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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.

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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

INSTRUCTIONS FOR AUTHORS

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

Discussion

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

Study Limitations

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

Conclusion

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

Conflict of Interest

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

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

Introduction, Case Report, Discussion and References.

References

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

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

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

Examples

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

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

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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

LETTER FROM THE PRESIDENT

Dear Turkish Gynecology and Obstetrics Family;

I am proud and happy to be back together in the June issue of the Turkish Journal of Obstetrics and Gynecology. In this issue, we are accompanied by valuable and scientifically strong national and international publications that we have presented to the scientific community with meticulous and rigorous research. I would like to express my gratitude to each member of the editorial board and the referees who were involved in the evaluation process.

As you know, we held the 20th National Turkish Congress of Gynecology and Obstetrics in Cyprus last week. We held a highly successful congress where we not only gathered with our colleagues but also shared our up-to-date information. The largest congress in our country, which was attended by 1742 gynecologists, featured a total of 65 scientific sessions, 9 satellite symposium, and 14 oral presentation sessions. I must also mention that our congress, which received significant interest from the medical sector, included a total of 123 sponsoring companies and 95 company booths present at the event.

My esteemed colleagues, unfortunately, this year we held the 20th National Congress of Gynecology and Obstetrics with a sense of sorrow and woundedness. The pain of the earthquake that occurred on February 6, 2023, and affected 11 provinces still remains within us as if it were the first day. Certainly, as the Turkish Society of Gynecology and Obstetrics (TJOD) family, while carrying out our scientific activities, we are doing everything in our power to help heal the wounded. I would like to emphasize that we have provided both financial aid and a support of 14 containers to the disaster-stricken regions. I also need to mention that we held a press conference during our congress to raise awareness about our colleagues in the earthquake-affected region.

Turkish Society of Gynecology and Obstetrics (TJOD) family is not just a professional organization but also operates as a social and scientific association, which sets us apart from others. As the president of such an association, I am filled with justified pride, and I look forward to being with you all in the next issue.

Bulent Tiras, Prof. MD
President of TJOD

TURKISH JOURNAL OF OBSTETRICS AND GYNECOLOGY

EDITORIAL

Dear Colleagues;

We are delighted to be with you in the June issue of our journal. In our June issue, we have prepared high-quality scientific studies for you, thanks to the dedicated and diligent work of our editorial board and referees.

Knowing that our journal's international recognition and prestige is increasing day by day, I would like to give you the good news that Turkish Journal of Obstetrics and Gynecology has been included in the "China Knowledge Resource Integrated (CNKI)" index. Since 2020, CNKI has been operating as an open-access database, receiving approximately 16 million daily clicks and registering around 2.33 billion downloads of its indexed academic content. Additionally, CNKI serves as a database for approximately 1,600 institutes from 60 different countries through agreements. Indeed, having our journal included in such a database is a significant achievement for our country.

Dear colleagues, our utmost effort is to elevate our journal, which is a national treasure, to its deserved highest level. As editors of the journal, we are indebted to the independent referees who have participated in the editorial board and the evaluation process. With best wishes, we look forward to meeting you in our next issue. With love and respect.

Ercan Yilmaz, Prof.MD
Fatih Sendag, Prof.MD
Editors in TJOG

Clinical Investigation / Araştırma

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The role of different Doppler parameters in predicting adverse neonatal outcomes in fetuses with late-onset fetal growth restriction

Geç başlangıçlı intrauterin büyüme kısıtlılığı saptanan fetuslarda farklı Doppler parametrelerinin olumsuz neonatal sonuçları öngörmedeki rolü

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Abstract

Objective: The aim of this study is to clarify the role of different Doppler parameters such as umbilicocerebral ratio (UCR), cerebroplacentouterine ratio (CPUR), aortic isthmus, renal artery, and umbilical vein flow Doppler in predicting adverse neonatal outcomes in fetuses with late -onset fetal growth restriction.

Materials and Methods: The study included all patients diagnosed with fetal growth restriction at 32-39 weeks' gestation between 01/02/2020 and 01/02/2022 and treated at the Department of Obstetrics and Gynecology, Inonu University School of Medicine.

