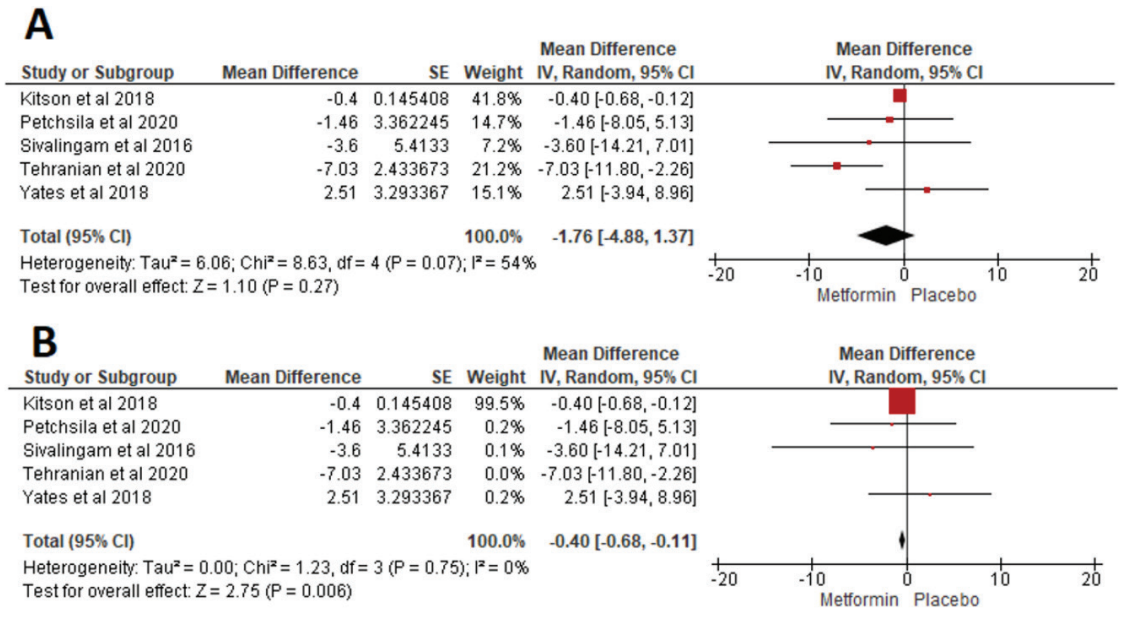

Figure 8. Shows the glucose (mg/dL) outcome

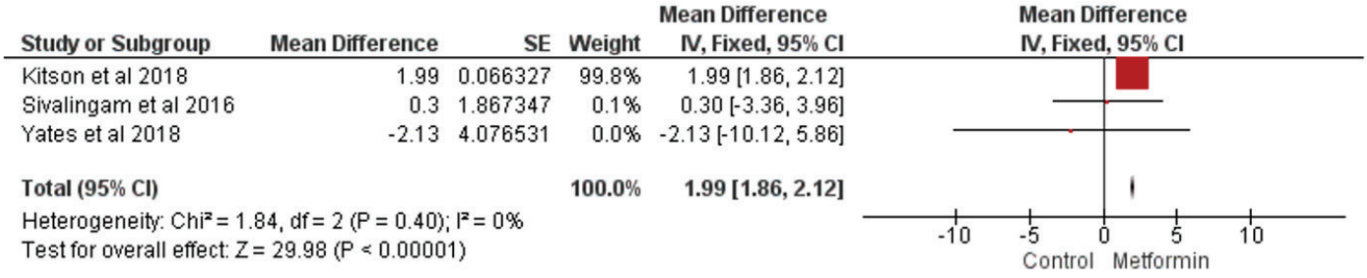

Figure 9. Shows the insulin (mIU) outcome

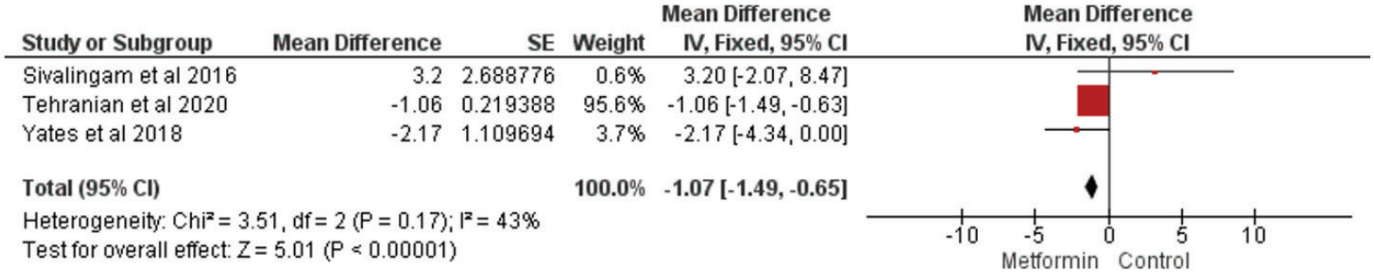

Figure 10. The BMI (kg/m^2) outcome

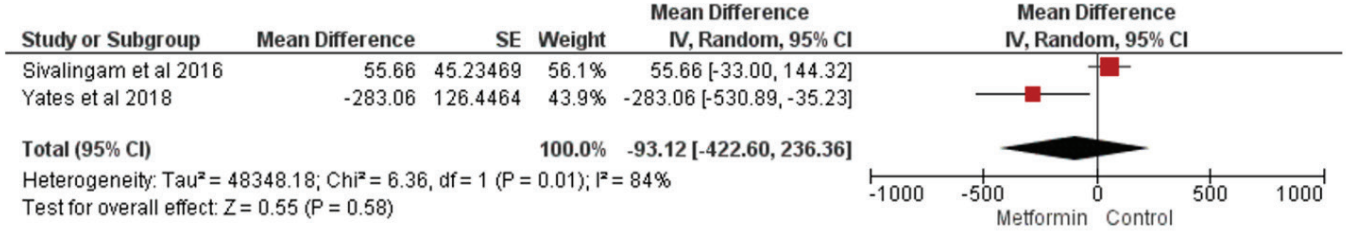

Figure 11. The c-peptide (pg) outcome

tolerance and insulin resistance may induce the initiation and progression of EC. Therefore, Adequate diabetes control by metformin is suggested to prevent EC[35]. There is a debate on whether metformin increases or decreases plasma insulin levels. Two studies reported that metformin decreases insulin levels[24,30,36], while another study found that metformin did not affect insulin-signaling pathways[27].

The main point of strength in our study is the inclusion of clinical trials only while excluding other observational evidence. It is well-known that data from clinical trials are considered the strongest evidence, according to Cochrane's handbook. We found an overall low risk of bias among the included trials, which further supports the accuracy of our findings. Most of the analyzed outcomes were homogeneous, and this favors the true interpretation of data.

Study Limitations

The major limitation of this study is the relatively small sample size (397 participants). Other limitations include some heterogeneous secondary outcomes and the fact that two trials were not randomized. Additionally, no data were reported regarding the safety parameters of administering metformin in patients with EC. So, we highly recommend the initiation and conduction of further clinical trials with a larger sample size and considering safety endpoints.

Conclusion

As a summary, the evidence from the included studies shows that metformin administration in patients with EC significantly decreases Ki-67 proliferation and expression, reduces serum glucose levels and p-S6.

Ethics

Ethics Committee Approval: The ethics statement is not applicable because this study is based exclusively on published literature.

Informed Consent: Not necessary.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: M.A.S., A.A.S., A.T.A., A.T.M., A.M.F., H.M., Design: M.A.S., A.A.S., A.T.A., A.T.M., A.M.F., H.M., Data Collection

or Processing: M.A.S., A.A.S., A.T.A., A.T.M., A.M.F., H.M., Analysis or Interpretation: M.A.S., A.A.S., A.T.A., A.T.M., A.M.F., H.M., Literature Search: M.A.S., A.A.S., A.T.A., A.T.M., A.M.F., H.M., Writing: M.A.S., A.A.S., A.T.A., A.T.M., A.M.F., H.M.

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

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

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Clinical Investigation / Araştırma

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Ovarian drilling down-regulates endometrial nuclear factor-κB p65 expression in women with PCOS: A prospective case-control study

Ovaryen drilling PKOS hastalarında endometrial nükleer faktör κB p65 ekspresyonunu downregüle eder: Prospektif olgu kontrol çalışması

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Abstract

Objective: To investigate the impact of laparoscopic ovarian drilling (LOD) on the expression of endometrial NFκB p65 (Rel A) in women with clomiphene-resistant polycystic ovary syndrome (PCOS).

Materials and Methods: The study group comprised 25 normal-weight women with PCOS undergoing LOD and 14 control women without PCOS. Endometrial NF-κB p65 levels evaluated before and after LOD following immunohistochemical staining. The semiquantitative method was used to evaluate the intensity of NF-κB p65 levels. NF-κB p65 was found to higher in the endometrium of patients with PCOS compared to controls. LOD leads to significant down-regulation in endometrial NF-κB p65 expression. NF-κB p65 expression of PCOS and fertile control were similar after LOD. After LOD, H-score values decreased approximately 3-fold. The H-score of the control subjects was lower than the preoperative and postoperative H-score values of the control women with ovarian cyst.

Results: Expression of endometrial NF-κB p65 did not change following ovarian cystectomy. The laterality of the ovarian cyst did not cause any change in preoperative H-score values.

Conclusion: By downregulating the endometrial NF-κB p65 expression LOD improved physiological inflammation in women with PCOS.

Keywords: PCOS, ovarian drilling, clomiphene, nuclear factor κB, endometrial inflammation

Öz

Amaç: Bu çalışmanın amacı laparoskopik ovaryen drilling (LOD) işleminin klomifen sitrat rezistansı olan polikistik over sendromlu (PKOS) hastalarda endometriyal NFκB p65 (Rel A) ekspresyon düzeylerini etkileyip etkilemediğinin araştırılmasıdır.

Gereç ve Yöntemler: Çalışma grubu LOD yapılan 25 tane normal kiloya sahip PKOS'li hastadan oluşmaktadır. Kontrol grubunda ise 7 tane PKOS olmayan sağlıklı fertil kadın ve 7 tane de benin kisti olan ancak endometriyoması olmayan toplam 14 hasta mevcuttur. Endometriyal NF-κB p65 ekspresyonu LOD öncesi ve sonrası mid-sekretuar fazda ölçülmüştür. Endometriyal örnekler immünohistokimyasal yöntemlerle boyanmıştır. Endometriyal NF-κB p65 ekspresyonu H-skor metodu ile değerlendirilmiştir.

PRECIS: In this paper, on the basis of our observations by down regulating the endometrial NF-κB p65 expession laparoscopic ovarian drilling (LOD) improve physiological inflammation and receptivity in women with clomiphene-resistant PCOS.

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Bulgular: Endometriyal NF-κB p65 ekspresyonu PKOS olan hastalarda LOD öncesi fertil hastalardan daha yüksek bulunmuştur. PKOS'li hastalarda LOD sonrası NF-κB p65 ekspresyonu belirgin olarak azalmıştır. LOD sonrası PKOS grubundaki hastalar ile fertil grupta NF-κB p65 ekspresyonu benzer bulunmuştur. LOD sonrası H-skoru 3 kat azalmıştır. Fertil grubun H-skoru pre-op ve post-op dönemde over kistli hasta grubunun H-skorundan düşük bulunmuştur. Yapılan benin kistektomi operasyonları sonrası endometrial NF-κB p65 ekspresyonunda belirgin değişiklik gösterilmemiştir. Ovaryen kist varlığı pre-operatif H-skor değerlerini etkilememektedir.

Sonuç: LOD, klomifen sitrat rezistansı olan PKOS'li hastalarda endometrial NF-κB p65 ekspresyonunu azaltarak fizyolojik enflamasyonu ve reseptiviteyi iyileştirmektedir.

Anahtar Kelimeler: PKOS, ovaryen drilling, klomifen sitrat, nükleer faktör κB, endometrial enflamasyon

Introduction

The physiological amount of inflammation is needed for the blastocyst to be held securely in the endometrium. Inflammation at the pathological level might block attachment and invasion of a blastocyst[1,2]. Pathological inflammation in endometrial tissue may lead to failed decidualization and implantation. Clear scientific data on pathological inflammation preventing implantation come from patients using intrauterine devices[3,4]. In addition to local endometrial pathology diseases located in extra-endometrial areas such as ovaries or fallopian tubes may adversely affect the endometrium receptive status[1,5]. In support of this hypothesis, in the presence of endometrioma or hydrosalpinx pathological inflammation in the endometrium is significantly increased. The reduction of inflammation following salpingectomy or endometrioma cystectomy further confirms the effects of extra-endometrial diseases on endometrial inflammation[1,6].

In addition to anovulation-failed receptivity due to increased endometrial inflammation may further complicate the implantation in subfertile subjects due to polycystic ovary syndrome (PCOS)[7]. Concordantly, it has been reported that PCOS- related inflammation may cause expression of both steroid hormone receptor and receptivity genes[8,9]. In line with this, the expression of homeobox genes, one of the most important receptivity molecules, decreased in PCOS[9]. Chronic inflammation is one of the most important factors leading to subfertility in PCOS[10]. Increased levels of NF-κB p65, an intracellular marker of inflammation, have been shown in women with PCOS[7]. NF-κB is a molecule that contains homo and heterodimers in its structure consisting of five different subunits: p50/p105, p52/p100, p65, c-Rel, and RelB. They are bound to its inhibitory protein IκBα and block the activation of NF-κB. Following any stimulus, IκBα is phosphorylated and the NF-κB is released. NF-κB dimers migrate to the nucleus where they activate many genes related to inflammation[1,11].

Laparoscopic ovarian drilling (LOD) is a treatment option in the case of clomiphene-resistant women with PCOS to increase ovulation and pregnancy rates[9]. The increase in the pregnancy rates following LOD was attributed to the increase in ovulation rate or decrease in serum androgen levels[12,13]. Senturk et al.[9] reported that LOD improves receptivity gene expression in women with PCOS. The increase in homeobox 10 and 11 mRNA after LOD, made us think that LOD may also improve the decreased implantation rates in patients with PCOS by regulating endometrial inflammation. To clarify our prediction we decided to determine NF-κB p65 expression in the endometrial tissues obtained from women with PCOS. We, therefore, attempted to investigate the effect of LOD on endometrial NF-κB p65 of infertile women with clomiphene-resistant PCOS.

Materials and Methods

Power analysis of the study was performed with Mann-Whitney U test with the effect size of 0.50% and power of 80%. Type 1 error was noted as 5%. In this case-controlled study endometrial NF-κB expression was evaluated during the mid-luteal phase from 25 infertile women with clomiphene-resistant PCOS. Patients were diagnosed with PCOS based on the revised Rotterdam criteria. Detail criteria can be found elsewhere. Women who do not respond to clomiphene treatment were accepted as clomiphene-resistant[9]. Failure to achieve ovulation with clomiphene, followed by letrozole, led to the decision of laparoscopy (L/S). Seven infertile women ovarian cysts and seven fertile women without PCOS undergoing tubal sterilization were recruited as controls. While benign ovarian cysts were unilateral in 4 cases, they were recorded bilaterally in 3 cases. In addition to fertile controls, taking patients with benign ovarian cysts as a control group enabled us to test the possible effects of both the presence and surgery of non-endometriotic ovarian cysts on the endometrium. LOD was performed using a monopolar hook and 3 to 5 injury of 1 to 2 mm in the cortex of ovary[14,15].

Benign ovarian cysts were removed surgically in control participants. Fertile participants underwent endometrial sampling during tubal sterilization. Women with PCOS were subjected to progesterone withdrawal to determine their secretory phases. Noyes criteria were also taken into consideration in endometrial specimens. Serum progesterone levels were measured in each case to determine ovulation. Venous blood was taken from PCOS and control subjects for biochemical and hormonal analysis. Homeostasis model assessment-insulin resistance index (HOMA-IR) was used to determine insulin resistance. Women with history of endometrioma, hydrosalpinx, chronic inflammatory diseases were excluded. The study was conducted approving by the

Local Ethics Committee (approval number: 10973, date: 10.03.2019).

NF-κB/p65 Staining

Endometrial samples were taken before drilling with a Pipelle cannula. The second samples were taken 3 months later and embedded in paraffin blocks. The immunostaining was performed using ready to use NF-κB/p65 antibody. Following washing with PBS, the slides were incubated with a peroxidase kit. The slides were developed in DAB, counterstained with hematoxylin. To determine the expression intensity of NF-κB p65, the H-score was used. H-score=ΣPi (i+1), where Pi is the percentage of stained cells in each intensity category (0-100%), and i is the intensity indicating weak (i=1), moderate (i=2) or strong staining (i=3).

Statistical Analysis

Statistical analyses were performed with the SPSS 20.0 software. Data were shown as mean ± standard deviation values or percentages. Percentages of demographic findings were compared using the Paired t-test. A $p<0.05$ was considered statistically significant. Kolmogorov-Smirnov test was used for normality of data. While continuous variables were analyzed by using the Mann-Whitney U test Pearson chi-square test was used for categorical data.

Results

Table 1 shows the demographic characteristics of both groups of participants. Fertile controls had no evidence of clinical and ultrasonographical manifestations of PCOS. The average age of the fertile group was recorded as higher than both PCOS and ovarian cyst groups. While the mean ages of the patients with PCOS and ovarian cyst groups were similar, the body mass index (BMI) was found to be higher in patients with PCOS. BMI of PCOS and control were found similar. BMI of subjects with PCOS was higher than the BMI of women with ovarian cysts. In addition to fasting insulin and total testosterone levels, HOMA-IR levels were significantly higher than those in the fertile control and ovarian cyst group. No difference was found between the three groups in terms of blood glucose values.

Expression levels of NF-κB p65 were higher in women with PCOS compared to controls. Likewise, endometrial NF-κB p65 expression was higher in patients with PCOS before LOD compared to women with benign ovarian cysts. The LOD of PCOS ovaries decreased the endometrial NF-κB p65 expression to the levels of fertile control subjects. Endometrial NF-κB p65 did not change significantly after ovarian cystectomy. A trend toward decreased endometrial NF-κB p65 expression was found after ovarian cystectomy compared with the preoperative values. H-score values of endometrial NF-κB p65 in the fertile group were significantly lower than those of women with PCOS before LOD. The pre-LOD H-score values were approximately twice higher than the H-score values of the ovarian cyst group. Following LOD, H-score values of patients with PCOS and fertile cases were found to be similar. After LOD, H-score values decreased approximately 3 times. The H-score value of the fertile group was significantly lower than the preoperative and postoperative H-score values of the ovarian cyst group. The laterality of the ovarian cyst did not cause any change in preoperative H-score values. Likewise, the fact that the ovarian cyst was unilateral or bilateral did not affect the change in H-score values due to surgery (Table 2). The immunoreactivity of NF-κB p65 was detected in the cytoplasm of luminal and glandular cells (Figure 1).