Results: Patients included in the study had a median gestational week at delivery of 37 (minimum 33+0-maximum 39+0), median CPR of 1.42 (minimum-maximum 0.43-3.57), and median UCR of 0.7 (minimum-maximum 0.28-2.3). Receiver operating characteristic analysis was performed to determine the performance of the measured obstetric Doppler parameters in predicting the development of adverse neonatal outcomes. Umbilical venous blood flow showed the best performance in predicting adverse neonatal outcomes [area under the curve 0.952, 95% confidence interval (CI) 0.902-0.981, p<0.001]. Multivariate logistic regression analysis showed that fetuses with abnormal CPUR had a 4.5-fold (95% CI 0.084-0.583, p=0.02) increased risk of adverse neonatal outcome, whereas fetuses with abnormal umbilical venous flow had a 1.07-fold (95% CI 0.903-0.968, p<0.001) increased risk of adverse neonatal outcome.

Conclusion: The results of this study demonstrate that the use of UCR, CPUR, umbilical venous flow, and aortic isthmus PI Doppler parameters along with umbilical artery PI and CPR are effective in predicting adverse neonatal outcomes in fetuses with late -onset fetal growth restriction.

Keywords: Doppler ultrasound, fetal growth restriction, pregnancy, adverse outcomes, umbilical artery, and umbilical vein

Öz

Amaç: Bu çalışmanın amacı geç başlangıçlı fetal büyüme kısıtlılığı saptanan fetuslarda rutin obstetrik Doppler parametrelerine ek olarak umbilikoserebral oran (UCR), serebroplasentouterin oran, (CPUR), aortik istmus, renal arter Doppler ve umblikal ven kan akımı gibi farklı Doppler parametrelerinin fetustaki olumsuz neonatal sonuçları öngörmedeki rolünü açıklamaktır.

Gereç ve Yöntemler: İnönü Üniversitesi Tıp Fakültesi Hastanesi, Kadın Hastalıkları ve Doğum Kliniği'ne 01.02.2020-01.02.2022 tarihleri arasında başvuran, gebeliğin 32-39. haftasında fetal büyüme kısıtlılığı saptanan, çalışma kriterlerine uygun tüm hastalar çalışmaya dahil edildi.

Bulgular: Çalışmaya dahil edilen hastaların doğumda median gestasyonel haftası 37 olup (en küçük 33+0-en büyük 39+0), median serebroplasental oran (CPR) 1,42 (minimum-maksimum 0,43-3,57) ve median UCR 0,7 (minimum-maksimum 0,28-2,3) saptandı. Ölçülen obstetrik Doppler parametrelerinin

PRECIS: We investigated the predictive value of different Doppler parameters for adverse neonatal outcomes in fetuses with late-onset fetal growth restriction.

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olumsuz neonatal sonuçların gelişimini öngörmesi açısından performansının saptanması için Receiver Operating Characteristic analizi yapıldı. Umblikal ven kan akımı olumsuz neonatal sonuçları predikte etmede en iyi performansı gösterdi [Area under curve 0,952, %95 güven aralığı (GA) 0,902-0,981, p<0,001]. Çok değişkenli lojistik regresyon analizi, anormal CPUR'ye sahip fetusların 4,5 kat (%95 GA 0,084-0,583, p=0,02) olumsuz neonatal sonuç riskine sahip olduğunu, anormal umbilikal ven kan akımı olan fetuslarda ise 1,07 (%95 GA 0,903-0,968, p<0,001) kat daha fazla olumsuz neonatal sonuç riski olduğunu gösterdi.

Sonuç: Bu çalışmanın sonuçları geç başlangıçlı fetal büyüme kısıtlılığı saptanan fetuslarda umblikal arter PI ve CPR ile birlikte UCR, CPUR, umblikal ven kan akımı ve aortik istmus PI Doppler parametrelerinin kullanımının neonatal olumsuz sonuçları predikte etmede etkin olduğunu göstermiştir.

Anahtar Kelimeler: Doppler ultrasonografi, fetal büyüme kısıtlılığı, gebelik, olumsuz sonuçlar, umblikal arter, umblikal ven

Introduction

The inability of a fetus to reach its intrauterine growth and developmental potential is known as fetal growth restriction (FGR). Fetuses affected by FGR may experience increased perinatal morbidity and mortality, as well as long-term risks associated with neurological, cardiovascular, endocrine and cognitive developmental disorders[1]. FGR occurs in 3-10% of pregnancies and in about 20% of stillbirths. FGR is often difficult to define because fetal growth cannot be determined by a single biometric measurement of fetal size and the idea of growth potential is speculative. FGR can be classified into two main types, each with different characteristics such as gestational age onset, ultrasound findings, and pathological features. In particular, early-onset FGR is associated with maternal vascular malperfusion of the placenta, characterized by abnormal transformation of the spiral arteries, pathological features of the placental villi, and multifocal infarcts. These factors contribute to placental insufficiency and are the major cause of placenta-associated FGR[2,3]. Late-onset FGR is typically identified after 32 weeks' gestation and has a different pathophysiology to early-onset FGR. Placental lesions in late-onset FGR tend to be less specific and milder, and there may be changes in the way oxygen and nutrients are diffused[4]. Although late-onset FGR is not as severe as early-onset FGR, it still results in unfavorable perinatal outcomes and long-term neurodevelopmental problems[5]. Although late-onset FGR is not yet fully understood, it may result in a lower diagnosis rate in fetuses with growth restriction near term[6]. Additionally, near-term fetuses have reduced tolerance to hypoxemia, possibly due to their higher metabolic rate compared to fetuses at earlier gestational ages. Therefore, frequent monitoring of pregnancies with late-onset FGR is essential, just as it is for pregnancies with early-onset FGR.

Various parameters from conventional sonography and color Doppler sonography have been used to diagnose FGR and monitor its progression. Doppler ultrasound plays an important role in the diagnosis, monitoring, and management of FGR as it is an essential tool for detecting uteroplacental insufficiency and fetal cardiovascular adaptation to hypoxemia. In early-onset FGR, deterioration in fetal well-being usually follows a predictable pattern, with Doppler parameters showing increasing abnormalities before biophysical parameters become abnormal. However, in late-onset FGR, normal or slightly elevated umbilical Doppler indices and mildly abnormal cerebral Doppler findings may be present but not easily detectable[7]. Changes in umbilical artery (UA) Doppler and venous fields are rare, and are not an effective means of identifying the majority of late-onset FGR or predicting adverse outcomes in affected fetuses. Many studies have found an association between adverse perinatal outcomes (such as stillbirth, increased risk of caesarean section, and increased neurodevelopmental disorders at two years of age) and middle cerebral artery (MCA) vasodilation. This vasodilation is indicated by a decrease in the MCA pulsatility index (PI) or an altered ratio of MCA to UA PI[8]. In addition to all these parameters, there are recent studies showing that evaluation of various Doppler parameters such as umbilicocerebral ratio (UCR), cerebroplacentouterine ratio (CPUR), aortic isthmus, and renal artery Doppler can help diagnose FGR and predict adverse perinatal outcomes in this group of patients. Although each parameter can predict adverse neonatal outcomes in women with late-onset FGR, there has been no evaluation of these parameters in combination[9]. The aim of this study was to elucidate the specific contributions of different Doppler parameters in predicting adverse neonatal outcomes in fetuses diagnosed with late-onset FGR.

Materials and Methods

This study included all patients diagnosed with late-onset FGR at 32-39 weeks' gestation at the Department of Obstetrics and Gynecology, Faculty of Medicine, Inonu University between 01.02.2020-01.02.2022 and was conducted in accordance with the World Medical Association's Declaration of Helsinki (including the 2013 amendments) and with the approval of the Clinical Research Ethics Committee of Inonu University (Ethics Committee approval number: 2020/04). In addition, informed consent was obtained from all participants prior to enrolment.

Pregnant women were included if they met the following criteria: (i) 18-39 years of age; singleton live pregnancy; (ii) 32+0- 39+6 weeks of gestation (gestational age confirmed by first trimester ultrasound); and (iii) normal fetal anatomy.

Exclusion criteria were: (i) multiple pregnancy; (ii) major fetal anomalies (anomalies that are fatal or require prenatal or postnatal surgery), chromosomal anomalies, genetic syndromes and macroscopic placental anomalies; (iii) fetal death; and (iv) patients with eclampsia, placental abruption, disseminated intravascular coagulation.