Table 1. Demographic and hormonal features of each group of participant

	I-PCOS (n=25)	II-Ovarian cyst (n=7)	III-Fertile control (n=7)
Age	26.9±8.39	27.4±4.30	29.2±4.50
Infertility duration (years)	3.16±1.01	2.81±0.31	-
Cyst size (mm)	-	6.12+0.16	-
Laterality	Bilateral	Unilateral in 4, bilateral in 3 cases	-
BMI (kg/m^2)	26.4±1.02*	24.4±4.04	26.7±3.35
Total testosterone (ng/dL)	74.0±1.23*	34.3±4.59	35.9±8.19
HOMA-IR	4.54±1.60*	2.60±1.29	2.77±1.02
Fasting insulin (Mu/mL)	18.1±0.11*	10.3±1.22	11.0±0.03
Fasting glucose (mg/dL)	84.1±1.22	81.4±3.45	86.3±0.50
P-value			
I vs II	BMI: $p<0.03$, testosterone: $p<0.01$, HOMA-IR: $p<0.03$, insulin: $p<0.01$.		
I vs III	Age: $p<0.023$, testosterone: $p<0.001$, insulin: $p<0.01$		

BMI: Body mass index, HOMA: Homeostatic model assessment, IR: Insulin resistance, PCOS: Polycystic ovary syndrome

Discussion

In this study, we showed that pathological endometrial inflammation before LOD in clomiphene-resistant PCOS cases increased significantly compared to non-PCOS controls. The LOD of PCOS ovaries decreased the endometrial NF-κB p65 expression to the levels of fertile control subjects. Endometrial NF-κB p65 did not change significantly after ovarian cystectomy. Providing normalization in endometrial inflammation following LOD is an important finding that supports the view that a cause of subfertility in PCOS is abnormal endometrial inflammation. Many hormone values of our patients returned to normal after LOD. The decrease in HOMA-IR and androgen values after LOD may be the reason for the decrease in endometrial NF-κB p65 levels. As a result, by performing ovarian drilling in PCOS cases we can improve hormonal values and NF-κB p65 expression close to natural cycles by achieving a reduction in ovarian volume, serum testosterone, and androstenedione levels. In a recent study conducted in clomiphene-resisting PCOS cases, it has been shown that ovarian drilling increases the expression of endometrial receptivity genes[9]. LOD does not require cycle monitoring, is performed once, is inexpensive and has no risk of hyperstimulation, making it an alternative approach to medical treatments. In addition to all these positive effects, it has disadvantages such as surgical complications and the risk of periovarian adhesion.

The pathophysiology of PCOS is examined under four interacting topics such as insulin resistance, hyperandrogenemia, and chronic low-grade inflammation. While clinical and laboratory findings of systemic inflammation are observed in some of the

Table 2. Pre-drilling and post-drilling H-score values of NF kappa B/65 (RelA) expression in PCOS and control groups

	H-score of NF kappa B/65 (RelA) expression		
Groups	Preoperative	Postoperative	p-value
PCOS (n=25)	0.33±1.27	0.11±2.32	<0.002
Unilateral ovarian cyst (n=4)	0.18±1.29	0.10±1.27	0.069
Bilateral ovarian cyst (n=3)	0.21±0.12	0.19±0.34	0.080
Fertile control (n=7)	0.09±4.78		-

PCOS: Polycystic ovarian syndrome

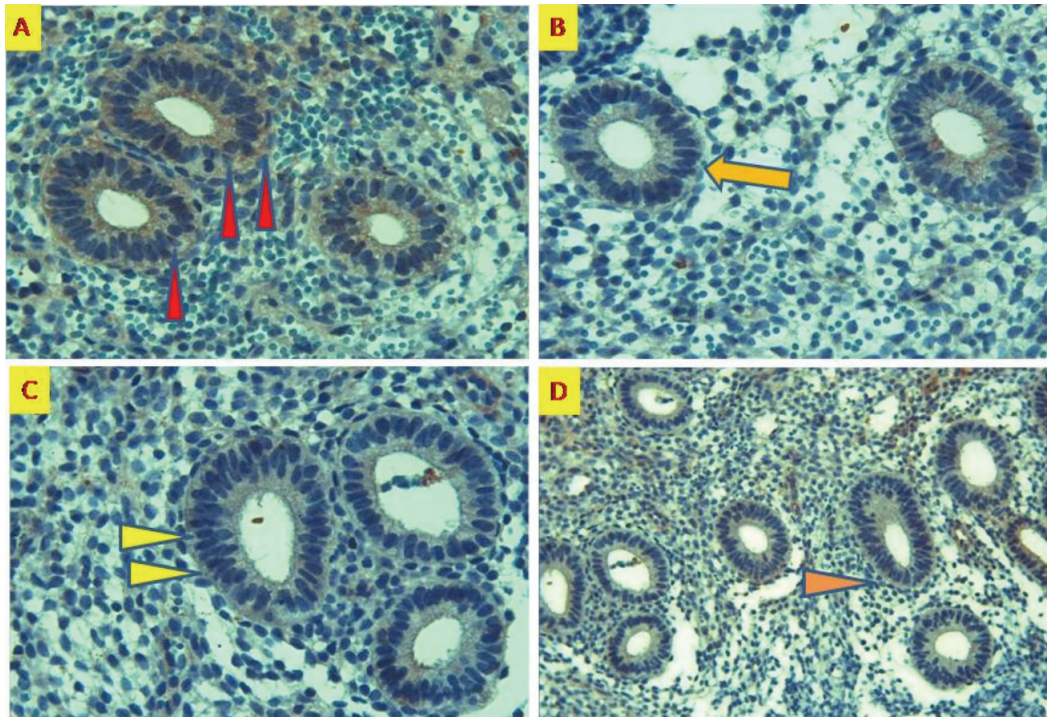

Figure 1. High endometrial NF-kB p65 immunoreactivity in women with PCOS localized in luminal and glandular epithelium before LOD (A, red arrowheads, X20). Significantly decreased NF-kB p65 expression after LOD (B, arrow, X20). Normal endometrial NF-kB p65 expression in ovarian cyst group before cystectomy (C, arrowheads X20). Insignificant decline in NF-kB p65 expression following cystectomy (D, arrowhead X10)

PCOS: Polycystic ovarian syndrome, LOD: Laparoscopic ovarian drilling

PCOS cases, there is no evidence of the presence of inflammation in some of them. Adipose tissue of patients with PCOS causes pathological changes in insulin sensitivity, lipid metabolism, reproductive system functions, and inflammation through adipokines and proinflammatory cytokines such as hs-C-reactive protein, interleukin-6, and tumor necrosis factor α, adiponectin, visfatin, and omentin[16,17]. Any systemic inflammatory condition located in the reproductive organs may affect the endometrium and then its receptive capacity[1,3]. In line with this, the presence of PCOS, hydrosalpinx, endometriosis, or endometrioma affects the endometrial inflammation irrespective of their location[1,5,9]. Since PCOS is a chronic and systemic inflammatory disease, the endometrium can be negatively affected by this process. Although many oocytes are collected in in vitro fertilization-embryo transfer cycles implantation rates are still relatively low in women with PCOS suggesting failed receptivity or deviation from physiological inflammation. NF-κB is the most studied molecule in the evaluation of endometrial inflammation. A recent study by Koc et al.[7] demonstrated that expression levels of endometrial NF-κB p65 increased in normal and overweight women with PCOS suggesting pathological inflammation. Nevertheless, studies investigating the effect of medical agents or ovarian surgery on PCOS-related pathological endometrial inflammation are lacking. Likewise, the role of LOD in endometrial NF-κB expression in PCOS-related subfertility has not yet been elucidated yet. Our findings are important in terms of the first demonstration of increased NF-κB p65 expression in infertile women with clomiphene-resistant PCOS. We clearly showed that expression levels of endometrial NF-κB p65 were higher in infertile patients with PCOS before LOD compared with healthy fertile controls. Significantly decreased NF-κB p65 expressions were deteceted in endometrial samples obtained three months after LOD.

LOD is a minimally invasive surgical procedure preferred in patients with PCOS who are resistant to clomiphene citrate administration, have BMI less than 30 kg/m^2 and LH levels above 10 IU/L. In infertile PCOS cases, a proper LOD normalizes impaired ovarian morphology, as well as endocrine properties[12,13,18]. A recent study demonstrated that endometrial receptivity genes were shown to be downregulated in clomiphene-resistant PCOS cases[9]. They also reported that LOD improves the expression of receptive genes. Although they did not evaluate the endometrial inflammation following LOD, they suggested that one of the most important causes of a decrease in receptivity molecules is pathological endometrial inflammation. In another work of the same author, it has been reported that NF-κB expression decreased significantly after endometrioma cystectomy[1]. When these results and our findings are evaluated together, we conclude that the endometrium can be indirectly affected in the presence of remote tissue disorders such as endometrioma or systemic diseases such as PCOS. Furthermore, because of the removal of the diseases, the indirect changes on the endometrium disappear. The decrease in endometrial NF-κB p65 expression after LOD in clomiphene-resistant PCOS cases is the biggest supporter of these claims. However, the absence of changes in endometrial inflammation in cases of a non-endometriotic benign cyst located in the ovary suggests a special communication between the ovarian tissue and the endometrium in PCOS cases.

Increased endometrial NF-κB p65 expression may lead to PCOS-associated subfertility and LOD restores pathological inflammatory events. We demonstrated for the first time that LOD improved the pathological endometrial inflammation in infertile women with clomiphene-resistant PCOS. We do not know the basic mechanism of the reduction of inflammation after LOD. However, it is possible to make some assumptions from the results obtained from previous studies. Endometrial inflammation and receptivity increase after salpingectomy is one of the best examples[6]. A significant reduction in the expression of endometrial NF-κB p65 after endometrioma resection is also a supportive example[1]. Similar to these two examples, LOD may have regulated inflammation on the endometrium by causing some morphological, metabolic and hormonal changes in the ovarian tissue. Since LOD does not have a direct effect on the endometrium, the results we obtained should depend on the change in ovarian tissue. Since inflammation is more detected in women with PCOS decrease in ovarian androgen production, stromal thickness and improvement of insulin resistance after LOD may indirectly normalize endometrial NF-κB p65 expression. Whatever the actual mechanism, the morphological and hormonal changes in the ovary after LOD affect the endometrium positively and correct the pathological inflammation. A reason for the improvement in fertility outcome after LOD may be the decrease of NF-κB p65 levels in the endometrium. Studies comparing medical treatment with LOD will further strengthen our knowledge of this issue.

Ethics

Ethics Committee Approval: The study was performed approving by the Local Ethics Committee (approval number: 10973, date: 10.03.2019).

Informed Consent: Inform consent was obtained.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: N.D.G., K.G., A.Y., K.C., Ş.H., Design: N.D.G., K.G., A.Y., K.C., Ş.H., Data Collection or Processing: N.D.G., K.G., A.Y., K.C., Ş.H., Analysis or Interpretation: N.D.G., K.G., A.Y., K.C., Ş.H., Literature Search: N.D.G., K.G., A.Y., K.C., Ş.H., Writing: N.D.G., K.G., A.Y., K.C., Ş.H.

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Review / Derleme

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Surgical benefits of bidirectional knotless barbed sutures over conventional sutures for uterine repair during cesarean section-A meta-analysis of randomized controlled trials

Sezaryen sırasında uterus onarımı için kullanılan çift yönlü düğümsüz dikenli sütürlerin geleneksel sütürlere göre cerrahi açıdan faydaları-Randomize kontrollü çalışmaların bir meta-analizi

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Abstract

To analyze the surgical benefits of bidirectional knotless barbed suture (BS) compared with conventional sutures for uterine closure during cesarean section. The databases were searched using the following keywords: "Cesarean Section," "Uterine closure," "Barbed suture" and "Conventional suture." Randomized control trials reporting the comparison of bidirectional knotless BS with conventional sutures for closing uterine incision were included. The outcome measures were closing time of uterine incision, the number of additional hemostatic sutures used, blood loss parameters, and the total duration of surgery. A random or fixed-effects model was used to obtain the pooled estimates using the inverse variance method. The heterogeneity was assessed using the I2 test and the GRADE approach was used to assess the quality of evidence. Out of 15 full-text assessed, three randomized controlled trials were included. We observed significantly short uterine incision closure time with BS [standardised mean difference -1.51; 95% confidence interval (CI): -1.97, -1.06; I2=64%; GRADE approach evidence: Moderate], significantly lesser need of additional hemostatic sutures (risk ratio: 0.39; 95% CI: 0.28, 0.54; I2=0%; GRADE approach evidence: High) and significantly less blood loss during uterine incision closure [-0.47 (95% CI:-0.75, -0.19); I2 =0%; GRADE approach evidence: moderate]. with no significant difference in total blood loss, the need of blood transfusion, and total duration of surgery. The use of bidirectional knotless BS for uterine closure can reduce suturing time and the additional suture requirement.

Keywords: Barbed suture, cesarean section, conventional suture, uterine closure

Öz

Bu meta-analiz, sezaryen sırasında uterus kapatma için geleneksel dikişlerle karşılaştırıldığında çift yönlü düğümsüz dikenli sütürlerin (DS) cerrahi faydalarını analiz etmek için yapılmıştır. Veri tabanları "sezaryen", "uterin kapatma", "dikenli sütür" ve "konvansiyonel sütür" anahtar kelimeleri kullanılarak tarandı. Uterus insizyonunu kapatmak için çift yönlü düğümsüz DS'nin konvansiyonel sütürlerle karşılaştırılmasını bildiren randomize kontrollü çalışmalar dahil edildi. Sonuç ölçütleri, uterus insizyonu kapanma zamanı, kullanılan ek hemostatik sütür sayısı, kan kaybı parametreleri ve toplam cerrahi süresiydi. Ters varyans yöntemini kullanarak havuzlanmış tahminleri elde etmek için rastgele veya sabit etkiler modeli kullanıldı. Heterojenlik, I2 testi kullanılarak değerlendirildi ve kanıt kalitesini değerlendirmek için GRADE yaklaşımı kullanıldı. Değerlendirilen 15 tam metinden üç randomize kontrollü çalışma dahil edildi. DS kullanımı ile daha kısa uterus insizyonunu kapatma süresi [standartlaştırılmış ortalama fark -1,51; %95 güven aralığı (GA): -1,97, -1,06; I2= %64; GRADE yaklaşımı kanıtı: orta] önemli ölçüde daha az ek hemostatik sütür ihtiyacı [risk oranı (RR): 0,39; %95 GA: 0.28, 0.54; I2= %0; GRADE yaklaşımı kanıtı: Yüksek] ve uterus insizyonunun kapatılması sırasında önemli ölçüde daha az kan kaybı (-0,47 [(%95 GA: -0,75, -0,19); I2 =0; GRADE yaklaşımı kanıtı: Orta) tespit edildi. Toplam kan kaybı, kan transfüzyonu ihtiyacı ve toplam ameliyat süresinde anlamlı bir fark gözlenmedi. Sonuç olarak, uterus kapatma için çift yönlü düğümsüz DS kullanımı, dikiş süresini ve ek dikiş ihtiyacını azaltabilir.

Anahtar Kelimeler: Dikenli sütür, sezaryen, konvansiyonel sütür, uterus kapatılması

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Introduction

Cesarean section is the most performed surgery in obstetrics. There are many variations in the technical aspect particularly uterine incision closing technique either a single layer or double layer, intermittent suturing or continuous suturing, locked or unlocked suturing. There is a lack of evidence to recommend one suturing technique over the other or one suturing material over the other regarding the risk of short-term or long-term complications[1,2]. Surgeons mostly use the technique and suture material based on their experience or preference. Conventional smooth sutures require knotting. A surgical knot simply helps in anchoring the smooth suture. Knotting causes uneven distribution of tension across the incision and reduces the tensile strength of the suture by thinning and stretching the suture material[3]. Studies have reported a 35%-95% reduction in tensile strength at the site of the knot or just adjacent to the knot. Also, there are chances of suture failure due to knot slippage. This concern leads to the over-tightening of knots with conventional sutures. Tighter knots are even worse for tissue healing as they can cause localized tissue hypoxia and reduced fibroblast proliferation leading to decrease strength in the healed tissue. The knot also acts as foreign body material and the amount of inflammatory response is related to the number and size of the knot. So, minimizing knot size or eliminating knots altogether by using bidirectional knotless BS should be beneficial, if tissue approximation of suture line is not compromised[4,5].

Knotless BS are a relatively new type of suture. BS have been approved by Food and Drug Administration since 2004. It consists of a standard monofilament suture with tiny barbs cut along the length, facing in opposite directions at approximately 1 mm intervals. The BS may be unidirectional with a needle at one end and a loop at the end of the suture or bidirectional with a needle at both the ends and barbs changing direction at the middle of the suture[3].

BS is frequently used in gynecological surgeries especially laparoscopic surgeries over a decade because of their beneficial role in reducing suturing time and blood loss. Later, BS was introduced in obstetrics to reduce operative time and blood loss in cesarean section. The current meta-analysis determines whether knotless BS can be considered a reasonable alternative to conventional sutures.

Materials and Methods

This meta-analysis was conducted as per the PRISMA checklist (Figure 1).

2.1. Study Identification

We searched published literature using the following electronic database- PubMed, Google Scholar, Clinical trial registry (clinicaltrials.gov.in, ctri.in), and Cochrane Database of Systematic Reviews. We also searched bibliographies of relevant research and review articles. A combination of the following search terms was used "Cesarean Section," "Uterine closure," "Barbed suture (BS)" and "Conventional suture." Studies published up to August 2020 were included. The last search was run on 25th March 2021. Studies were selected on the basis of a review of the title and abstract by 2 independent investigators. There were no Language restrictions while searching for studies. The meta-analysis was registered on PROSPERO (CRD42020207029).

2.2. Selection Criteria

Articles reporting the comparison of a bidirectional knotless BS a conventional suture for closing uterine incision during cesarean section were assessed. Randomized control trials, which have provided data on the closing time of uterine incision with the use of bidirectional knotless BS and conventional sutures were included. Polyglactin and Catgut sutures were considered conventional sutures. All observational (cross-sectional, case-control, and cohort designs), non-comparative studies, review articles, and duplicate studies were excluded.

2.3. Risk of Bias Assessment of Included Studies

Three investigators assessed the methodological quality of the included studies as per revised Cochrane "risk of bias assessment tool for the randomized controlled clinical trials (ROB-II)"[6]. Each included studies were assessed for following parameters: the process of randomization, deviations from the intended interventions, missing outcome data, outcome measurement, and selective outcome reporting. Any disagreements were resolved by discussion and consensus among the authors.

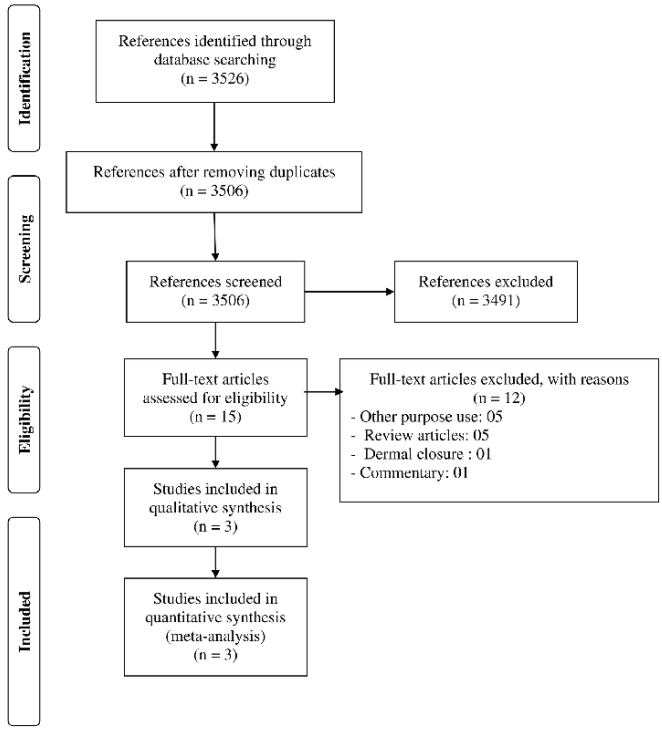

Figure 1. Study selection- The preferred reporting items for systematic reviews and meta-analysis flow diagram

2.4. Data Extraction

The following data were extracted in a Microsoft Excel sheet, 2019: The first author, publication year, study design, the place of study, age, the indication of cesarean sections, number of previous cesarean sections, and outcome data as per intention-to-treat analysis. The data were cross-checked to ensure the accuracy of extraction.