Procedure

The diagnosis of late-onset FGR was made according to the Delphi procedure defined in 2016. All pregnant women underwent both gray-scale imaging and pulsed-wave Doppler ultrasonography with a spatial peak temporal average intensity of <100 mW/cm^2 using a 5-MHz probe (Voluson E6, GE Medical Systems). Routine fetal biometry was performed in all pregnant women with late FGR according to the International Society of Ultrasound in Obstetrics and Gynecology standard protocols, and the estimated fetal weight (EFW) was calculated using the Hadlock 4 formula[10,11]. All Doppler ultrasonography recordings were performed without fetal respiration or fetal movement. The average of three consecutive Doppler velocity waveforms from each vessel was used for analysis. To ensure that blood flow in the larger vessels was uniform and free of aliasing, the maximum speed of the color Doppler was set to high velocities. The high-pass filter was adjusted to 50 Hz, and the energy output was kept below 50 mW/cm^2. The sample volume size was adjusted to match the vessel diameter so that it was completely covered. In addition to fetal biometric measurements, UA PI, MCA PI, MCA-peak systolic velocity (PSV), cerebroplacental ratio (CPR), UCR, CPUR, uterine artery (Ut A) PI, ductus venosus PI, umbilical venous blood flow, aortic isthmus PI, and renal artery PI Doppler parameters were measured and recorded according to standards. The UA was sampled from the free loop, and PI was used to analyze the waveforms. The Doppler parameters of the MCA were measured so that the direction of blood flow and the angle between the ultrasound beams were close to 0°. An UA PI above the 95th percentile for gestational week, loss of end-diastolic flow, or end-diastolic reverse flow on UA Doppler was considered abnormal. MCA waveforms were assessed by PI and PSV. CPR was calculated by dividing MCA PI by UA PI, while UCR was calculated by dividing UA PI by MCA PI. A transabdominal approach was used to measure Ut A Doppler, with the probe angled medially in the parasagittal plane and positioned longitudinally in the lower lateral part of the abdomen. Color Doppler mapping was used to determine the point of intersection of the external iliac artery and the uterine artery, and the position of the probe was adjusted according to the orientation of the uterine artery to obtain the best insonation angle. The crossing point was used as a reference, and the sample volume was located one centimeter downstream. The contralateral uterine artery was subjected to the same procedure. The average of the two Ut A PIs was calculated as the mean Ut A PI. For Doppler measurement of the ductus venosus, color Doppler mapping was used to locate the vessel where the intra-abdominal portion of the umbilical vein joins the left inferior vena cava just below the diaphragm. Alternatively, the presence of aliasing in the midsagittal longitudinal plane of the fetal trunk or in the transverse oblique section of the upper abdomen, indicating high flow velocity in the ductus venosus, was used to identify the vessel. Triphasic ductus venosus waveforms were obtained from these standard sampling sites, and Doppler measurements were performed. To determine the umbilical blood flow, measurements were taken from the intra-abdominal portion of the umbilical vein before the first portal branches. The diameter of the umbilical vein was measured by vertically insonating and imaging the abdominal section of the vein. To calculate the internal diameter of the umbilical vein, three measurements were taken at an insonation angle that was perpendicular to the wall of the vessel and then averaged. Time-averaged maximum velocity (TAMXV) was calculated during fetal rest and apnea by measuring the maximum dimension of the waveform in steady flow for at least 10 s in another plane with an insonation angle <20°. Venous waveforms were obtained, and the maximum waveform size was measured on the basis of flow uniformity (absence of pulse). The blood flow in the umbilical vein (measured in milliliters per minute) was determined by the formula $\pi \times$ (umbilical vein diameter/2) × 0.5 × TAMXV. The measurements for aortic isthmus Doppler were obtained from either the longitudinal aortic arch or the sonographic plane of the three vessels and trachea. For renal artery Doppler measurements, a coronal section of the fetal abdomen was used to obtain a horizontal view of the aorta. The color Doppler mapping identified the renal arteries, and the scan plane was adjusted to minimize the angle of insonation. Both the right and left renal arteries were examined, and Doppler flow velocity waveforms were obtained from two different locations: near the proximal end of the aorta and before any visible major bifurcation at the distal end of the vessels. The average of the two renal artery PIs was calculated as the mean renal artery value PI. Ultrasound were examined by a single clinician who had received extensive training in fetal ultrasonography and held the Fetal Medicine Foundation Certificate of Competence in Doppler Ultrasound Examinations.

Pregnant women with late-onset FGR were scheduled for delivery at 37-39 weeks' gestation, unless there was a pregnancy complication requiring delivery. Labor management of pregnant women enrolled in the study was performed according to the routine follow-up and delivery protocols used in our hospital. The frequency of follow-up was individualized according to the week of gestation and the presence of associated circumstances (oligohydramnios, abnormal Doppler findings, maternal risk factors, comorbidity).

The following parameters were recorded; Age, gravidity, parity, abortion, body mass index (BMI), presence of FGR in obstetric history, smoking, presence of hypertensive disease/preeclampsia, abdominal circumference (AC), EFW, amniotic fluid index (AFI), UA systol/diastole (S/D) ratio, UA Doppler PI, UA Doppler RI, MCA Doppler PI, MCA PSV, CPR, UCR, Ut A Doppler PI, CPUR, ductus venosus Doppler PI, umbilical venous blood flow (mL/min), aortic isthmus and renal artery Doppler PI, day between delivery and last ultrasound scan, meconium-stained amniotic fluid, induction of labour, mode of delivery, birthweight, gender, need for active resuscitation at

birth (respiratory support with endotracheal tube), APGAR score 1st minute, APGAR score 5th minutes, umbilical cord blood pH, umbilical cord blood base excess, umbilical cord blood partial oxygen pressure (pO_2), umbilical cord blood partial carbon dioxide pressure (pCO_2), need for neonatal intensive care unit, length of stay in neonatal intensive care unit, use of respiratory support (mechanical ventilation or continuous positive airway pressure or both), neonatal death. An adverse neonatal outcome was defined if one of the following criteria was met: Apgar score less than 7 at 5 min, umbilical artery pH less than 7.10, need for neonatal intensive care, or neonatal death.

Power analysis: The area under the curve (AUC) of the umbilical venous flow estimation score (0.85) was used as the alternative hypothesis, and an AUC value of 0.5 was used as the null hypothesis. The expected incidence of the primary outcome was 32.5%[12]. The sample size was calculated as 90, which was calculated by taking the amount of type I error (alpha) 0.05 and the power of the test 0.85. MedCalc version 20 was used for sample size calculations.

Statistical Analysis

Statistical analysis of the study was performed using SPSS 20.0 software and included the Kolmogorov-Smirnov test to ensure normality of distribution. The relevant statistical analyses conducted included Mann–Whitney U, Pearson chi-squared test, the chi-squared test with Yates correction, and Fisher's exact chi-squared test. ROC analysis was used to determine the diagnostic performance and to identify the optimal cut-off points for relevant variables. Logistic regression analysis was used to estimate the odds ratios. Statistical significance was set at a p-value <0.05 for all tests performed.

Results

In this study, 149 patients with late-onset FGR in the fetus between 32-39 weeks of gestation were identified at the Department of Obstetrics and Gynecology, Inonu University Medical Faculty Hospital between 01/02/2020 and 01/02/2022. As the measurement of all Doppler parameters could not be completed in 8 patients due to the fetal position, 141 patients were included in the study. The median gestational week at delivery was 37 [minimum (min) 33+0- maximum (max) 39+0], the median EFW was 2224 g (min-max 1086-2764), the median UA PI was 1.02 (min-max 0.66-2.85), median CPR 1.42 (min-max 0.43-3.57), median UCR 0.7 (min-max 0.28-2.3), median ductus venosus PI 0.77 (min-max 0.32-1.58), and median birthweight 2345 g (min-max 1070-2840). The rate of cesarean section for fetal distress was 31.5%, and the rate of cesarean delivery was 93.7% in the study cohort. When neonatal outcomes were analyzed, the need for neonatal intensive care was 54.6% and the neonatal mortality rate was 0.7%. The clinical, sonographic, and Doppler sonographic data and neonatal outcomes of the study cohort are summarized in Table 1.