2.5. Outcome Measures

The main outcome measure chosen for this meta-analysis was the closing time of uterine incision. Other outcome measures were the number of additional hemostatic sutures used, blood loss parameters, and the total duration of surgery.

2.6. Data Synthesis

All continuous outcome variables were presented as standard mean difference (SMD) with a 95% confidence interval (CI). The SMD of 0.2 was considered a small effect, 0.5 a moderate, and 0.8 a large effect as described previously[7]. All dichotomous variables were presented as a risk ratio (RR) and its 95% CI. The I^2 index was used to look for heterogeneity among included studies. Fixed-effects model was used if there is no significant heterogeneity. If the I^2 index >50% among analyzed studies, then a random-effects model was used. The funnel plot method was used to report publication bias. The meta-analytic summary was measured using the inverse-variance method.

A sensitivity analysis of all outcomes was performed based on the risk of bias assessment. The outcome measures were estimated by excluding studies having "some concern" or "high concern" on the risk of bias assessment.

The GRADE approach was used to present the quality of the evidence for each outcome variable. The following parameters were considered: study design, study limitations, inconsistency, indirectness of evidence, imprecision, and publication bias[8]. The meta-analysis was performed using Review Manager software version 5.4.

Results

3.1. Study Characteristics

A total of 3.526 articles were found after a meticulous search using the search strategy. As shown in Figure 1, three intervention trials (comprising of 136 bidirectional knotless BS cases and 136 conventional suture cases) were included out of 15 full-text articles assessed as per selection criteria in this meta-analysis. Relevant study characteristics of included trials (study design, size and types of sutures, and the number of participants) are summarized in Table 1[9-11]. Baseline characteristics of the patients from all included trials are summarized in Table 2. The most common indication for cesarean was failed/refused Trial of labor followed by arrest disorders, multiple gestations, cephalopelvic disproportion, etc. in all included studies.

Grin et al.[9] conducted a randomized controlled trial on 70 participants (35-35 in each group). The participants, data analysts, and postpartum staff were kept blinded to the treatment allocation. Surgeons were unmasked to randomization after scrubbing for the surgery, as it is impossible to keep them blinded due to the different appearance of suture materials. Baseline demographics, medical history, and antepartum characteristics were comparable in both groups. A standard operative technique was used in the cases. Uterine incision length and maximal myometrial thickness were measured using a sterile disposable ruler as they can be the potential confounding factors. In one group, the uterine incision was closed using bidirectional knotless BS in a two-layer continuous, non-locking technique. In another group, uterine closure was done using polyglactin in two layers, the first layer a continuous locking and the second layer a continuous non-locking manner.

Peleg et al.[10] Conducted an open-labeled, randomized controlled trial, 102 women were randomized, 51-51 in each group. Randomization was kept masked till the time of surgery to minimize provider bias. Demographic and clinical characteristics were similar in both groups. Four experienced surgeons performed all the cesarean sections using a similar technique. In the bidirectional knotless BS group uterus was closed in two-layers continuous, unlocked fashion. In the polyglactin group, the first layer was in continuous locking with knotting on both ends and the second layer in a continuous unlocked fashion. The outcome data were assessed by blinded assessors.

Zayed et al.[11] Conducted an allocation concealed, randomized controlled trial, 100 women were randomized into 2 groups in a 1:1 ratio. The clinical profile of the included women (gravida, parity, gestational age at the time of cesarean, number

Table 1. Characteristics of included studies

Study	Study design	Barbed suture	Conventional suture	Barbed suture group (N)	Conventional suture group (N)
Grin et al. 2019[9]	RCT	Size-1.0 "Stratafix"	Size-1.0, Polyglactin suture, "vicryl"	35	35
Peleg et al. 2018[10]	RCT	Size-2.0 "Stratafix"	Coated size-1.0 polyglactin 910 suture, "Vicryl Plus"	51	51
Zayed et al. 2017[11]	RCT	Size-1.0 "Stratafix"	Size-1.0, polyglactin 910 suture, "vicryl"	50	50

RCT: Randomised controlled trial

Table 2. Baseline characteristics of patients in the included studies

Study	Number		Age (years) Mean ± SD		BMI (kg/m²) Mean ± SD		Gravidity Mean ± SD		Parity Mean ± SD		Gestational age (weeks) Mean ± SD		Previous cesarean n. percentage/Mean ± SD*	
	Barbed	Conventional	Barbed	Conventional	Barbed	Conventional	Barbed	Conventional	Barbed	Conventional	Barbed	Conventional	Barbed	Conventional
Grin et al. 2019[9]	35	35	32.4±5.4	32.9±6.1	30.7±5.9	32.6±6.4	3.1±2	2.9±1.2	1.6±1.8	1.5±1	38[1]±2[2]	38[1]±2[2]	17 (48.6)	18 (51.4)
Peleg et al. 2018[10]	51	51	32.2±6.2	33.0±5.0	30.07±5.08	30.50±5.01	2.8±1.5	2.9±1.3	1.5±1.3	1.5±1.0	NR	NR	31 (60.8%)	39 (76.5%)
Zayed et al. 2017[11]	50	50	NR	NR	NR	NR	2.3±1.7	2.1±1.1	1.5±1.2	1.5±1.2	37.8±0.74	37.8± 0.95	1.9±1*	1.7±0.7*

SD: Standard deviation

of previous cesarean sections, indications of cesarean section, etc.) was comparable in both arms. Though, they did not mention baseline characteristics like age, BMI in their study. All the cesarean was done by a single surgeon. In the bidirectional knotless BS group, the uterus was closed in a two-layer continuous suturing technique. In the polyglactin group, the first layer was closed using the continuous suturing technique and the second layer was closed with interrupted sutures. The study did not comment on the blinding of outcome assessors.

3.2. Risk of Bias Assessment

The risk of bias assessment in individual trials is in Figure 2. Two randomized controlled trials were considered of having low[9,10] and one having "some concern"[11] as per the ROB-II tool. Zayed et al.[11] was considered to have 'some concern' for measuring outcomes.

3.3 Outcomes

3.3.1. Uterine Incision Closing Time

All three included studies took uterine incision closing time as their primary outcome. Two studies mentioned closing time in seconds while the third one gave results in minutes. For comparing the data, results given in minutes were converted to seconds. The uterine incision closing time was significantly shorter in a bidirectional knotless BS group than that in the conventional suture group based on a pooled SMD of -1.51[(95% CI: -1.97, -1.06); I^2= 64%]. (Figure 3). As shown in Table 3, the GRADE approach suggests moderate-quality evidence of the uterine incision closing time outcome. The funnel plot appeared asymmetrical on visual inspection. On sensitivity analysis, a similar trend was observed. The result was found to be favoring BS group [SMD: -1.30 (95% CI: -1.69, -0.91); I^2= 26%].

3.3.2. Additional Suture Requirement

Similarly, the need for additional sutures for hemostasis was found to be significantly less in the bidirectional knotless BS compared to the conventional suture group. The pooled RR for additional suture was 0.39 [(95% CI: 0.28, 0.54); I^2 = 0%] (Figure 4). The GRADE approach suggests high-quality evidence of this outcome. On sensitivity analysis, the trend flavored BS group [RR: 0.40 (95% CI: 0.29, 0.57); I^2= 0%].

3.3.3. Blood Loss Parameters

3.3.3.1. Blood Loss During Uterine Incision Closure

Two studies contributed to blood loss during uterine incision closure analysis. The pooled SMD for blood loss during uterine closure was -0.47 [(95% CI: -0.75, -0.19); I^2= 0%] (Figure 5). The GRADE approach suggests moderate-quality evidence of this outcome. On sensitivity analysis, the results favoured BS group [SMD: -0.56 (95% CI: -0.96, -0.16); n=1].

3.3.3.2. Total Blood Loss During Surgery

On comparing total blood loss during surgery, the bidirectional knotless BS was not found to have an additional advantage over conventional sutures. Only two studies contributed to total blood loss analysis. The pooled SMD for total blood loss during surgery was -0.25 [(95% CI: -1.01, 0.51); I^2 =84%] (Figure 6).

3.3.3.3. Need for Blood Transfusion

Two studies reported the need for blood transfusion. The pooled RR for the need of blood transfusion was 1.00 [(95% CI: 0.11, 9.45); I^2= 0%] (Figure 7). The GRADE approach suggests moderate-quality evidence of this outcome (Table 3).

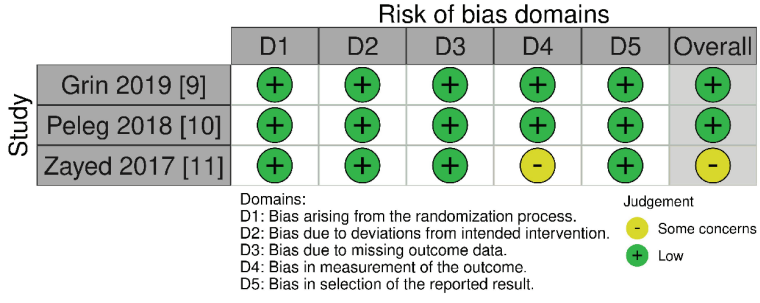

Figure 2. Risk of bias assessment as per "Revised Cochrane risk-of-bias tool for randomized trials (ROB-II)"

3.3.3.4. Perioperative Hemoglobin Change (Delta Hemoglobin)

Only one study by Grin et al.[9] reported a change in hemoglobin between preoperative and postoperative blood count. The authors found no significant difference in delta hemoglobin levels between both the groups at various time intervals (6, 18, 72 h postoperative).

3.3.3.5. Need of Additional Uterotonics and Need of Hemostatic Agents

Only one study by Grin et al.[9] reported a comparison on the need for additional uterotonics (misoprostol, methylergonovine, and carboprost tromethamine) and the need for hemostatic agents (Surgicel Nu-Knit Absorbable Hemostat, Ethicon). They found a significant reduction in the hemostatic agent used in the BS group (RR-0.33) with no difference in the uterotonic requirement (p=0.8).

3.3.4. Other Outcomes

All 3 included studies contributed to the total duration of surgery analysis. The pooled SMD for a total duration of surgery was -0.43[(95% CI: -2.08, 1.21); I^2 =97%] (Figure 8). BS did not show any advantage compared to conventional sutures. The GRADE approach suggests low-quality evidence of this

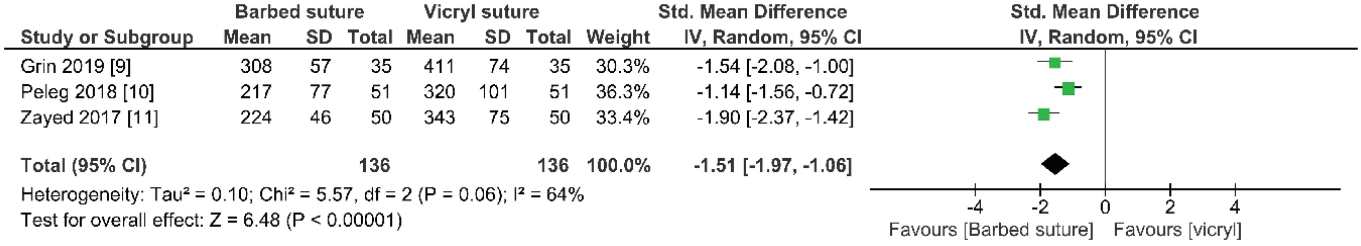

Figure 3. Meta-analytic summary of uterine incision closing time through the random effect of model

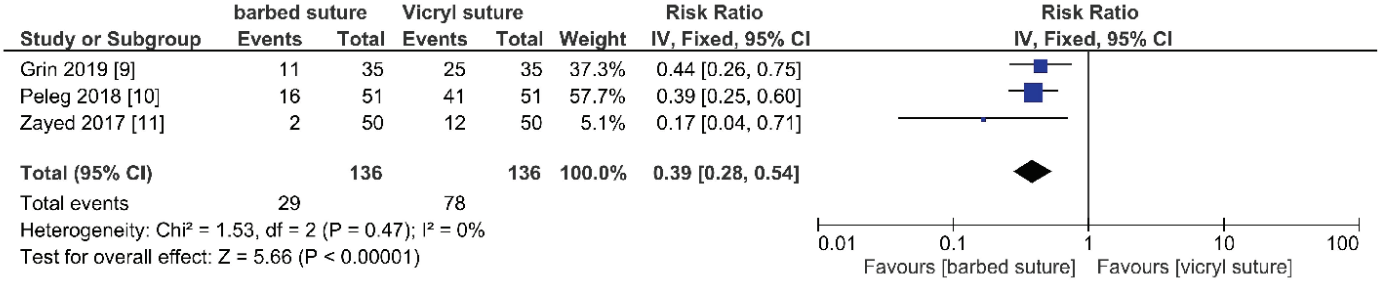

Figure 4. Meta-analytic summary of additional suture requirement through the fixed effect of model

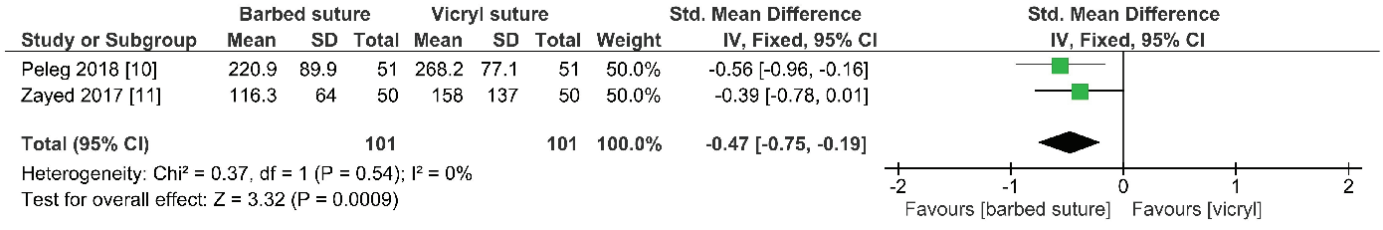

Figure 5. Meta-analytic summary of blood loss during uterine incision closure through the fixed effect of model

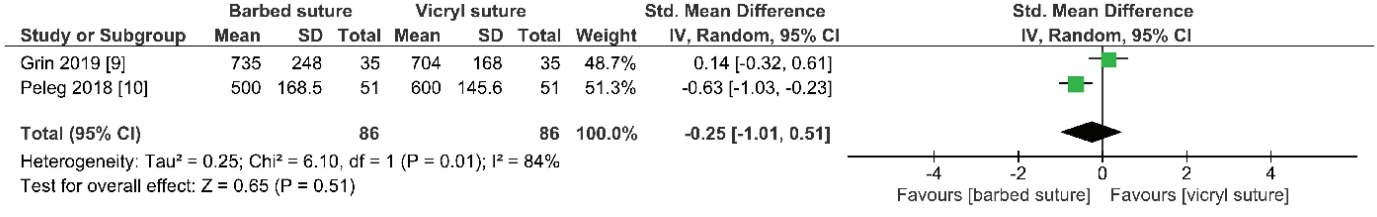

Figure 6. Meta-analytic summary of total blood loss during surgery through the random effect of model

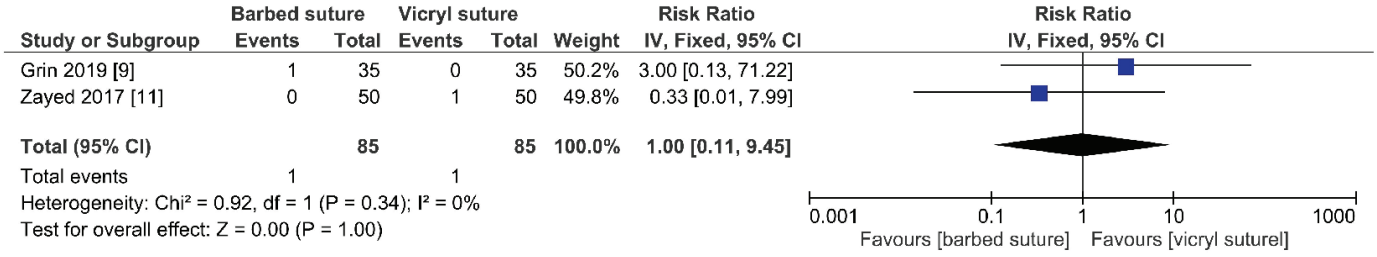

Figure 7. Meta-analytic summary of the need of blood transfusion through the fixed effect of model

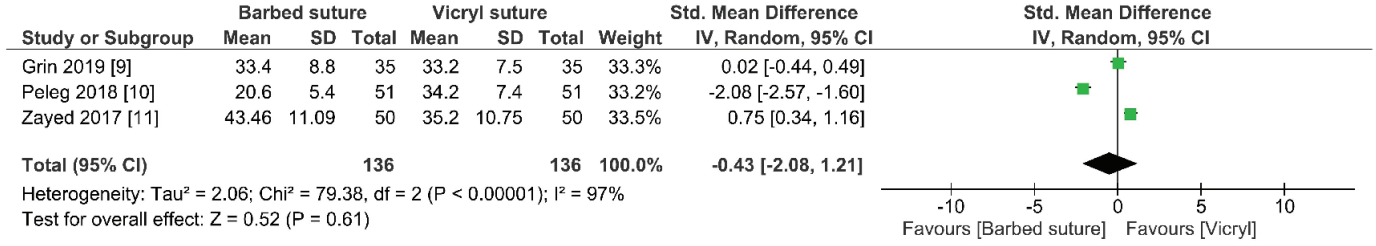

Figure 8. Meta-analytic summary of the total duration of surgery through the random effect of model

outcome (Table 3). The cost of sutures was reported in one study. Zayed et al.[11] mentioned the cost of sutures used in uterine incision closure, which was significantly higher in the BS group (22.75±0 versus 11.025±1.61 U.S. dollars, p<0.001, mean difference 11.725 U.S. dollars). But the number of sutures required was almost three times in the vicryl group, reducing the cost difference (1±0.00 in the barbed group versus 2.94±0.43 in the vicryl group).

Discussion

To summarize, BS offers a significant surgical advantage over conventional smooth sutures in terms of uterine incision closing time, the need for additional hemostatic sutures, and the amount of blood loss during uterine incision closure. GRADE approach suggests moderate to high quality of evidence; However, BS were not found to provide any benefit in terms of total blood loss during surgery, total duration of surgery, the need for blood transfusion, and perioperative complications. A similar trend was observed in the sensitivity analysis.

Suturing is one of the important steps during the cesarean section. Suturing techniques and choice of suture material can influence the healing of the cesarean section scar[12,13]. BS has been introduced in obstetrics recently, as they provide a combined advantage of continuous and interrupted sutures and reduce the suturing time and bleeding without causing tissue ischemia. Several factors are associated with BS, which contribute to a better outcome. First, it might be due to a reduction in suturing time of uterine incision. The reduction in suturing time can be because BS do not require knots as well as there is no backsliding of the suture[14,15]. Compared to conventional continuous sutures, BS do not require tension to be applied to the suture thread by the assistant. Once the BS has been pulled taut, the points of commissure will not loosen even if the tension is not maintained on the suture thread by the assistant[16]. The self-anchoring property of BS contributes significantly in reducing the suturing time. Second, BS result in a good approximation of tissues at the start of suturing resulting in early hemostasis and reduced blood loss[17]. Third, much evidence suggests that BS are associated with better tissue healing. This may be because the presence of barbs on the suture thread at an equal distance result in an equal distribution of tension along the suture line and causes less ischemia to the tissues as well as an absence of knots reduces inflammatory reaction, which harms the healing of tissues[3,18].