Table 1. Clinical characteristics and Doppler velocimetry data of the study cohort

Variable		Patients with late-onset FGR (n=141)
Age (years)*		29 (18-43)
Gravidity*		2 (1-6)
Parity*		0 (0-5)
Abortus*		0 (0-4)
Body mass index (kg/m²)**		27.97±3.72
Estimated fetal weight (gr)**		2153.38±381.91
Amniotic fluid index (cm)*		11.4 (0-22)
Maxiumum vertical pocket (cm)*		4 (0-8)
Umbilical artery S/D*		2.82 (1.23-7.92)
Umbilical artery PI*		1.02 (0.66-2.85)
Umbilical artery RI*		0.67 (0.48-1.22)
MCA PI*		1.44 (0.78-2.57)
MCA-PSV*		51.2 (30.8-72.0)
CPR*		1.42 (0.43-3.57)
UCR*		0.7 (0.28-2.3)
Uterine artery PI*		0.93 (0.42-2.05)
CPUR*		1.64 (0.21-5.64)
Ductus venosus PI*		0.77 (0.32-1.58)
Umbilical vein flow*		120.8 (19.94-180.13)
Aortic isthmus PI*		2.45 (1.8-3.57)
Renal artery PI*		2.14 (1.47-3.16)
Day between birth and last ultrasound scan*		1 (0-6)
Gestational age at birth (week)*		37 (33-39)
Birthweight (gr)**		2273.09±456.03
APGAR 1*		8 (5-8)
APGAR 5*		10 (6-10)
Cord blood pH*		7.33 (7.12-7.49)
Cord blood base excess*		-4.2 (-16.3-15.1)
Cord blood pO_2**		22.02±9.02
Cord blood pCO_2**		41.34±8.74
Duration of hospital stay in NICU (day)*		0 (0-54)
FGR in obstetric history***		32 (22.7)
Smoking***		11 (7.8)
Hypertension***		36 (25.5)
BPD percentile***	**<3p**	81 (57.4)
	3p-10p	18 (12.8)
	>10p	42 (29.8)
HC percentile***	**<3p**	94 (66.7)
	3p-10p	21 (14.9)
	>10p	26 (18.4)

AC percentile* **	**<3p**	131 (92.9)
	3p-10p	10 (7.1)
	>10p	0 (0.0)
FL percentile* **	**<3p**	102 (72.3)
	3p-10p	23 (16.3)
	>10p	16 (11.4)
EFW percentile* **	**<3p**	96 (68.1)
	3p-10p	45 (31.9)
	>10p	0 (0.0)
Meconium stained amniotic fluid* **		27 (19.1)
Cesarean section for fetal distress* **		45 (31.9)
Labor induction* **		32 (22.7)
Mode of delivery* **	**Vaginal Delivery**	9 (6.4)
	Cesarean section	132 (93.6)
Gender* **	**Female**	73 (51.7)
	Male	68 (48.3)
Need for active resuscitation at birth* **		5 (3.5)
NICU requirement* **		64 (45.4)
Neonatal respiratory support* **		40 (28.4)
Neonatal mortality* **		1 (0.7)

* Median (Min-Max) **Mean ± SD ***n (%)
FGR: Fetal growth restriction, BPD: Biparietal diameter, HC: Head circumference, AC: Abdominal circumference, FL: Femur length, EFW: Estimated fetal weight, FGR: Fetal growth restriction, S/D: Systole/diastole, PI: Pulsatility index, RI: Resistive index, MCA-PSV: Middle cerebral artery-Peak Systolic Velocity, CPR: Cerebroplacental ratio, UCR: Umbilicocerebral ratio, CPUR: Cerebroplacentouterine ratio, pO_2: Partial oxygen pressure, pCO_2: Partial pressure of carbon dioxide, NICU: Neonatal Intensive Care Unit. Statistically significant p values are indicated in bold

The results of multivariate logistic regression analysis with forward selection of characteristics to determine independent predictors for the adverse neonatal outcome group with clinical data (age, parity, BMI, smoking, hypertensive disease/preeclampsia, gestational age at birth) are shown in Table 2. After the forward feature selection, the variables gestational week and age were included in the model. According to the regression equation created, it was observed that the gestational week had a decreasing effect on the need for neonatal intensive care [odds ratio (OR): 0.205, 95% confidence interval (CI) 0.116-0.362; p<0.001] and the age variable had an increasing effect on the need for neonatal intensive care (OR: 1.113, 95% CI 1.011-1.225; p=0.030). In addition, the results of multivariate logistic regression analysis with forward selection of characteristics to determine independent predictors for the adverse neonatal outcome group with sonographic data (UA PI, MCA PI, CPR, UCR, Ut-A PI, CPUR, umbilical vein flow, aortic isthmus PI, renal artery PI, AFI, AC, EFW) are shown in Table 3. After the forward feature selection, the variables CPUR, umbilical vein flow, and EFW were included in the model (OR: 0.222, 95% CI 0.084-0.583; p=0.002, OR: 0.935, 95% CI 0.903-0.968; p<0.001 and OR: 0.996, 95% CI 0.993-0.998; p=0.001, respectively). According to the regression equation, it was observed that all three parameters had a decreasing effect on the need for neonatal intensive care. While fetuses with abnormal CPUR had a 4.5-fold increased risk of adverse neonatal outcome, fetuses with abnormal umbilical venous flow had a 1.07-fold increased risk of adverse neonatal outcome.

Table 2. Multivariate logistic regression analysis of clinical data for adverse neonatal outcomes

	Odds ratio	95% Confidence interval	p-value
Age	1.113	(1.011-1.225)	0.030
Parity	0.879	(0.555-1.391)	0.581
BMI	0.979	(0.857-1.120)	0.761
Smoking	0.720	(0.101-5.153)	0.744
Hypertensive disease/ preeclampsia	0.587	(0.206-1.669)	0.318
Gestational age at birth	0.205	(0.116-0.362)	**<0.001**
FGR in obstetric history	0.844	(0.243-2.937)	0.790

FGR: Fetal growth restriction, BMI: Body mass index. Statistically significant p values are indicated in bold

Table 3. Multivariate logistic regression analysis of the sonographic parameters for adverse neonatal outcomes

	Odds ratio	95% Confidence interval	p-value
CPUR	0.222	(0.084-0.583)	0.002
Umbilical vein flow	0.935	(0.903-0.968)	<0.001
EFW	0.996	(0.993-0.998)	0.001

CPUR: Cerebro-placental-uterine ratio, EFW: Estimated fetal weight. Statistically significant p values are indicated in bold

The presence of at least one of the following parameters in the neonates of pregnant women in the study cohort was considered an adverse neonatal outcome: APGAR 5 minute score <7, umbilical artery pH <7.10, need for neonatal intensive care or neonatal death. An adverse neonatal outcome was observed in 77 (54.6%) of the pregnant women enrolled in the study. It was found that MCA PI, CPR, CPUR, umbilical venous blood flow, and birthweight were significantly lower (compared to normal) in infants with adverse neonatal outcomes (p<0.001, p<0.001, p<0.001, p<0.001, and p<0.001, respectively). However, compared with neonates without adverse neonatal outcomes, UA PI and aortic isthmus PI were significantly higher in neonates with adverse neonatal outcomes (p<0.001 and p<0.001, respectively), whereas no significant difference was found between the two groups for ductus venosus PI, MCA PSV, and renal artery PI (p=0.392, p=0.401, and p=0.304, respectively). The clinical, sonographic, and Doppler sonographic data and neonatal outcomes of patients with and without adverse neonatal outcomes are compared in Table 4.

Table 4. Comparison of clinical characteristics, Doppler velocimetry, and neonatal outcome data of patients with and without adverse neonatal outcome