The significantly lesser time in uterine closure with the use of BS has important implications in terms of generalizability. Because, the skill and experience of the surgeon might play a crucial role regarding the uterine incision closing time. Conventional sutures being more widely used, replicability of this meta-analysis result requires prior surgeon training in suturing with BS as done in the included studies. However, long-term risks and benefits such as myometrial healing and effect on subsequent pregnancy are still unknown. Peleg et al.[10] compared uterine incision closing time among all operating surgeons. Three of four surgeons had significantly shorter closing times with BS, which suggest results should be easily replicable. Grin et al.[9] did further stratification in the primary cesarean group and repeat cesarean group. The time required to complete the uterine repair was significantly lower in both the strata when BS was used. This shows BS is equally effective in previous cesarean section patients.

Table 3. Quality assessment for outcome parameters as per GRADE approach

No. of studies (Study design)	Study limitations (Risk of bias)	Inconsistency	Indirectness	Imprecision	Publication bias	Quality	Outcome
Closing time - uterine incision							
Three (RCT)	Unclear (No serious limitations)	Substantial heterogeneity (I^2=64%), but of questionable importance (No serious inconsistency)	No serious indirectness	Sample size less than 400 (serious imprecision)	Asymmetric Funnel plot	Moderate	SMD: -1.15 (-1.97, -1.06)
Need for additional suture							
Three (RCT)	Unclear (No serious limitations)	No heterogeneity -I^2=0% (No serious inconsistency)	No serious indirectness	Sample size less than 400 (Serious imprecision)	Asymmetric Funnel plot	High*	RR - 0.39 (0.28, 0.54)
Blood loss during uterine incision closure							
Two (RCT)	Unclear (No serious limitations)	No heterogeneity -I^2=0% (No serious inconsistency)	No serious indirectness	Sample size less than 400 (Serious imprecision)	Asymmetric Funnel plot	Moderate	SMD: -0.47 (-0.75, -0.19)
Total blood loss							
Two (RCT)	Unclear (No serious limitations)	Substantial heterogeneity (I^2=84%), of unequivocal importance (Serious inconsistency)	No serious indirectness	Sample size less than 400 (Serious imprecision)	Asymmetric Funnel plot	Low	RR: -0.25 (-1.01, 0.51)
Need of blood transfusion							
Two (RCT)	Unclear (No serious limitations)	No heterogeneity -I^2=0% (No serious inconsistency)	No serious indirectness	Sample size less than 400 and wide confidence interval (Serious imprecision)	Asymmetric Funnel plot	Moderate	RR: 1.00 (0.11, 9.45)
Total duration of surgery							
Three (RCT)	Unclear (No serious limitations)	Substantial heterogeneity (I^2=97%), of unequivocal importance (Serious inconsistency)	No serious indirectness	Sample size less than 400 (Serious imprecision)	Asymmetric Funnel plot	Low	SMD: -0.43 (-2.08, 1.21)

*Rating updated from moderate to high due to large magnitude of effect (RR<0.5); RCT: Randomized controlled trial, CI: Confidence interval, SMD: Standardized mean difference

Our meta-analysis supports the findings of an earlier meta-analysis showing the beneficial effects of using BS during laparoscopic hysterectomy and myomectomy[19-21]. Over time, the use of BS has expanded in gynecological surgeries. Various studies have shown comparable efficacy to conventional sutures with the added advantage of decreasing suturing time, total operative time, and blood loss during uterine defect closure in Myomectomies[14-16,18,22-24] and during vaginal cuff repair during laparoscopic hysterectomies[25-27].

Our meta-analysis showed that the BS group required a significantly lesser number of additional sutures compared to the conventional group for closing uterine incision during

cesarean section (RR-0.39). The higher cost of BS can be offset as conventional suturing often requires additional hemostatic sutures, which eventually reduces the cost difference[22]. According to a USA study if we look at the average total charges of cesarean delivery, using BS only increases the total charges by 0.05%, which is an insignificant amount[28]. But the scenario could be different in developing countries. Future studies should compare the cost-effectivity of BS in developing countries.

Our meta-analysis could not detect a significant difference in the total duration of surgery as this may be influenced by multiple factors such as the presence of intraabdominal adhesions, adherent bladder in cases of previous cesarean sections, time consumption during the baby delivery, the time required for delivery of the placenta and achieving hemostasis. Furthermore, the experience and expertise of a primary surgeon can also play a role. Because using BS only affects uterine repair, the rest of the factors remain unchangeable, so its effect on the total duration of surgery could not be found. But a recent review of BS versus conventional suture at cesarean delivery found a significant reduction in total surgical duration. This could be because the authors also included a study comparing BS and conventional sutures for skin closure[29]. It is difficult to ensure comparability between uterine musculature closure and skin closure. In contrast with the meta-analysis on gynecological surgeries[19-21], our meta-analysis failed to demonstrate a reduction in the blood loss during uterine incision loss and total blood loss during surgery. As there are many possible reasons for bleeding during cesarean delivery. The common causes are uterine atony, uterine incision extensions, adhesions, placental site bleeding, etc.[30]. Another reason could be the small number of studies included in this analysis.

Due to data limitations, a meta-analytic summary could not be calculated for the peri-operative complications. However, the use of BS did not cause any increase in the incidence of perioperative complications. There was no case of wound infection/endometritis or any other maternal morbidity in all included studies. This finding is also supported by a study by Alessandri et al.[31], who used a fishbone technique with BS for uterine incision closure and compared residual myometrial thickness, incidence, and depth of isthmocele in both groups up to 12 months of follow-up. They found a significantly better result with BS. But till now there is uncertainty on long-term complications such as adhesion formation, poor wound healing leading to increased risk of wound dehiscence or uterine rupture, and morbidly adherent placenta in the next pregnancy. Future studies are needed to resolve such issues.

Study Limitation

This meta-analysis has several limitations. Our findings on BS should be interpreted cautiously due to the inclusion of the open-labeled and small number of randomized studies

Conclusion

The moderate to high-quality evidence suggests the use of bidirectional knotless BS can reduce suturing time and the additional suture requirement for uterine closure.

Ethics

Peer-review: Internally and externally peer-reviewed.

Authorship Contributions

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

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

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Review / Derleme

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Oocyte maturation abnormalities - A systematic review of the evidence and mechanisms in a rare but difficult to manage fertility pheneomina

Oosit maturasyon anormallikleri - Nadir fakat çözümü zor fertilite fenomeninde kanıt ve mekanizmaların sistematik derlemesi

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Abstract

A small proportion of infertile women experience repeated oocyte maturation abnormalities (OMAS). OMAS include degenerated and dysmorphic oocytes, empty follicle syndrome, oocyte maturation arrest (OMA), resistant ovary syndrome and maturation defects due to primary ovarian insufficiency. Genetic factors play an important role in OMAS but still need specifications. This review documents the spectrum of OMAS and to evaluate the multiple subtypes classified as OMAS. In this review, readers will be able to understand the oocyte maturation mechanism, gene expression and their regulation that lead to different subtypes of OMAs, and it will discuss the animal and human studies related to OMAS and lastly the treatment options for OMAs. Literature searches using PubMed, MEDLINE, Embase, National Institute for Health and Care Excellence were performed to identify articles written in English focusing on Oocyte Maturation Abnormalities by looking for the following relevant keywords. A search was made with the specified keywords and included books and documents, clinical trials, animal studies, human studies, meta-analysis, randomized controlled trials, reviews, systematic reviews and options written in english. The search detected 3,953 sources published from 1961 to 2021. After title and abstract screening for study type, duplicates and relevancy, 2,914 studies were excluded. The remaining 1,039 records were assessed for eligibility by full-text reading and 886 records were then excluded. Two hundred and twenty seven full-text articles and 0 book chapters from the database were selected for inclusion. Overall, 227 articles, one unpublished and one abstract paper were included in this final review. In this review study, OMAS were classified and extensively evaluatedand possible treatment options under the light of current information, present literature and ongoing studies. Either genetic studies or in vitro maturation studies that will be handled in the future will lead more informations to be reached and may make it possible to obtain pregnancies.

Keywords: Oocyte maturation abnormalities, oocyte maturation arrest, empty follicle syndrome, in vitro maturation, oocyte maturation gene expression/pathways

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Öz

İnfertil kadınların küçük bir kısmında tekrarlayan oosit matürasyon anormallikleri (OMAS) görülür. OMAS, dejenere ve dismorfik oositleri, boş folikül sendromunu, oosit olgunlaşma arrestini (OMA), rezistan over sendromunu ve primer over yetmezliğine bağlı olgunlaşma kusurlarını içerir. OMAS'de genetik faktörler önemli rol oynamaktadır ancak yine de spesifikasyonlara ihtiyaç duyulmaktadır. Bu derleme, OMAS'nin spektrumunu göstermeyi ve OMAS olarak sınıflandırılan çoklu alt türleri değerlendirmeyi amaçlamaktadır. Bu derlemede okuyucular, farklı OMA alt tiplerine yol açan oosit olgunlaşma mekanizmasını, gen ekspresyonunu ve bunların düzenlenmesini ve OMAS ile ilgili hayvan ve insan çalışmalarını ve son olarak OMA'ların tedavi seçeneklerini anlayabileceklerdir. Literatür taraması; OMAS'ye odaklanan İngilizce yazılmış makaleleri saptamak için; PubMed, MEDLINE, Embase, National Institute for Health and Care Excellence kullanılarak ve aşağıdaki ilgili anahtar kelimeleri arayarak yapıldı. Belirtilen anahtar kelimelerle arama yapılmış ve İngilizce yazılmış kitap ve makaleler, klinik deneyler, hayvan çalışmaları, insan çalışmaları, meta-analiz, randomize kontrollü araştırmalar, derlemeler, sistematik derlemeler tarandı. 1961'den 2021'e kadar yayınlanmış 3.953 kaynak tespit edildi. Çalışma türü, uygunluk ve kopya olan çalışmalar için başlık ve özet taraması yapıldı ve 2.914 çalışma hariç tutuldu. Kalan 1.039 kayıt, tam metin okuma ile uygunluk açısından değerlendirildi ve ardından 886 kayıt hariç tutuldu. Veri tabanından 227 tam metin makale ve 0 kitap bölümü dahil edilmek üzere seçildi. Son olarak, 227 makale, bir yayınlanmamış ve bir özet makale bu nihai incelemeye dahil edildi. Bu derleme çalışmasında, güncel bilgiler, mecvut literatürler ve yürütülmekte olan çalışmalar ışığında osit matürasyon anormallikleri kapsamlı olarak değerlendirilmiş, sınıflanmış ve olası tedavi seçenekleri incelenmiştir. Gelecekte yapılacak olan gerek genetik gerekse in vitro matürasyon çalışmaları, OMAS ile ilgili daha çok bilgiye ulaşılmasına imkan sağlayacak ve gebeliklerin elde edilmesi mümkün olabilecektir.

Anahtar Kelimeler: Oosit matürasyon anormallikleri, oosit matürasyon arresti, boş folikül sendromu, in vitro matürasyon, matürasyon gene ekspresyonu/yolaklar

Introduction

Oocytes undergo a passive loss beginning from approximately 24 weeks gestational age until puberty and then a transition to active loss combined with passive loss during the reproductive life[1,2]. In brief, oocytes are prone to the apoptotic processes until menopause[1,2]. Those oocytes that escape apoptosis are selected as the follicular cohort in each menstrual cycle. It is the pubertal hormonal changes that trigger the resumption of oocyte meiosis. Meiotic resumption is crucial in oocyte maturation and fertilization[3].

Intrafollicular or extrafollicular factors may interfere with the selection of the follicular cohort and can result in various oocyte pathologies including oocyte degeneration, early oocyte loss, oocyte maturation arrest and impaired embryonic development. All these pathologies are defined as oocyte maturation abnormalities (OMAS)[4].

Each one of the OMAS studied in the literature were accepted as separate pathologies, until recently. Our ongoing studies demonstrated that OMAS is often related and there may exist intercycle and intracycle variability resulting in the heterogenic presentation of OMAS. Animal studies have enlightened the mechanisms of human OMAS although, there are some differences.

Traditionally, couples with repetitive OMAS were offered oocyte donation or cytoplasmic-nuclear transfer from donor oocytes, which is unpalatable to some patients and in some societies, impermissible ethically or religiously. In the last decade, studies on the mechanisms and treatment options made it possible to overcome some forms of OMA.

In this review, we aimed to evaluate all known aspects of OMAS.

Methods

Literature searches using PubMed, MEDLINE, Embase, National Institute for Health and Care Excellence were performed to identify articles written in English focusing on the Oocyte Maturation Abnormalities by looking for the following keywords:

Oocyte maturation arrest, Oocyte Maturation failure, In vitro maturation (IVM) arrest, intrinsic oocytes maturation arrest, genetic causes of oocytes maturation arrest, meiotic arrest, meiotic resumption, empty follicle syndrome (EFS), resistant ovary syndrome (ROS), immature oocytes, degenerated oocytes, immature oocytes, zona pellucida mutation, germinal vesicle arrest, GV arrest, germinal vesicle breakdown, M1 arrest, M2 arrest, fertilization failure, fertilization arrest, mikst arrest, premature ovarian failure, premature ovarian insufficiency, somatic cell nükleer transfer, spindle transfer, pronuclear transfer, polar body transfer, DNA heteroplasmy, GV transfer, mitochondrial replacement therapy, CAPA IVM, drugs for oocytes maturation arrest, treatment of oocytes maturation arrest, in vitro activation, transvaginal ovarian injury, ovarian prp. An advanced search was made with the specified keywords by selecting the article type, books and documents, clinical trial, animal studies, meta-analysis, randomized controlled trial, review, systematic review options. The search included 3,953 studies from 1961 to 2021. After title and abstract screening for study typewith duplicates, 2,914 studies were excluded. The remaining 1,039 records were assessed for eligibility by full-text reading and 806 records were then excluded. Two hundred and twenty seven full-text papers selected from database were selected. Overall, 227 articles, one unpublished and one abstract paper were included in this final extensive review.

Literature written in English complying with our search criteria were included in this review while others, either written in languages other than English or incompliant with search criteria were excluded.

Physiology of Meiotic Arrest, Meiotic Resumption and Oocyte Maturation

Until the first LH surge at the beginning of puberty, oocytes coated with a single layer of granulosa cells remain in an arrested state at the diplotene stage of the first meiotic division

as primordial follicles[5]. This preservation of oocytes is crucial for the maintenance of the future reproductive potential. However, the apoptosis of the follicles and oocytes started at approximately 24 weeks gestation age and results in the loss of 90% of the ovarian follicular cohort by initiating puberty. Roughly, 1-2/1000 follicles have a potential to be fertilized, by having undergone maturation and ovulation. Those follicles are derived from a select follicular cohort[6].

Those follicles should be in a dormant state until puberty to survive and reach reproductive potential[7]. In estrous cycles of most animal species and in menstrual cycles of humans, maturation promoting factor (MPF) is a crucial cytoplasmic factor that initiates meiotic resumption. MPF induces germinal vesicle breakdown and promotes the subsequent maturation processes in response to the LH surge[8-10]. Without the introduction of LH analogs or an endogenous LH surge, mature oocytes would be collected at IVF cycles. Mechanisms and pathways of meiotic arrest and resumption are depicted in Figure 1.

High levels of cGMP and cAMP and low levels of PDE 3A enzyme within the oocyte are *sine qua non-oocyte* in meiotic arrest[5,9] cAMP is produced in the oocyte but cGMP is produced in the somatic cells surrounding oocytes and through Nppc/Npr2 system diffuses into the oocyte. MPF is inhibited by the increased cGMP and cAMP and eventually oocytes remained in an arrested state. Until the release of oocytes with surrounding cumulus

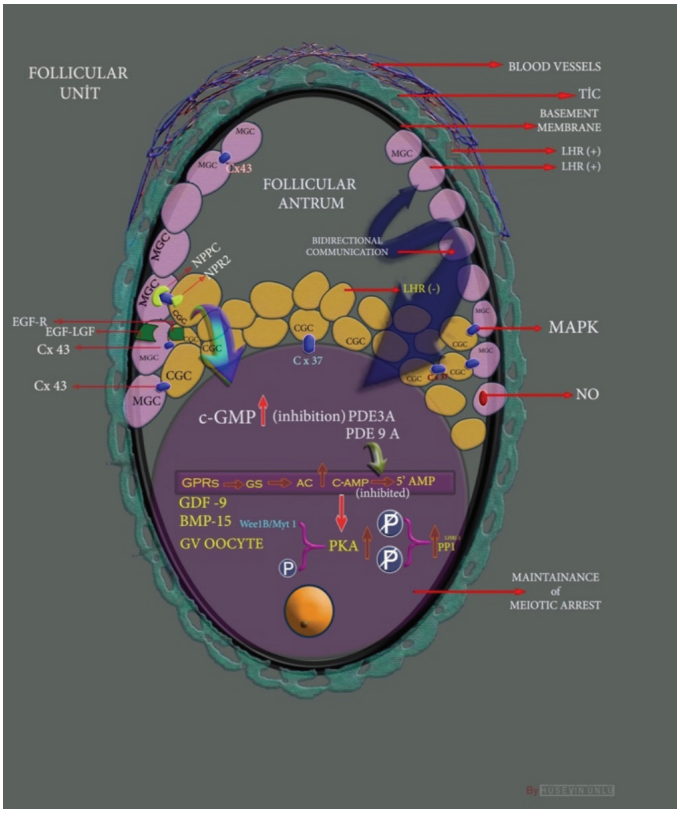

Figure 1. Orchestration of maintenance of meiotic arrest and resumption

cells, there are strong bounds between mural granulosa cells and oocytes. This interaction is bidirectional and interruption of this communication result in spontaneous meiotic resumption in mammals[9,11]. This bidirectional communication is orchestrated by oocyte itself[5,12,13]. LH exerts its action on the resumption of meiosis. Mural granulosa cells carry LHR while cumulus cells and oocytes are lack LHR[14,15]. Thus LH exerts its action on COC indirectly. The LH peak stimulates the expression of endothelin-1, leptin, epidermal growth factor-like ligands, and insulin like-3 transcript in the process of meiotic resumption[5]. Key role in meiotic resumption is the decline in the concentration of cAMP in the oocyte cytoplasm[16]. Nppc/Npr2 pathway is present in granulosa cells[17]. This pathway is strongly expressed in mural granulosa cells and almost not expressed in cumulus cells and oocytes[18,19] Nppr/Npr2 pathway plays important role in follicular competence, formation of healthy cumulus oophorus and maintenance of oocyte meiotic arrest[20]. Human intrafollicular C- type natriuretic peptide (CNP) expression, along with Nppc/Npr2 precursors was determined in human follicular fluid and follicles having mature oocytes were found to have less FF CNP and less Nppc/Npr2 m RNA expresssion[21]. LH, cAMP and Nppc/Npr2 pathway related cGMP are key factors for oocyte meiotic arrest and oocyte meiotic resumption. LH related meiotic resumption happens either using gap junction-related processes or by non-gap junction-related process. In gap junction related process, mural granulosa cell to oocyte cGMP transport is blocked by the closure of gap junctions, thus intracytoplasmic cGMP and cAMP concentrations decrease which increases PDEA3 enzyme concentrations and initiates meiotic resumption[22]. For EGFR to exert its action on maintaining oocyte maturation arrest in zebrafish, Pgrmc1 signaling was reported essential[23]. In non-gap junction process, LH induced EGFR directly inhibits Nppc/Npr2 pathway and decreases cGMP levels[24-26]. FSH stimulation elevates cAMP in mural granulosa cells and make gap junctions more permeable and changes the intracellular distribution of connexin43 (Cx43). This in turn maintain cAMP levels in a certain threshold[27-29]. Additionally, G protein, its Gs protein-coupled receptor (GPR3) was found to play an important role in maintenance of oocyte meiotic arrest by the maintenance of basal cAMP concentrations[30]. Spontaneous meiotic resumption was reported in GPR3 knockout mice[30-32].

Up regulation of GPR3 in Xenopus oocyte resulted in increased intracytoplasmic cAMP levels and inhibition of meiotic resumption process[33]. Lincoln et al.[34] studied the role of Cdc25b phosphatase in meiotic resumption in mice. They used Cdc25b knockout female mice and reported that they were sterile and that the oocytes remained arrested at prophase with very limited MPF activity. Cytosolic malate dehydrogenase (Mor2) injected mouse oocytes demonstrated significant decreases in oocyte maturation[35].

Mor2 mRNA levels were signficantly decreased in immature oocytes. Mor2 has been reported as an essential component

of oocyte maturation and embryo development in the mouse and microinjection of Mor2 mRNA decreases the IVM of mouse oocytes[35]. Mor2 mRNA is highly expressed in MII stage mouse oocytes during maturation cytoplasmic maturation.The disruption of Mor2 mRNA results in inability of the mouse oocyte to use the malate-aspartate shuttle that is crucial for regulating the balance between cytoplasmic and mitochondrial metabolism.

Ovarian follicles, with their mural granulosa cells, cumulus cells and the oocyte is a unit with bidirectional communications. All these communications are orchestrated by the oocyte itself. Theca cells provide structural support for follicles together with synthesis of androgens that are used as susbtrates for aromatase enzyme activity[9]. Since cGMP levels do not change in concentration during meiotic resumption in theca cells, it can be stated that theca cells are not involved in the meiotic resumption, which is energy intensive. This may be attributed to the absence of gap junction between theca and granulosa cells[9].

Oocyte-specific Genes and Their Expression and Regulation During Oocyte Maturation

The oocyte exhibits an unusual pattern of gene expression regulation, with separate transcription and translation profiles. Whereas some oocyte RNA are translated for cellular metabolism, others are deadenylated and stored in cytoplasm. In most mammalian species RNA content of the fully grown oocyte is estimated to be 0.3-0.5 ng (mouse, human)[36]. After maturation and fertilization, the transition from the maternal to embryonic control of genome expression occur gradually.

mRNA deadenylase shortens the poly(a) tail of mRNA via deadenylation and this event slows down or prevent mRNA translation.Thus regulation of posttranscriptional gene expression is ensured.

Oocyte maturation is related to reproductive potential and understanding the gene regulation of human oocyte maturation is important for understanding oocyte physiology and to advance IVM technology, determination of upregulated and downregulated genes, during oocyte maturation can help identify markers of competent oocytes. To examine how the genes are regulated at different maturation stages of human oocytes, Yu et al.[37] evaluated genes at three human oocyte maturation stages (GV, MI, MII) within the same individual. Single-cell mRNA sequencing and single-cell whole genome bisulfite sequencing was performed (WGBS). They also focused on the possible role of non-CpG methylation and the DNA methylome in oocyte maturation. DNA methylation plays important roles in gene expression, regulation and chromatin structure/modifications. They demonstrated that when comprising MII and MI oocytes, 1,077 genes were upregulated in mature oocytes (MII) and 3,758 were downregulated[37].

In general the upregulated genes or pathways play significant roles in RNA degradation, splicing and transport, the cell cycle, ubiquitin-mediated proteolysis and oocyte meiosis. The downregulated pathways were primarily the metabolic pathways, such as TCA (tricarboxylic acide) cycle and oxidative phosphorylation[37] these data matches with the results of other studies[38-41].

TET proteins play an important role in the regulation of DNA-methylation as related to the regulation of gene expression during early zygote formation, embryogenesis, and neuronal differentiation[42-45].

TET3 is significantly upregulated in MII oocytes compared to MI oocytes. Both TET3 and TET2 genes are expressed in all stages of oocyte maturation (GV, MI and MII) and they are important in removing methylation in the genome of zygotes because of fertilization.

The downregulated pathways mostly involve the alternative glucose metabolic pathways which is required during the oocyte cytoplasmic maturation stage. A group of signal transduction (WNT) pathways have been implicated in ovarian development, oogenesis, and early embryonal development[46,47].

Zheng et al.[48] demonstrated an overall downregulation of genes encoding important components of the WNT signaling pathway during preimplantation development.

Chermuta et al.[49] analysed more than twenty genes involved in the cellular response to hormone stimuli during the oocyte maturation process. Ten of these genes *(ID2, FOS, CYR61, BTG2, AR, ESR1, CCND2, TACR3, TGFBR3,* and *EGR)* were downregulated by the IVM conditions.

Insulin-Like Growth Factor I Receptor; IGF1R

Insulin stimulates glucose uptake by regulating the transporter activities at both the transcriptional and post-translational levels. IGF1R mRNA is abundant in oocytes and early stage embryos, but decreased dramatically upon the formation of early blastocysts. The INSR, IRS1 and IRS2 mRNAs were expressed in oocytes and throughout preimplantation development. The gene for the catalytic subunit of PI3K, PIK3CA, displayed a maternal expression pattern, with its mRNA abundance decreasing by the morula stage. Similar to PIK3CA, the AKT1/AKT2 cDNA probe revealed down-regulation in morula and blastocyst stages, although the overall amount of transcript was low. Oocyte-specific gene expressions are presented in Table 1.

Meta-analysis have found that genes involved in oocyte maturation are highly conserved in flies (drosophila) and distantly related vertebrates including the mouse. Among them, BMP15 and GDF9, which plays an important role in bidirectional communication between the oocytes and the granulosa cells, in vertebrate species[36]. This could be explained by the fact that, recombinant GDF9 (oocyte-derived growth differentiation factor-9) inhibits KITL mRNA expression in mouse preantral granulosa cells[64], whereas BMP15 (bone morphogenetic protein-15) promotes KITL expression in monolayers of granulosa cells from rat early antral follicles[65].

Other important conserved genes that play a role in the quality of IVM oocytes are: GREM1, HAS2, COX2/PTGS2, EGFR,

Table 1. Oocyte-specific gene expressions (upregulation or downregulation) during maturation

	GV	MI	MII	Reference
DAZL	+	+	+	Yu et al. 2020[37]
BMP15	++	++	++	Yu et al. 2020[37]
GDF9	++	++	++	Yu et al. 2020[37]
RBBP7	+	+	+++++	Yu et al. 2020[37]
PTTG1	+	+	+++	Yu et al. 2020[37]; Assou et al. 2006[50]
[50]TUBB9	+	++	++++	Huang et al. 2017[51]; Feng et al. 2016[52]
PTTG3	+	+	+	Assou et al. 2006[50]
AURKC	+	+	+	Assou et al. 2006[50]
TET2	+	+	+	Wossidlo et al. 2011[44]; Gu et al. 2011[45]
TET3	+	+	+++	Wossidlo et al. 2011[44]; Gu et al. 2011[45]
DNMT3B	+	+	+++	Yu et al. 2020[37]
DNMT3A	+++	+++	+	Yu et al. 2020[37]
ECAT1	+++++	++	+	Liu et al. 2016[53]; Parry et al. 2011[54]
AR	++++++	++	-	Gleicher et al. 2011[55]
TMEFF2	++++++	++	+	Markholt et al. 2012[56]; Yu et al. 2020[37]
TEs	+	+	+	Guo et al. 2014[57]; Georgiou et al. 2009[58]; Smith et al. 2014[59]
LİNE1	+	+	+	Smith et al. 2014[59]; Luo et al. 2016[60]
MPF	+++	++	++	Assou et al. 2006[50]
CDC25A-CDC25B-CDC25C	++	++	++	Assou et al. 2006[50]
CDC1-CDC2	++	++	++	Assou et al. 2006[50]
CCNB1-CCNB2	++	++	++	Assou et al. 2006[50]
BUB1-BUBR1	++	++	++	Assou et al. 2006[50]
MAD2-MAD2L1	++	++	++	Assou et al. 2006[50]
CENP-A - CENP-E	++	++	++	Assou et al. 2006[50]
APC/C	+	+	+	Assou et al. 2006[50]
CDC20	+	+	+	Assou et al. 2006[50]
STAG3	+	+	+	Assou et al. 2006[50]
ZP1-ZP2-ZP3-ZP4	++	++	++	Assou et al. 2006[50]
BMP6	+	+	+	Assou et al. 2006[50]
FGFR2 (HDAC9,DNMT1, DNMT3B,H1FOO)	+	+	+	Assou et al. 2006[50]
CENPH	+	+	+	Assou et al. 2006[50]
ANAPC1, ANAPC10	+	+	+	Assou et al. 2006[50]
FBXO5	+	+	+	Assou et al. 2006[50]
Emi 1	+	+	++	Assou et al. 2006[50]
MOS	+	+	+	Assou et al. 2006[50]
SOCS7	+	++	+++++	Krebs and Hilton, 2000[61]; Assou et al. 2006[50]
Oogenesins	+	+	+	Dalbies-Tran et al. 2020[36]
Nlrp	+	+	+	Dalbies-Tran et al. 2020[36]
Khdc 1	+	+	+	Dalbies-Tran et al. 2020[36]
Ooep	+	+	+	Dalbies-Tran et al. 2020[36]
Nlrp5	+	+	++	Tong et al. 2000[62]
Nlrp14	+	+	++	Hamatani et al. 2004[63]

cAMP, CDC42, GDF-9, PTX3, ACSL, CYP19A1, BMP15, Caspase 9 and FAS[66].

The large number of oocyte-specific genes and the complex gene regulation that are involved in oocyte maturation, makes it difficult to plan the clinical use of genetic tests. Therefore, it is necessary to perform gene sequence analysis, as well as carrying out gene expression tests and evaluating methylation patterns. Thanks to NGS platforms, it is possible to perform extended panels or WES (Whole Exome Sequence) analysis. However, the important problem with these techniques is that some variations detected are not classified sufficiently. In the future, as more cases are reported and new studies are done, a clearer interpretation of the variations of unknown significance of these genes will emerge.

What does any of the apragrtaphs in the above section have to do with OMAS. Either tie it in or remouve it.

Definitions of Oocyte Maturation Abnormalities and Early Descriptions

Rudak et al.[67] reported four cases with OMAS including GV arrest, MI arrest and EFS,

Levran et al.[68] reported on OMAS in eight women with unexplained infertility including one patient with GV arrest, four with MI arrest and three women with MII arrest. OMAS was first used as a term in the literature by Hourvitz et al.[69], and included patients with EFS together with OMA in their series of seven women with repeated IVF failures. They achieved pregnancies in two women with genuine EFS (G-EFS) by IVM but failed to manage other causes of OMA.

Three months after Hourvitz publication, Beall et al.[4] published the first classification of oocyte maturation failure. They classified OMA into four types (Type I; GV arrest, Type II; MI arrest, Type III; MII arrest and Type IV; Mixed arrest).

Their publication was based on animal studies and several human case reports. Galvão et al.[70] studied 28 cases (9 cases with ROS and 19 cases with oocyte maturation arrest) with OMAS. The nine women with ROS underwent 24 IVM cycles. The IVM resulted in 5 healthy livebirths. The nineteen women with OMA underwent 25 IVM cycles. However, none of the 24 cycles resulted in fertilized 2PN oocytes after ICSI. In one patient a high quality embryo was transferred, but failed to result in a pregnacy? In this study, ROS was presented as one of the causes of OMAS.

Our group published a classification system combined with a series of the highest number of OMA cases at that point listed in the litterature which we termed the Hatirnaz and Dahan Classfication[71]. In that study FSH-hCG primed follicular phase IVM cycles were performed in all patients. In the Hatirnaz and Dahan classification system, GV arrest is accepted as OMA Type I, MI arrest is accepted as OMA Type II, MII arrest is accepted as OMA Type III, GV, MI arrest is accepted as OMA Type IV and Mixed Arrest (GV, MI and and MII) is accepted as Type V. Type IV arrest (GV-MI arrest) was added as a subtype of OMA since such patients were noted to occur. In this classification and that Beall cases of ROS, G-EFS, and degenerated oocytes were excluded. IVM cycle outcomes were compared with their previous IVF cycle outcomes. Though an improved outcomes in term of the collection of MII oocytes and embryo development was observed, no pregnancy were achieved in this study group.

Studies of *in vitro* activation of ovarian tissue in premature ovarian failure cases[72,73] lead to the idea of using transvaginal ovarian needle injury (TVOI)[74] in cases with OMAS. Our group perfomred a study on the comination of transvaginal ovarian needle injury together with dual stimulation IVM (letrozole priming and hCG triggering) and obtained the first succesful pregnancies and livebirths in women with type II and type V OMAS (Hatirnaz et al, 2020ASRM and Hatirnaz et al, 2021ESHRE abstracts).

Upon investigating patients with OMAs we noted that there were inter-cycle variability in their previous IVF attemts (ranginfrom 2-11 IVF cycles with different cycle managements). We also confront intracycle variability of cases during Duostim IVM (luteal phase stimulaton and follicular phase stimulation). Intracycle distribution of in vitro matured oocytes in DuoStim IVM cycles are presented in Table 2.

Types of Oocyte Maturation Abnormalities

Issues remain related to the two classification systems of OMA. Both classification systems described so far used oocyte maturation arrest by excluding other factors that cause OMAS[71,4].

Curently we are investingating whole genome exomic testing for women with OMAS to evaluate the underlying genetic pathologies. Potential etiopathogenesis of OMAS subtypes are depicted in Figure 2.

Gene expressions related to OMAS in animal and human species are presented in Table 3.

a. Dysmorphic and/or Degenerated Oocytes

Oocyte dysmorphism in assisted reproduction is not uncommon and can be observed either extracytoplasmic or intracytoplasmic manner. Indented oocytes may be persistent in some cases (Soussa et al., 2013) or may be seen as part of the apoptosis/degeneration process in follicular waves. One rare form of dysmorphic/degenerated oocytes is necroptosis[75,76].

b. Empty Follicle Syndrome (EFS)

Coulam et al.[77] reported on four women with five IVF attempts without retrieved oocytes and described this entity as EFS. Awadalla et al.[78] accepted EFS as technical failure rather than a syndrome which was felt to be related to stimulation error causing defects in final maturation resulting in an oocyte that cannot repsond to the hCG surge. Zreik et al.[79] studied 200 cycles of 35 women with EFS (single cycle with EFS n=27 and more than one IVF cycle with EFS n=8). They reported the incidence of EFS was 1.8% and the recurrence rate was 24% in 34-39 year old patients and 57% in women over 40 years of age, suggesting that EFS may be associated with decreased

Table 2. Intracycle variability of in vitro matured oocytes of OMAS cases (Data from ongoing, unpublished study by Hatirnaz S et al)

		Luteal phase stimulation and follicular phase stimulation oocytes of duostim IVM									
		Numb. Oocyte	Dys Deg	GV	MI	MII	IVM (Fertilized by ICSI)	PN	Embryo Develop.	Freezing	Type of OMAS
Case 1	Follicular	11	0	0	5	6	4	0	4	2	MI arrest
	Luteal	12	0	0	7	5	3	0	2	2	
Case 2	Follicular	9	4	1	4	0	0		0	0	Mixed arrest
	Luteal	9	0	4	2	2	2	0		0	
Case 3	Follicular	8	0	2	3	3	2	0	2	0	Mixed arrest
	Luteal	5	0	2	1	1	0	0		0	
Case4	Follicular	4	0	0	4	0	0	0	0	0	MI arrest
	Luteal	5	0	0	5	0	0	0		0	
Case 5	Follicular	12	0	0	11	1	0	0	0	0	EFS-OMA
	Luteal	7	0	4	3	0	0	0		0	
Case 6	Follicular	2	1	1	0	0	0	0	0	0	GV arrest
	Luteal	2	0	1	1	0	0	0		0	
Case 7	Follicular	2	0	1	1	0	0	0	0	0	GV arrest
	Luteal	2	0	1	1	0	0	0		0	
Case 8	Follicular	4	0	0	4	0	0	0	0	0	MI arrest
	Luteal	4	0	0	4	0	0	0		0	
Case 9	Follicular	2	0	0	0	2	2	0	2	0	Mixed arrest
	Luteal	5	0	3	0	2	1	1			
Case 10	Follicular	3	0	1	0	2	2	1	1	0	Mixed arrest
	Luteal	1	0	0	0	1	0	0		0	
Case 11	Follicular	8	0	3	0	5	3	1	2	0	Mixed arrest
	Luteal	3	0	1	0	2	2	2		0	
Case 12	Follicular	2	2	0	0	0	0	0	0	0	MI arrest
	Luteal	3	0	0	3	0	0	0		0	
Case 13	Follicular	7	0	1	0	6	2	2	2	0	Mixed arrest
	Luteal	7	0	4	0	3	2	0		2	
Case 14	Follicular	5	0	2	1	2	2	2	0	0	Mixed arrest
	Luteal	8	0	5	0	3	1	1		0	
Case 15	Follicular	4	0	2	2	0	0	0	0	0	Mixed arrest
	Luteal	3	0	0	3	0	0	0		0	
Case 16	Follicular	2	0	1	0	1	1	1	0	0	Mixed arrest
	Luteal	2	0	1	1	0	0	0		0	
Case 17	Follicular	2	0	1	0	1	1	1	0	0	GV-MI arrest
	Luteal	4	4	0	0	0	0	0		0	
Case 18	Follicular		2	2	1	0	0	2	0	0	Mixed arrest
	Luteal	9	2	4	1	2	0	2		0	

IVM: In vitro maturation, OMAS: Oocyte maturation abnormalities, EFS: Empty follicle syndrome

ovarian reserve, oocyte potential, errors in stimulation or hCG triggering. Possibly as women age the number of LH receptors on the follicles decrease, resulting in a deminished responce to the LH surge (or hCG trigger) and an increase in EFS. However, Uygur et al.[80] reported a case of recurrent EFS in a young woman with normal ovarian reserve and hypothesized about altered folliculogenesis or early oocyte atresia as the cause of EFS in this case. Whether it is a technical artifact or G-EFS was discussed by Bastillo[81] in her review in 2003. She associated EFS with problems of oocyte aspiration without flushing and other problems of aspiration or as a result of a premature LH surge. However, reports of some recurrent cases arguies against the hypothesis of technical failures at the oocyte collection in all cases. Clearly, the cause of EFS is multifactorial. hCG dosing, inappropriate administration and bioavailability of hCG should also be investigated as causes of false EFS (F-EFS) cases.

Van Heusden et al.[82] sent a letter to the editor disucsing EFS and claimed that EFS was virtually nonexistent and not evidence based due to lack of metaanalysis However, Vutyavanich et al.[83] reported a case of G-EFS where they were able to identify immature oocytes in the filtrate of follicular aspirates which wer initialy missed by the embryologist. The prevalence of EFS

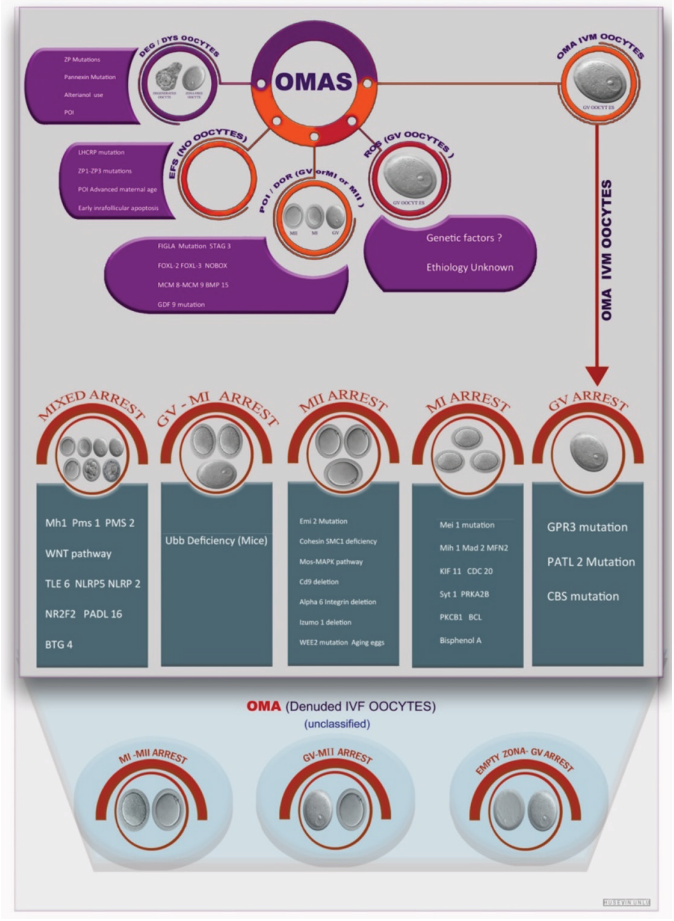

Figure 2. Schematic representation of etiopathogenetic mechanisms of OMAS

was studied by Mesen et al.[84] and found to occur in 0.016% of collection. They found 11 cases (2 G-EFS and 9 F-EFS) in 12 359 IVF cycles between 2004-2009 at there center. The estimated incidence of EFS ranges from 0.016-7% in the literature[85-87].

Baum et al.[86] reported in their study that EFS is more prevalent in older aged women with prolonged infertility and diminished ovarian reserve Yakovi et al.[88] was prospectively studied EFS in 95 women with between 2011 and 2015 and found four G-EFS cases with advanced maternal age (4.2%).

They concluded that G-EFS complicates infertile women with a low number of mature oocytes stimulated in their IVF cycles and classified it as two form of EFS. F-EFS related to hCG bioavailability and G-EFS[89,90].

EFS can be seen alone or a part of oocyte degeneration or maturation arrest In our OMAS series of patients, there are cases which were three times diagnosed as G-EFS. However, in their subsequent round of IVF with double dose HCG trigger, oocytes were retrieved and a pregnancy was obtained.

EFS can also be secondary to agonist triggering in antagonist IVF cycles. Deep luteolysis and a lack of repponce with an LH surge are causes of EFS[91].

Castillo et al.[92] retrospectively studied the incidence of 2034 donation cycles and 1433 IVF cycles EFS and reported EFS rate were 3.5% and 3.1% respectively.

Though statistically insignificant, EFS can be observed in agonist trigger cycles. Deepika et al.[93] studied 271 women affected by polycystic ovary syndrome (PCOS) with agonist triggering in GnRH-antagonist IVF cycles and found a 3.3% incidence of EFS.

In case studies dual trigger with GnRH analog and hCG triggering in GnRH-antagonist cycles with healthy livebirths were reported[94,95]. Blazquez et al.[96] studied 12.483 oocyte donation cycles and found 0.59% EFS cases in their study group and they found no difference in the gonadotropin stimulaton and triggering et al first achieved two livebirths by IVM in two women suffering from G-EFS[69]. Al-hussaini et al.[97] reported repeated immature oocyte retrieval in IVF cycles but failed to mature them *in vitro*[97]. In conclusion, EFS can be cathegorised as a subtype of OMAS and oocyte retrieval, oocyte maturation, clinical pregnancy and livebirths are possible following IVM in women suffering from EFS.

c. Oocyte maturation arrest (OMA)

c.1. GV Arrest (Type I OMA)

Meiotic resumption depends on meiotic competence and acquires some key steps including protein production, localization, phosphorilation and degradation[98,99]. Knockout mice studies and inhibitory drug use in animal studies enlighten meiotic arrest and resumptiom mechanisms but their impact on human OMA and resumption yet to be clarified. Other factors related with GV arrest were listed in Table 3.

Table 3. Gene expressions (up regulation or down regulation) related to subtypes of OMAS in animal and human species

	Dys Deg	EFS	POI DOR	GV Arrest	MI Arrest	MII Arrest	GV-MI Arrest	Mixed Arrest	Ref
ZP1	+++	++	NA	NA	NA	NA	NA	NA	Chen et al.[100], Zhang et al., 2017[100];Cao et al, 2020[101]; Xu et al., 2020[102]; Sun, 2019[103]; Yang et al., 2017[104]; Luo et al., 2020[105]; Okutman et al., 2025[106]
ZP2	+++	++	NA	NA	NA	NA	NA	NA	Yang et al., 2017[104]; Luo et al., 2020[105]
ZP3	++	++	+/-	+/-	+/-	+/-	+/-	NA	Chen et al., Zhang et al., 2017[100,107]; Zhang et al., 2020[107]
ZP4	+	+	+/-	+/-	+/-	+/-	+/-	NA	
PNX1	++	+	NA	NA	NA	NA	NA	NA	Sang et al., 2019[108]
LHCGR	NA	+++	NA	NA	NA	NA	NA	NA	Chen et al., 2018[109];Yuan et al., 2017[110]
GPR3	NA	NA	NA	++	NA	NA	NA	NA	Mehlman, 2005[31]
PDE3A	NA	NA	NA	++	NA	NA	NA	NA	Masciarelli et al., 2004[111]
PATL2	NA	NA	NA	++	NA	NA	NA	NA	Maddirevula et al., 2017[112]; Chen et al., Zhang et al., 2017[100,107]; Huang et al., 2018[113]; Wu et al., 2019[114]
CBS	NA	NA	NA	++	NA	NA	NA	NA	Liang et al., 2007[11]
Mad2	NA	NA	NA	NA	+++++	NA	NA	NA	Madgwick and Jones., 2007[115]
MFN2	NA	NA	NA	NA	+++++	NA	NA	NA	Wang et al., 2020[116]
KIF11	NA	NA	NA	NA	+++	NA	NA	NA	Wan et al., 2018[117];Santella et al., 2018[118]
CDC20	NA	NA	NA	NA	++	+/-	+/-	NA	Yang et al., 2014[119];Sang et al., 2018[108]
Syt1	NA	NA	NA	NA	-----	NA	NA	NA	Zhu et al., 2012[120]; Zhang et al., 2010[121]
PRKAR2B	NA	NA	NA	NA	-----	NA	NA	NA	Yoon et al., 2018[122]
PKCB1	NA	NA	NA	NA	------	NA	NA	NA	Yi et al., 2009[123]
Bcl2110	NA	NA	NA	Overinjection	++++	NA*	NA	NA	Yoon et al., 2009[124]
TUBB8	NA	NA	NA	NA	++++++++	NA	NA	NA	Feng et al., 2016[52]; Feng et al., 2016[125]; Chen, Li et al., 2019[126]; Huang et al., 2017[51]; Wang et al., 2018[127]; Xiang et al., 2018[128]; Chen et al., 2019[126]
TRIP13	NA	NA	NA	NA	++	NA	NA	NA	Li and Schimenti., 2007[129]; Zhang et al., 2020[130]
Emi2	NA	NA	NA	NA	NA	++	NA	NA	Araki et al., 1996[131]
APC/C-PKC	NA	NA	NA	NA	NA	++	NA	NA	Lorca et al., 1993[132]
CamKII	NA	NA	NA	NA	NA	++	NA	NA	Lorca et al., 1993[132]
SMC1	NA	NA	NA	NA	NA	++	NA	NA	Hodges et al., 2005[133];Revenkova et al., 2004[134]
MOS-MAPK	NA	NA	NA	NA	NA	++	NA	NA	Inoue et al., 2007[135]
Erp1	NA	NA	NA	NA	NA	++	NA	NA	Inoue et al., 2007[135]
P90Rsk	NA	NA	NA	NA	NA	++	NA	NA	Maller et al., 2002[136]
CD9	NA	NA	NA	NA	NA	++	NA	NA	Kaji et al., 2000[137]; Bianchi et al., 2014[138]; Inoue and Sagata, 2005[139]
A6 Integrin	NA	NA	NA	NA	NA	++	NA	NA	
Izumo1	NA	NA	NA	NA	NA	++	NA	NA	

Table 3. Continued

	Dys Deg	EFS	POI DOR	GV Arrest	MI Arrest	MII Arrest	GV-MI Arrest	Mixed Arrest	Ref
WEE2 CDC2	NA	NA	NA	NA	NA	+++	NA	NA	Dai et al., 2019[140]; Sang et al., 2018[141]; Han and Conti, 2006[142]
WEE1B	NA	NA	NA	NA	NA	++	NA	NA	Kim et al., 2015[143];Zielinska et al., 2019[144]
Ubb Mlh1	NA	NA	NA	NA	NA	NA	++	NA	Ryu et al., 2008[145]; Lipkin et al., 2002[146]
Pms1	NA	NA	NA	NA	NA	NA	NA	++	Lipkin et al., 2002[146]
Pms2	NA	NA	NA	NA	NA	NA	NA	++	Lipkin et al.,2002[146]
BTG4	NA	NA	NA	NA	NA	NA	NA	++	Zheng et al., 2020[147]
WNT	NA	NA	NA	NA	NA	NA	NA	++	Paonessa et al., 2021[148];Assou et al., 2011[149]
NR2F2	NA	NA	NA	NA	NA	NA	NA	++	Paonessa et al., 2021[148]; Zhang et al., 2009[150]
TLE6	NA	NA	NA	NA	NA	NA	NA	++	Wang et al., 2018[151]; Mu et al., 2019[152]; Wang et al., 2018[151]
NLRP5	NA	NA	NA	NA	NA	NA	NA	++	
NLRP2	NA	NA	NA	NA	NA	NA	NA	++	
PAD16	NA	NA	NA	NA	NA	NA	NA	++	
FIGLA	**NA**	**NA**	++	**NA**	**NA**	**NA**	**NA**	**NA**	Zhao et al., 2008[153]
FOXL2 FOXO3	**NA**	**NA**	++	**NA**	**NA**	**NA**	**NA**	**NA**	Chatterjee et al., 2007[154];Wang et al., 2010[155]
MCM8	**NA**	**NA**	++	**NA**	**NA**	**NA**	**NA**	**NA**	Tenenbaum-Rakover et al., 2015[156];Al Asiri et al., 2015[157]
MCM9	**NA**	**NA**	++	**NA**	**NA**	**NA**	**NA**	**NA**	Wood-Trageser et al., 2014[158]; Fauchereau et al., 2016[159]
STAG3	**NA**	++ (Hatirnaz et al., ongoing study)	++	**NA**	**NA**	**NA**	**NA**	**NA**	Caburet et al., 2014[160]; Le Quesne Stabej et al., 2016[161]; He et al., 2018[162]
NOBOX	**NA**	**NA**	++	**NA**	**NA**	**NA**	**NA**	**NA**	Bouilly et al., 2011[163];Bouilly et al., 2015[164]
FSHR	**NA**	**NA**	++	**NA**	**NA**	**NA**	**NA**	**NA**	Masui and Markert, 1971[165]; Doherty et al., 2002[166]; Meduri et al., 2003[167]; Nakamura et al., 2008[168]
GDF9	**NA**	**NA**	++	**NA**	**NA**	**NA**	**NA**		Dixit et al., 2005[169]; França et al., 2018[170]
BMP15	**NA**	**NA**	++	**NA**	**NA**	**NA**	**NA**	**NA**	Di Pasquela et al., 2006[171], Di Pasquela et al., 2004[172]

c.2. MI Arrest (Type II OMA)

MI to MII transition is different from GV to MI transition wherein chromosomal condensation, spindle formation and chromosomal alignment on the equatorial plate happens before segregation of homologous chromosomes. Morphologicaly the transition of MI to MII can be noted with extrusion of the first polar body (PBI). Any factors blocking this transition to MII could cause MI arrest. If MI arrest is present alone, it is called Type II OMA by the Hatirnaz and Dahan classification system. MI arrest is observed in the absense of Mei1 and Mlh1 in mices, both have role in recombination during completion of meiosis[173]. The role of Mei1 in female infertility is not clear[174]. Meiotic spindle formation is crucial step in MI to MII transition. Other factors related with MI arrest are listed in Table 3.

Bisphenol A (BPA), a well known plastic material also used in laboratory materials was shown to damage spindle configuration and chromosomal alignment at the MI stage. BPA resulted in MI arrest in high doses in mouse oocytes[175].

Dietylstilbestrol (DES) leads to oocyte meiotic dysfunction and oocyte maturation arrest by impairing spindle formation and chromosomal malalignment in mouse oocytes[176].

A widely used chemotherapeutic drug, doxorubicin (DOX) was shown to arrest oocyte maturation by reducing PBI extrusion and by triggering early oocyte apoptosis[177].

c.3. MII Arrest (Type III OMA)

MII oocytes are accepted as mature morphologically and presumed to be fertilizizable. Fertilization is a complex process involving the transition from meiosis to mitosis and from oocyte to zygote. This process includes sperm egg binding, the release of corticle granules (also a measure of oocte maturation), Polar body II (PBII) extrusion and pronuclear formation[178]. However, normal aprearing MII oocytes may fail to form viable embryos and may present with fertilization failure, whcih could be caused by imaturity of the MII oocyte. Oocyte maturation is a continuous process and after the extrusion of the first polar body (PB1), should be completed in order for the oocyte to be capable of fertilization. This unique pathology is quite uncommon and presents as mixed OMAs and presents with fertilization failure (FF). The main difference between FF and MII arrest is that FF can be overcome in repeated cycles or ICSI while MII arrest is persistent. More collected data is needed to clearly differantiate this abnormality from FF. MII imuaturity is normaly present before fertilization and this is thought to be regulated by cytostatin factor (CSF). MII maturation arrest may be caused by dysregulation in levels of cyclin B. Meng et al.[179] studied the role of Cyclin B (Ccnb3) on MII arrest in mouse oocytes and found that gradual decreases in Ccnb3 levels is required for meiotic maturation to ocur.

Ccnb3 participates in the separation of homologous chromosomes during the first meiotic process by forming a complex with Cyclin dependent kinase (CDK1).

In mammals, MPF play an important role in oocyte maturation[180]. MPF concentration increases during oocyte maturation and reaches maximum level at MII stage and than mature oocyte is arrested at this phase. This arrest stage is exclusive for oocytes and is regulated by the cytostatic factor (CSF). This factor stabilizes the MPF, keeps chromosomes condensed, therefore, allows avoiding a second round of DNA replication during transition from MI to MII[181,182].

For the successful in vivo or in vitro fertilization, both oocyte and sperm cells need to have some essential elements. For example phospholipase C zeta protein in the sperm cell is essential for the reactivation of the oocyte arrested at MII stage through the induction of intracellular calcium oscillation.

However the oocyte activation failure is not the only reason of the fertilization failure. Sperm nuclear descondensation failure and premature chromosome condensation (PCC) have been showed as reasonable events in fertilization failure studies (Sedo CA, 2015). Of course both nuclear and cytoplasmic maturation of the oocyte have important effects on the fertilization rate.

Other factors related with MII arrest are listed in Table 3.

c.4. GV and MI Arrest (Type IV OMA) May Also Include Some MIIs

In this subset of OMA, oocytes mature to the MI and GV stages and were found to be arrested during the meiotic resumption process. Beall et al.[4] included this group in mixed arrest OMA. However, in the Hatirnaz and Dahan system we classified this as a seperate group from Mixed OMA where GV, and MI oocytes were observed together[71]. Rarely, imature MII oocytes are also noted in this group, which fail fertilization with ICSI. Many studies in animal models revealed that mixed oocyte maturation arrest are related to MutL homolog 3 (Mlh3) and Ubiquitin b (Ubb) proteins[145,146].

Mlh3 plays a dual role in DNA mismatch repair and meiosis. Mlh3–/–oocytes fail to complete meiosis I after fertilization[146]. Deficiencies of Ubb, a member of the ubiquitin family results in infertility and GV and MI arrest in mices[145]. Ubb geneis essential for postnatal gonadal maturations and fertility. Ubb_/_ oocytes do not proceed beyond metaphase I. Loss of one Ubb family member may be compensated by the expression of other Ubb family members (Uba 52, Uba80 though this compensation is not enough to sucessfully compete the meiotic resumption)[145].

c.5. Mixed Arrest (Type V OMA)

This is the most commonly encountered subtype of OMA and has the highest chance of ET and clinical pregnancy[71] arnt rates of pregnacy rare how wer they achieved you need to disucss this. In this subtype, only a small proportion of collected oocytes (rougly less than 25%) are MII and most others are immature, either GV or MI in repeated cycles. Mhl3 belongs to a family including Mlh1, Pms1 and Pms2. Deficiency of Mhl in mice result in MI and MII arrest[146].

In this subtype of OMA, compensatory mechanisms hypothetised to play a role in maturing oocytes but their maturation rate and clinical significance are not well known. Zygotic cleavage failure (ZCF) is also commonly seen in this subtype. Homozygous mutations in BTG4 (B cell translocation gene 4) is reported to cause ZCF[147].

MII oocytes complete their maturation mostly improperly thus embryonic development may be arrested at the PN stage or at the cleavage stage. Most developed embryos are observed as bad quality embryos. Embryos can be transferred in this subtype. Early embryonic arrest is common in OMA type V. In this subtype MII oocytes are immature and FF, PN arrest and bad quality nembryo development are the consequences of treatment. Letrozol IVM together with TVOI improved the maturation and fertilization and pregnancy was achieved. This is the form where zygotic cleavage failure can happen and sperm related factors may also have impact but all case with previous attemts had failed to achieve pregnancy and in most of the case fertilization and cleavage of embryos.

Other factors related with mixed arrest are listed in Table 3.

d. Premature Ovarian Failure (POF)/Premature Ovarian Insufficiency (POI)

The spectrum of OMAS frequently manifested in IVF cycles of women with POI[183]. POI is characterized by oligo/amenorrea and high serum gonadotropin levels, POI affects 1-3% of women before the age of 40[183]. POI is a heterogenous disorder both phenotypically and genetically[184]. Novel candidate genes were reported in POI[185,186]. More than one genetic variation was reported in one woman with POI[187].

Menstrual Dynamics in women with POI is abnormal and there is a failure of development of regular follicular waves in POI cases[188]. Both follicle recruitment and follicular apoptosis frequencies are diminished in POI. Oocyte collected in POI may range from EFS, dysmorphic/degenerated oocytes to MII oocytes which can be normaly functioning (ongoing study).

Other factors related with POI are listed in Table 3.

e. Resistant ovary syndrome (ROS)

ROS is a rare entity where there is ovarian resistance to both endogenous or exogenous gonadotropins, elevated FSH and LH are observed, although AMH levels and antral follicle counts are in the normal range[70].

Persistent immature oocytes are often obtained in cases with ROS. Thus ROS is evaluated as part of the OMAS spectrum. The etiology of this condition is uncertain but immunological and genetic factors[70] may have role in occurence.FSHR mutations play role in the pathogenesis of ROS.Heterozygous mutation of FSHR: c.182T>A (p.Ile61Asn) and c.2062C>A (p.Pro688Thr) was found pathogenic for ROS in the siblings of a chinese family[189]. IVM is the selected mode of treatment to overcome this clinical entity. Livebirths of babies from IVM of ROS was reported[190-192].

f. Unclassified

f.1. Empty Zona-GV Arrest

f.2. GV-MII Arrest

f.3. MI and MII Arrest

So far we had two cases with this subtype and both cases were seen long after our classification system was published. In the first case, three IVF attempts were performed. Collected oocytes in the first attempt consisted of 16 MI and 1 MII with 1 fertilization and ETof day 3 8 cell grade II embryo without pregnancy. In the second IVF attempt 4 MI oocytes whcih failed in-vitro maturation were obtained. In the third IVF attempt 16 MI oocytes and1 MII oocyte was retrieved and the MII developed into an embryo and was vitrified at the cleavage stage for pooling. Due to financial reasons, the frozen-thawed ET was performed without a pregnancy occuring. This patient stoped care.

The second case had four previous IVF cycles. The patient had 6 MI and 1MII oocyte in her first IVF cycle with fertilization of the MII, 8 cell grade I ET was performed on day 3 of embryo development and a pregnancy with a biochemical loss occured. Whole genome exomic analysis performed and TUBB8 (c.535G>A) mutation was determined in her genetic testing. This mutation is related with MI arrest in OMAS. Her second attempt yielded 6 MI and 1 MII oocyes with fertilization failure with ICSI. The third IVF attemt yielded 4 MI and 4 MII oocytes, three 2PN fertilization developed and only one 7 cell grade III embryo transfer was performed without a pregnancy. The fourth IVF attempt resulted in the collection of 6 MI and 1 MII oocytes with fertilization but ET cancelled due to arrested embryos only whihc had degenerated by day 3.

Treatment Modalities of Oocyte Maturation Abnormalities

Treatment options other than oocyte donation for OMAS are below;

1. Organelle Transfer

Until recently, the only recommended treatment for women with OMAS was oocyte donation (OD). In the last two decades, the use of somatic cell nuclear transfer, pronuclear transfer and polar body transfer have been used and succesful pregnancies and livebirths have been reported both in humans and animal species[193].

Pronuclear transfer of oocytes from OMAS patient to subzonal area of enucleated donor oocytes resulted in blastocyst development and healthy livebirth which is the demonstration of the role of oocyte cytoplasm on embryogenesis and implantation[193]. Nuclear genetic codes were matching with mother but mitochondrial DNA were coming from donor oocytes. The use of this tehchnique may be beneficial in women with mitochondrial DNA related diseases However the application of this method is ethically questioned.

Germinal vesicle transfer is the removal of germinal vesicle from GV oocyte and reimplantation of GV into the subzonal perivitelline area of donor oocyte[194]. GV transfer gives opportunity to investigate the interrelation of nucleus and cytoplasm in the oocyte maturation process. GV removal from the cytoplasm is a less invasive procedure as compared to chromosomal removal from mature oocytes and GV transferred mouse oocytes have reached blastocyst stage[195]. GV transfer into discarded human oocytes revealed normal PBI extrusion and MII transition[196]. However GV transfer carries the risk of mitochondrial DNA heteroplasmy[197]. Moffa et al.[198] reported the use of GV transfer between fresh and frozen mouse oocytes and GV transfer from frozen immature oocytes produced chromosomally normal oocytes. Nuclear transfer in primates were studied and challenged because of molecular requirements and for nonhuman primates, nuclear transfer was reported unsuccesfully[199].

There is only one rhesus monkey birth after embryonic cell nuclear transfer[200].

Pronuclear transfer embryos of nonhuman primates had more spindle defects and had higher aneuploidy rate[201]. GV transfer can be a unique option for women having meiotically arrested oocytes or ovarian resistance to gonadotropins[194].

For all above mentioned treatments, the availability of IVM setup in IVF laboratories is mandatory.

IVM for OMAS

Until the most recent commitee opinion from the ASRM[202]. the use of IVM in humans has drasticaly declined because of the previous ASRM opinion that IVM is experimental in the era of agonist triggering for PCOS[203]. Tremendous efforts have been devoted to the development of culture media for better clinical outcomes in IVF. However, there has been much less effort put into the development and advancement of IVM culture medias.
Capacitation IVM(CAPA IVM) Recently, a novel approach, CAPA IVM was introduced with favorable oocyte maturation in IVM cycles[204-206]. In this treatment, immature oocytes with cumulus complexes were put in a prematuration culture medium including C type natriuretic peptide for up to 24 hours before standart IVM culture media use. CAPA IVM was studied in minimally stimulated mice and results showed that both cumulus function and oocyte quality were improved[207]. In a clinical trial conducted by Vuong et al.[208], 40 women with CAPA IVM were compared with 40 women with standart IVM and they reported significantly higher clinical pregnancy rates (63.2%, 38.5%, respectively). Although this is an interesting result in a small study, the role of CAPA IVM in OMAs is unclear and not studied yet. There is no evidence of the use of CAPA IVM in women with OMAS.
Coenzyme Q10 supplementation in IVM culture media was shown to increase maturation rates in human oocytes and decrased aneuploidy rates in the oocytes of elderly women[209]. There is no evidence of teh use of Coenzyne Q10 as a supplement in IVM cultur media for the maturation of oocytes from women with OMAS.
A more advanced IVM culture media in the future may improve oocyte maturation in IVM cycles. The addition of autocrine and paracrine factors were reported to significantly influence the embryonic development in animal models[73] of IVM. While brain derived neurotropic factor, colony stimulating factor (CSF), granulocyte macrophage CSF,epidermal growth factor, artemin and insulin-like growth factor increased the blastocyst rate 2.5 fold, growth hormone increased the blastocyst rate two folds[73]. Whether to use these add ons in IVM culture media for OMA has yet to be clarified and studies are needed.
Putrescine supplementation in IVM culture media of elderly mouse oocytes yielded better quality blastocyst development[210,211]. LH induces a temporary rise of ornithine decarboxylase (ODC) activity and its enzymatic product, putrescine in mamalian ovaries during the ovulation and the implantation period[212]. Periovulatory rise of putrescine in mouse ovaries resulted in more blastocyst development, less embryonic loss and more livebirths[210]. Human use of putrescine may improve periovulatory diminished ODC activity and targets oocyte maturation[213]. Putrescine use, if alloved for human studies may help to improve oocyte maturation in IVM cycles and may be beneficial for OMAS in future.
IVM cycles have demonstrated promising results in women with G-EFS and ROS[69,70].
FSH-hCG priming IVM FSH-hCG priming IVM in women with intrinsic OMA resulted in a slight improvement in maturation however, no pregnancy was achieved in these cases[214]. Letrozole priming IVM was reported to have favorable outcomes in PCOS and cancerphobic women[214,215] without maturation arrest. The use of letrozole for OMA has been investigated by our group.
Letrozole is an aromatase inhibitor, which increases androgen levels in the ovary and triggers endogenous FSH secretion. Hte increased intra follicular androgen levels will also increase FSh receptors and ltalamtely LH receptors. By this mechanism, letrozole stimulates follicular and oocyte development[216]. Letrozole primed IVM was performed by our group in 25 women with OMAS and the first two healthy livebirths were achieved by this treatment modality (Hatirnaz et al., Ongoing study).
DuoStim IVM was selected for almost all cases to obtain more oocytes and to have more embryos to transfer. Duostim IVM also enabled us to evaluate the oocytes yielded in follicular and luteal phase and to determine the intracycle variability of oocytes. The clinical and laboratory outcomes of treatment modalities used and attempted in OMAS cases are depicted in Table 4.
In vitro activation of primordial follicles (IVA). Patients with diminished ovarian reserve and with POI commonly present with OMAS. Therefore, IVA by disrupting the hipposignaling pathway and Akt stimulation may be an option for treatment. The hypothesis of this approach was inspired from ovarian tissue injuries (wedge resection or drilling) in women with PCOS[73,217]. Grafting and reimplantation of ovarian cortical slices produced rapid follicle development and Kawamura et al.[72] reported first livebirth with this method in an women with POI. Drug free IVA of diminished ovarian reserve patients was studied by Kawamura et al.[72] and 9 out of 11 women with DOR responded well to the IVA with 68.7% fertilization and 56.9% high quality embryo development repoorted with one livebirth and two ongoing pregnancies occuring. IVA has not been studied in women with OMAS and there is no evidence fort he efficacy of IVA in OMAS.
Transvaginal ovarian needle injury (TVOI) may have similar action with drug free IVA on the ovaries and need to be evaluated in women with POI or DOR. TVOI was studied in women with PCOS[74] but not studied alone in cases with OMAS. In our ongoing study, we use TVOI as to trigger the primordial follicle pool and follicular and oocyte activation but in our protocol TVOI is added to Duostim IVM and we can not prove that TVOI alone is a good option for OMAS. An interesting finding is that laparoscopic ovarian tissue stripping, a similarly damaging procedure may overcome ROS[218] in one study.
There are various causes and mechanisms of fertilization failure. Some of these have been shown to benefit from piezoelectric application.

Piezoelectricty was introduced to be a valuable option in patients with fertilization failure[140]. The electromagnetic field created by applying electric current increases the number of pores and calcium conductivity in the cell membrane by enabling the movement of proteins. This situation increases the calcium concentration in the cell[219].

This high concentration of calcium triggers oocyte fertilization. Fertilization and pregnancies have been reported especially in cases of unspecified fertilization failure cases or in cases with spermatogenic disorders (structural disorders such as globozoospermia)[220,221].

There is currently no evidence of the use of piezoelectricity in women with OMAS but it could be studied to trigger cytoplasmic maturation in OMAS.

Immature oocyte vitrification before IVM was found slightly increased high quality embryo rates[222]. Immature oocyte vitrificiation can be used to store oocytes for future studies and for future treatment modalited developed. Similar experiment was performed by Molina et al.[223] and they reported promising outcomes. The rationale behind this is the rapid transmembrane ionic changes which may trigger cytoplasmic maturation and thus can be used in women suffering from OMAS. There is currently no evidence for the use of vitrification of oocytes from OMAS.We tried this in two cases after their permission and vitrified and thaved the immature oocytes but failed to mature oocytes by this modality.

Before concluding the review, we would like to present some future perspectives related to OMAS:

1. Bypassing OMAS and offering OD as the firstline treatment should be rethought by the clinicians.
2. Although promising results reported, organele transfers have some limitations, either ethical or genetical.
3. Succesfull treatment of EFS and ROS and some OMAS by IVM is possible with clinical pregnancies and healthy livebirths.
4. A new classfication system of OMAS including degenerated oocytes, dysmorphic oocytes, G-EFS, POI and ROS should be considered in future.
5. EFS is neither a syndrome nor empty and this pathology should be redefined and included into OMAS as subtype which will clear the confusions.
6. Type V OMA (Mixed arrest) has genetic roots with bodily compensatory mechanisms and can be managed by TVOI DuoStim IVM with letrozole priming.
7. For those women with genetic factors, future studies may reveal production of defective proteins and adding these proteins in IVM culture media may overcome arrested meiotic resumption, especially MI arrest.
8. Thorough investigation of OMAS in fact has great impact on the understanding of meiotic resumption and oocyte maturation and understanding the mechanisms may postpone menopouse and may open a new field of contraception. Besides, these developments may control the abnormal apoptotic process that led to OMAS.

Table 4. Clinical and laboratory outcomes of treatment modalities of OMA subtypes

	OMA / Dysmorphic Degenerated	OMA /G-EFS	OMA Type I(GV)	OMA Type II(MI)	OMA Type III(MII)	OMA Type IV(GV-MI)	OMA Type V(Mixt)	OMA / POI	OMA / ROS
FSH-hCG IVM	+/-	+	+/-	-	-	+/-	+	+/-	+
Duostim IVM*	+	+	+/-	+/-	?	+/-	+++	+/-	+
CAPA IVM	?	+	?	?	?	?	++?	?	+
Spindle transfer	-	-	-	+	?	+/-	-	-	-
Nuclear transfer	-	-	+	+	?	+/-	-	-	-
PB I transfer	-	-		+	?	+/-	-	-	-
PB II transfer	-	-		+	?		-	-	-
Coenzyme Q 10	?	?	?	?	?	?	++?	?	?
Putrescine	?	?	?	?	?	?	?	?	?
Oocyte maturation	+/-	+	+/-	+/-**	-	+/-	+	+/-	+
Fertilization by ICSI	+/-	+	+/-	+/-	-	+/-	+	+/-	+
Embryonic development	+/-	+	+/-	+/-	-	+/-	+	+/-	+
Embryo transfer	+/-	+	+/-	+/-	-	+/-	+	+/-	+
Pregnancy	None	+	-	+/-	-	-	+	+	+
Livebirth	None	+	-	+/-	-	-	+	+	+
Embryo freezing	-	+	-	+	-	-	+	+	+

IVM: In vitro maturation, OMAS: Oocyte maturation abnormalities, ROS: Resistant ovary syndrome, POI: Premature ovarian insufficiency

9. Clinical protocols using physiological mechanisms, ovarian tissue trauma by TVOI or drug free IVA and mechanisms of action of letrozole (local androgenic effect, endocrine and paracrine effect and endogenous FSH release) together with advanced in vitro culture media should be studied. A study of TVOI DuoStim IVM with letrozole priming conducted by our group is ongoing and preliminary results are promising.
10. Add on's for IVM culture media including CAPA IVM, Coenzyme Q-10 and putrescine should be studied in OMAS both in human and animal species.
11. Since immature oocyte freezing is reliable, women with OMAS should be offered for oocyte freezing because of future developments may overcome their pathologies. Some oocytes, with the written permission of patients should be frozen for electronmicroscopic evaluations.

Conclusion

Complete oocyte maturation has many steps including follicular and granulosa maturation, zona pellucida maturation, nuclear maturation, cytoplasmic maturation, genetic maturation and epigenetic maturation. Among these processes, cytoplasmic maturation is the most important step. Until last decade, animal studies led the human OMAS but datas on human OMAS accumulated and factors related to human OMAS become much clear though there are a lot to be done. Present data shows that OMAS are a spectrum and there are intercycle and intracycle variabilities which may be attributed to changing dynamics of apoptosis. Some genetic pathologies have certain impact on meiotic resumption while other genetic factors may be compensated by the other genes of the same family. In this review study, OMAS were classified and extensively evaluatedand possible treatment options under the light of cuurent information, present literature and ongoing studies. Either genetic studies or IVM studies that will be handled in the future will led more informations to be reached and may make it possible to obtain pregnancies.

Ethics

Peer-review: Internally and externally peer-reviewed.

Authorship Contributions

Concept: Ş.H., E.S.H., A.E.K., K.H., C.S.Ç., Ö.S., N.D.G., C.D., V.B., S.T., M.D., Design: Ş.H., E.S.H., A.E.K., K.H., C.S.Ç., Ö.S., N.D.G., C.D., V.B., S.T., M.D., Data Collection or Processing: Ş.H., E.S.H., A.E.K., K.H., C.S.Ç., Ö.S., N.D.G., C.D., V.B., S.T., M.D., Analysis or Interpretation: Ş.H., E.S.H., A.E.K., K.H., C.S.Ç., Ö.S., N.D.G., C.D., V.B., S.T., M.D., Literature Search: Ş.H., E.S.H., A.E.K., K.H., C.S.Ç., Ö.S., N.D.G., C.D., V.B., S.T., M.D., Writing: Ş.H., E.S.H., A.E.K., K.H., C.S.Ç., Ö.S., N.D.G., C.D., V.B., S.T., M.D.

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Case Report / Olgu Sunumu

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Epidermal growth factor receptor-mutated lung adenocarcinoma diagnosed from endometrial polyp metastasis: A case report and literature review

Endometrial polip metastazıyla tanısı konulan epidermal büyüme faktörü reseptörü-mutasyonlu akciğer adenokarsinomu: Bir olgu sunumu ve literatür incelemesi

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Abstract

Endometrial metastasis from the lung primary remains is rare. Moreover, the literature only contains case reports of endometrial metastasis from the primary lung cancer. An 83-year-old female patient presented with postmenopausal uterine bleeding and anemia. Endometrial thickening was detected using transvaginal ultrasound and endometrial curettage was performed. Histopathology revealed adenocarcinoma infiltration on an endometrial polyp surface. On histologic examination, high-grade serous carcinoma and clear cell carcinoma diagnoses were initially considered. The tumor cells were immunohistochemically negative for Wilms' tumor 1 and wild-type for p53 expression; however, it was positive for Napsin A. Primary lung adenocarcinoma (LUAD) metastasis was also included in the differential diagnosis. Thyroid transcription factor 1 was positive, whereas paired box gene 8 (Pax8) was negative in tumor cells. Primary LUAD metastasis was diagnosed since a lung mass was radiologically confirmed. Furthermore, epidermal growth factor receptor-exon 19 mutation was detected by molecular analysis. In addition to the clinical and morphological features, this case report emphasizes the importance of multiple immunohistochemical panel applications for the correct diagnosis.

Keywords: EGFR protein, adenocarcinoma of lung, metastasis, endometrium, metrorrhagia

Öz

Akciğer primerinden endometriyuma metastaz literatürde ağırlıklı olarak olgu raporları bildirilmiş olup oldukça nadirdir. Kliniğimize 83 yaşında kadın hasta postmenopozal uterin kanama ve anemi ile başvurdu. Transvajinal ultrason ile endometriyal kalınlaşma tespit edildi ve endometriyal küretaj yapıldı. Histopatolojik incelemede endometriyal polip yüzeyinde adenokarsinom infiltrasyonu saptandı. Histolojik incelemede ilk olarak yüksek dereceli seröz karsinom ve berrak hücreli karsinom tanıları düşünüldü. Tümör hücreleri immünohistokimyasal olarak Wilms tümör 1 proteini için negatif, p53 ekspresyonu için wild tip ve Napsin-A için pozitif olduğundan, primer akciğer adenokarsinomu metastazı da ayırıcı tanıya dahil edildi. Tümör hücrelerinde tiroid transkripsiyon faktör-1'in pozitif, Pax8'in ise negatif çıkması ve de radyolojik olarak akciğerde kitlenin doğrulanması üzerine primer akciğer adenokarsinomu metastazı tanısı konuldu. Ayrıca moleküler analizde epidermal büyüme faktörü reseptöründe ekson 19 mutasyonu tespit edildi. Bu olgu sunumu, klinik ve morfolojik özelliklerin yanı sıra doğru tanı için çoklu immünohistokimyasal panellerin uygulanmasının önemini vurgulamaktadır.

Anahtar Kelimeler: EGFR proteini, akciğer adenokarsinomu, metastaz, endometriyum, metroraji

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Introduction

Postmenopausal uterine bleeding (PUB) accounts for approximately 5% of gynecology outpatient clinic visits[1]. Usual or atypical endometrial hyperplasia and polyps, as well as endometrial cancers, are well-known causes of postmenopausal bleeding in women[2]. Uterine carcinomas are the most common gynecologic cancers in United States accounting for approximately 13,000 annual deaths[3]. Hence, endometrial sampling is a crucial step for evaluating patients who present with PUB.

Lung and bronchial cancers are the second most common cause of cancer-related death in both women and men and the most common type of cancer in both groups[3]. Lung adenocarcinoma (LUAD) is the most frequent subtype of lung carcinomas[4]. Most patients with LUAD are at an advanced stage at the time of diagnosis and lose the best chance of surgical resection due to the relatively insidious early symptoms of LUAD[5]. Tobacco smoking accounts for most lung cancer etiology, except for LUAD. The Swedish Cancer Registry (involving approximately 18,000 patients with lung cancer) revealed that the nervous system, bone, liver, respiratory system, and adrenal gland as the most common sites for metastasis[6]. An autopsy study involving 175 patients with primary lung cancer showed 0.6% metastasis to the ovary[7]. Metastasis from primary lung cancer to the female genital tract remains rare.

This report presents a primary LUAD case with uterine bleeding. The definitive pathological diagnosis was received from endometrial curettage material. Molecular study analyses were performed and epidermal growth factor receptor (EGFR)-exon 19 deletion was detected. Additionally, this study also includes a literature review of endometrial metastasis that originates from primary lung carcinomas.

Case Report

An 83-year-old female patient came to the outpatient gynecology clinic presenting vaginal bleeding, which lasted for one week. She had a similar vaginal bleeding complaint six years ago, and the biopsy specimen was diagnosed as an endometrial polyp. Her past medical history included pulmonary emboli, hypertension, osteoarthritis, gout, and cardiac pacemaker. Physical examination revealed active bleeding from cervix. Transvaginal ultrasound showed a 13 mm endometrial thickness, multiple cystic degeneration foci, and endometrial polyp formations. However, both ovaries were atrophic and without mass lesions.

Diagnostic hysteroscopy, polypectomy, and endometrial curettage were performed. Histologic examination revealed an adenocarcinoma infiltration on the endometrial polyp surface. The tumor was sharing papillary and micropapillary formations with eosinophilic cytoplasm (Figure 1). No necrosis was seen. "High-grade serous carcinoma" and "clear cell carcinoma" diagnosis was considered for the first morphological evaluation. Immunohistochemical (IHC) stains were negative for Wilms' tumor 1 (WT1) and wild-type for p53 (30% of the tumor cells were positive with p53), thus high serous carcinoma was ruled out (Figure 2). However, Napsin A was diffusely positive, which was requested for clear cell carcinoma (Figure 2). Napsin A is simultaneously a strong predictor of primary LUAD[8]. The medical reports were retrospectively reviewed from the hospital information system. The previous thoracic computed tomography (CT) reported a lung mass lesion at the right lower lobe and multiple additional metastatic lesions, which were consistent with primary lung carcinoma, with mediastinal lymph node and bone metastasis. However, the patient did not previously receive a pathological diagnosis of lung lesion. Therefore, "LUAD metastasis" is also included in the differential diagnosis and the IHC panel was expanded. Clear cell carcinoma was excluded by negative paired box gene 8 (Pax8) staining (Figure 2). Moreover, thyroid transcription factor 1 (TTF1) staining was performed for LUAD diagnosis, which was positive (Figure 2). Thereafter, the diagnosis of "LUAD metastasis to endometrial polyp" was determined.

Chemotherapy was the first treatment option due to the advanced stage, thus molecular testing studies were conducted from the curettage material. Deoxyribonucleic acid (DNA) isolation was performed using the "AmoyDx® FFPE DNA Kit." DNA quantity and quality were measured using the "Nanodrop 2000" device. The A260/A280 value of the DNA sample ranges

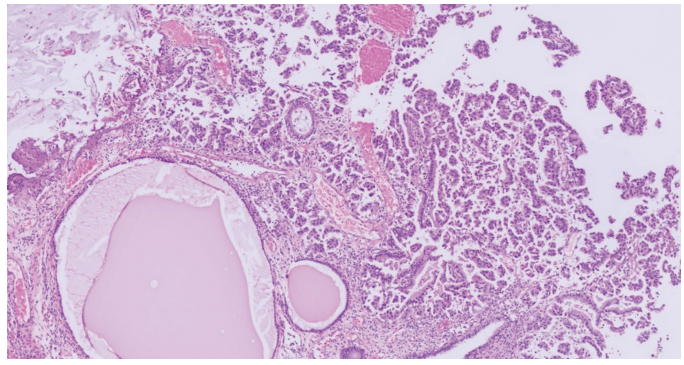

Figure 1A. Adenocarcinoma infiltration is seen on the endometrial polyp surface in papillary and micropapillary architecture (4x; hematoxylin and eosin)

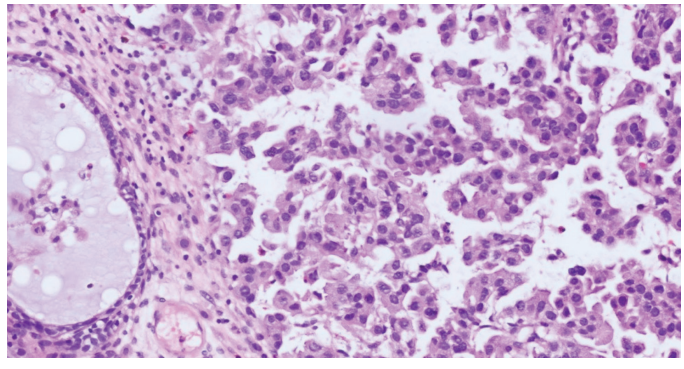

Figure 1B. Tumor cells that constitute the adenocarcinoma have mild to moderate nuclear atypia and eosinophilic cytoplasm without significant pleomorphism (hematoxylin and eosin)

from 1.8 to 2.0. The polymerase chain reaction was performed using the "BIO-RAD CFX96 Real-Time Detection System + C1000 Touch Thermal Cycler" device and the "AmoyDx® EGFR 29 Mutations Detection Kit" as specified in the kit protocol. Internal and external positive and negative controls were used in each study. Twenty-nine different mutations frequently seen in *EGFR* gene were evaluated, and exon 19 deletions of the EGFR were detected, which is known to be associated with the susceptibility to anti-EGFR-acting tyrosine kinase inhibitors in the tumor. T790M mutation was also evaluated but no mutation was found. Ventana ALK (D5F3) IHC antibody was used for the anaplastic lymphoma kinase (ALK) mutation analysis.

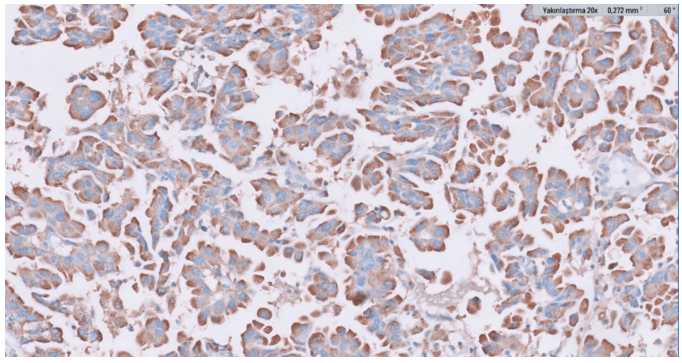

Figure 2A. Tumor cells diffuse granular cytoplasmic positivity with Napsin A

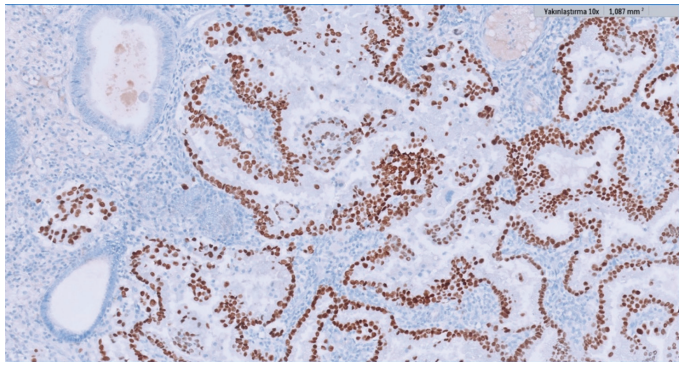

Figure 2B. Tumor cells diffuse nuclear positivity with thyroid transcription factor-1 (TTF1)

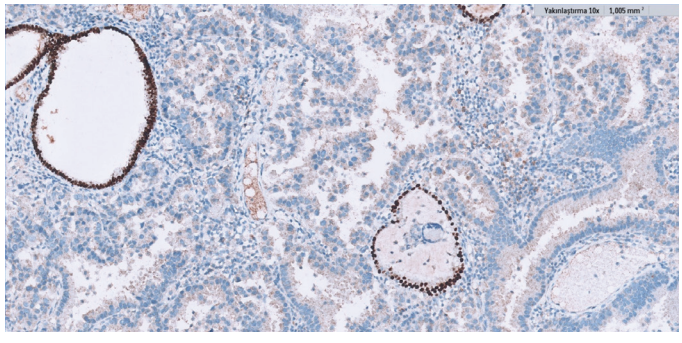

Figure 2C. Tumor cells are negative with paired box gene 8 (Pax8). As an internal control, the endometrial glandular cells are positively stained with Pax8

Ventana ROS (SP384) IHC antibody was used for reactive oxygen species (ROS) mutation analysis. The study was automatically conducted using the OptiView DAB IHC Detection Kit and the OptiView Amplification Kit on the Ventana Benchmark XT device. Appropriate staining was observed in the control tissue. No staining was observed with either ALK or ROS IHCs in the tumor.

Positron emission tomography-CT and brain CT showed metastatic mass lesions other than the endometrium. The patient received first-line erlotinib therapy for one month and currently has been using osimertinib for two months. Regression was not detected with these anti-EGFR therapeutic agents yet.

Discussion

Metastasis from the primary lung cancer to the female genital tract, including ovaries, myometrium, endometrium, vagina, cervix, and vulva is rare. Here, we present a case of EGFR-mutated LUAD with endometrial metastasis.

A literature search of endometrial metastasis from primary lung cancer yielded 11 case reports (Table 1)[9-18]. The patients' age ranged from 37 to 73 years. Most reported cases (81.82%) were non-small cell lung cancer. Endometrial biopsy due to abnormal vaginal bleeding (n=5, 45.46%) [9,11,12,14,17] and abnormal uterine or endometrial imaging during lung cancer follow-up (n=5, 45.46%)[10,14-16,18] were the leading causes for endometrial metastasis detection in this small cohort. Five cases (45.46%) were investigated for EGFR mutation status, where one case was negative[11], one case has had EGFR L858R and T790M mutation[14], one case was positive with E746_A750del mutation in exon 19 and T790M mutation in exon 20[16], and two cases were wild-type for EGFR mutation[17,18]. The EGFR status of the other cases is unknown. The ALK was detected in two cases and was treated with ALK inhibitors[16,18]. One of the ALK mutated cases sequentially had EGFR mutation[16].

Thyroid transcription factor-1 (TTF-1) and Napsin A are highly sensitive and specific markers for LUAD diagnosis, especially when used together[8]. Evaluation of 1,674 cases of lung cancer revealed that Napsin A was more sensitive (87% vs. 64%; p<0.001) and more specific (p<0.001) marker than TTF-1 in the differential diagnosis of LUAD[19]. TTF-1-positive

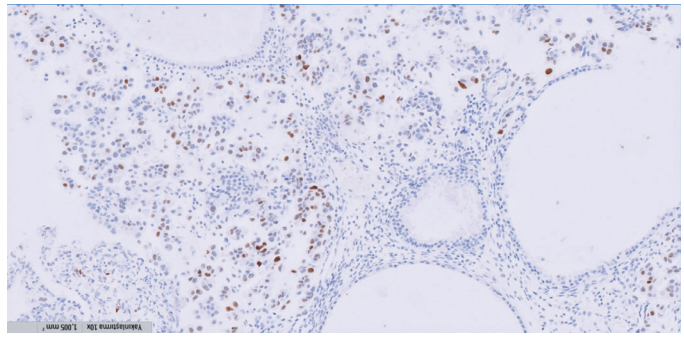

Figure 2D. Tumor cells with a wild-type pattern p53 staining

Table 1. Summary of the case reports with lung cancer metastasis to endometrium

Case no.	Age (years)	Race	Menopause	Smoking status	Discovery of metastasis to endometrium	Primary cancer	IHC findings (Lung)	IHC findings (Endometrium)	Report
1	68	NR	Yes	NR	Endometrial biopsy due to postmenopausal bleeding	well-differentiated neuroendocrine lung carcinoma (SCLC)	NR	NR	Jordan et al.[9]
2	56	NR	NR	No	After the diagnosis of lung cancer, hysterectomy was performed due to suspected mass that was detected in imaging	Small cell lung carcinoma (SCLC)	Chromogranin (+) Synaptophysin (+) TTF-1 (+)	Chromogranin (+) Synaptophysin (+) CD56 (+)	Chargari and Vedrine[10]
3	50	NR	NR	Yes	After the diagnosis of lung cancer, endometrial biopsy due to metrorrhagia	Lung adenocarcinoma (NSCLC)	TTF-1 (+) CEA (+) SPA (–)	TTF-1 (+) CEA (+) SPA (–) ER (–) PR (–)	Hibi et al.[11]
4	58	Caucasian	NR	Yes	After the diagnosis of lung cancer, endometrial biopsy due to vaginal bleeding	Lung adenocarcinoma (NSCLC)	TTF-1 (+) cytokeratin 7 (+) cytokeratin 20 (–) thyroglobulin (–) S100 (–) HMB-45 (–) melan A (–) ER (–)	TTF-1 (+) cytokeratin 7 (+) cytokeratin 20 (–) myogenin (–) S100 (–) ER (–)	Tiseo et al.[12]
5	70	White	Yes	NR	After the diagnosis of lung cancer, endometrial biopsy due to abnormal thickening of the endometrium	Pulmonary carcinoid tumor (NSCLC)	TTF1 (+) Chromogranin (+) Synaptophysin (+) Cytokeratin AE1/AE3 (+)	TTF1 (equivocal) Chromogranin (+) Synaptophysin (–) Cytokeratin AE1/AE3 (–)	Momeni et al.[13]
6	55	NR	NR	NR	Endometrial biopsy due to PET-CT showing hypermetabolic activity in the endometrium	Lung adenocarcinoma (NSCLC)	TTF-1 (+) cytokeratin 7 (+) Napsin (+) cytokeratin 20 (–) cytokeratin 5/6 (–) CDX2 (–)	TTF-1 (+) cytokeratin 7 (+) Cytokeratin AE1/AE3 (+) Vimentin (+) cytokeratin 20 (–) ER (–) PR (–)	Ahmad et al.[14]
7	51	NR	NR	No	After the diagnosis of lung cancer, endometrial biopsy due to heavy vaginal bleeding	Lung adenocarcinoma (NSCLC)	TTF-1 (+) Napsin A (+) cytokeratin 7 (+) MOC-31 (+) cytokeratin 5/6 (–) WT-1 (–) ER (–) PR (–) CDX-2 (–) cytokeratin 20 (–)	TTF-1 (+) ER (–) PR (–)	Ahmad et al.[14]

Table 1. Continued

Case no.	Age (years)	Race	Menopause	Smoking status	Discovery of metastasis to endometrium	Primary cancer	IHC findings (Lung)	IHC findings (Endometrium)	Report
8	73	NR	NR	NR	Uterus curettage due to PET/CT showing hypermetabolic activity in the uterus and spotting	Lung adenocarcinoma (NSCLC)	NR	TTF-1 (+) ER (–) PAX-8 (–)	Patel et al.[15]
9	37	NR	No	NR	After PET/CT showing uterine uptake, endometrial curettage was performed	Lung adenocarcinoma (NSCLC)	CK-7 (+) TTF (+) ER (–) PR (–) HER2 (–) NEU (–) GCDFP-15 (–)	TTF1 (+) (clone 8G7G3/1)	Anjali et al.[16]
10	47	NR	NR	No	Endometrial biopsy due to vaginal bleeding	Lung adenocarcinoma (NSCLC)	TTF1 (+) ALK (+) PDL1 (+)	TTF1 (+) CK7 (+) PAX-8 (+) ALK (+) PDL1 (+) CK20 (–) ER (–)	Sevinyan et al.[17]
11	54	NR	NR	NR	After abdominal CT revealing uterine mass, endometrial curettage was performed	Lung adenocarcinoma (NSCLC)	NR	NR	Kobayashi et al.[18]
12	83	White	Yes	NR	endometrial biopsy due to AUB	Lung adenocarcinoma (NSCLC)	NR	TTF-1 (+) Napsin A (+) WT-1 (–) p53 (+) PAX-8 (–)	Bulutay et al. (Current Case)

AUB: Abnormal uterine bleeding, CEA: Carcinoembryonic antigen, CT: Computed tomography, ER: Estrogen receptor, IHC: Immunohistochemistry, PET: Positron emission tomography, NR: Not reported, NSCLC: Non-small cell lung cancer, PR: Progesterone receptor, SCLC: Small cell lung cancer, SPA: Surfactant protein A, TTF-1: Thyroid transcription factor-1

tumoral infiltration in extrapulmonary tissues is accepted as a primary LUAD metastasis if the possibility of thyroid primary is not clinically predicted. TTF-1 is considered a relatively specific marker for lung and thyroid neoplasms; however, TTF-1 positivity was reported in a subset of endometrial carcinomas and this rate was much lower, especially in well-differentiated types[20]. Therefore, occasional TTF-1 expression of endometrial and endocervical carcinomas should be kept in mind when evaluating neoplasms of uncertain origin, especially on the gynecological tract. Napsin A is a highly sensitive marker for LUADs, thus studies showed that tumors as ovarian clear cell (71.7%), endometrial clear cell (42.8%), papillary renal cell (40.2%), clear cell (tubular) papillary renal cell (16.7%), endometrial serous (9.3%), papillary thyroid (9.3%), and clear cell renal cell carcinomas (8.2%) can similarly express Napsin A[21]. Therefore, studying multiple markers as a panel for targeted tumors would be beneficial, particularly if a tumor of unknown primary origin is seen. In this case, both TTF-1 and Napsin A positivity, as well as Pax8 negativity with radiologically defined lung mass lesions, were strong indicators of the endometrial metastasis of the primary LUAD.

Conclusion

The endometrium is a rare site for primary lung cancer metastasis; however, an increasing number of cases of endometrial metastases from lung cancer could be reported due to the increasing incidence of primary lung cancer. In addition to the endometrial-originating lesions in patients with abnormal uterine bleeding, clinicians should keep in mind the metastatic tumors. Furthermore, while evaluating tumors that are observed in endometrial curettage materials by pathologists, the possibility of metastatic tumors should always be considered.

Ethics

Informed Consent: Retrospective study.
Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Ş.Y., B.A., Concept: P.B., E.B., Ş.Y., B.A., Design: P.B., E.B., Ş.Y., B.A., Data Collection or Processing: P.B., E.B., Ş.Y., Analysis or Interpretation: P.B., E.B., B.A., Literature Search: P.B., E.B., Writing: P.B., E.B., Ş.Y.
Conflict of Interest: The authors declare no conflict of interest.
Financial Disclosure: The authors declared that this studyreceived no financial support.

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Maternal PARK7 (DJ-1) levels and the preterm premature rupture of membranes: Correspondence

Maternal PARK7 (DJ-1) seviyeleri ve erken membran rüptürü: Yazışma

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Keywords: PARK7 (DJ-1), preterm premature rupture of membrane, maternal
Anahtar Kelimeler: PARK7 (DJ-1), membran preterm erken yırtığı, anne

Dear Editor,

We would like to share ideas on the publication "The association between increased maternal Parkinson disease protein 7 (PARK7) (DJ-1) levels and the occurrence of preterm premature rupture of membranes (PPROM) - A randomized prospective study[(1)]." Turhan and Tatar[(1)] concluded that "PARK7 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[(1)]." We agree that PARK7 might be useful for management of PPROM. However, as Turhan and Tatar[(1)] noted, further studies are required. Other obstetric complication such as pre-eclampsia also affect level of DJ-1[(2)]. Also, quality control in analysis of the DJ-1 is necessary. In clinical chemistry, different centrifugation condition can significantly affect measurement of DJ-19[(3)]. These factors are important considerations in using maternal DJ-1 as biomarker.

Ethics

Peer-review: Internally peer-reviewed.
Financial Disclosure: The authors declared that this study received no financial support.

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