		Adverse neonatal outcome (–) (n=77)	Adverse neonatal outcome (+) (n=64)	p-value
Age (years)*		27 (18-43)	29 (17-42)	**0.029**
Gravidity*		2 (1-5)	2 (1-6)	0.947
Parity*		1 (0-3)	0 (0-5)	0.072
Abortus*		0 (0-1)	0 (0-4)	**0.007**
Body mass index (kg/m²)**		27.70±3.91	28.39±3.49	0.247
Estimated fetal weight (gr)**		2380.90±197.50	1878.28±374.41	**<0.001**
Amniotic fluid index (cm)*		12 (4.5-22)	11 (0-22)	0.747
Maximum vertical pocket (cm)*		4 (1.6-8)	3.84 (0-7.4)	0.126
Umbilical artery S/D*		2.42 (1.23-4.67)	4.03 (2.05-7.92)	**<0.001**
Umbilical artery PI*		0.92 (0.66-1.55)	1.38 (0.71-2.85)	**<0.001**
Umbilical artery RI*		0.6 (0.5-0.96)	0.77 (0.51-1.22)	**<0.001**
MCA PI*		1.53 (1.03-2.57)	1.28 (0.78-2.06)	**<0.001**
MCA-PSV*		48.6 (30.8-72.0)	54.8 (30.8-72.0)	0.401
CPR*		1.68 (0.89-3.57)	0.92 (0.43-2.29)	**<0.001**
UCR*		0.6 (0.28-1.12)	1.09 (0.44-2.3)	**<0.001**
Uterine artery PI*		0.75 (0.42-1.42)	1.32 (0.55-2.05)	**<0.001**
Ductus venosus PI*		0.82 (0.32-1.58)	0.72 (0.32-1.58)	0.392
CPUR*		2.48 (0.66-5.64)	0.75 (0.21-2.54)	**<0.001**
Umbilical venous flow*		136.8 (33.13-180.13)	101.63 (19.94-122.75)	**<0.001**
Aortic isthmus PI*		2.31 (1.8-2.99)	2.67 (2.15-3.57)	**<0.001**
Renal artery PI*		2.12 (1.47-3.16)	2.16 (1.47-3.16)	0.304
Day between birth and last ultrasound scan*		0 (0-6)	1 (0-6)	**0.011**
Gestational age at birth (week)*		38 (36-39)	36 (33-39)	**<0.001**
Birthweight (gr)**		2542.08±265.09	1949.45±427.22	**<0.001**
APGAR 1st minute score*		8 (6-8)	6 (5-8)	**<0.001**
APGAR 5th minute score*		10 (8-10)	8 (6-10)	**<0.001**
Cord blood pH*		7.33 (7.14-7.41)	7.33 (7.12-7.49)	0.561
Cord blood base excess*		-4.2 (-13.5-8.7)	-3.3 (-16.3-15.1)	0.729
Cord blood pO_2**		21.14±7.39	23.08±10.63	0.740
Cord blood pCO_2**		40.35±7.17	42.53±10.26	0.189
Duration of hospital stay in NICU (day)*		0 (0-0)	9.5 (0-54)	**<0.001**
FGR in obstetric history***		16 (20.78)	16 (25.00)	0.694
Smoking***		5 (6.49)	5 (7.81)	1.000
Hypertension***		16 (20.78)	20 (31.25)	0.220
BPD percentile ***	**<3p**	42 (54.55)	37 (57.81)	0.544
	3p-10p	12 (15.58)	6 (9.38)	
	>10p	23 (29.87)	21 (32.81)	

HC percentile* **	**<3p**	52 (67.53)	41 (64.06)	0.644
	3p-10p	9 (11.69)	11 (17.19)	
	>10p	16 (20.78)	12 (18.75)	
AC percentile* **	**<3p**	68 (88.31)	62 (96.88)	0.111
	3p-10p	9 (11.69)	2 (3.13)	
	>10p	0 (0.00)	0 (0.00)	
FL percentile* **	**<3p**	62 (80.52)	39 (60.94)	**0.021**
	3p-10p	7 (9.09)	16 (25.00)	
	>10p	8 (10.39)	9 (14.06)	
EFW percentile* **	**<3p**	36 (46.75)	58 (90.63)	**<0.001**
	3p-10p	41 (53.25)	6 (9.38)	
	>10p	0 (0.00)	0 (0.00)	
Meconium stained amniotic fluid* **		6 (7.79)	21 (32.81)	**<0.001**
Cesarean section for fetal distress* **		14 (18.18)	31 (48.44)	**<0.001**
Labor induction* **		27 (35.06)	5 (7.94)	**<0.001**
Mode of delivery* **	**Vaginal Delivery**	9 (11.69)	0 (0.00)	**0.004**
	Cesarean section	68 (88.31)	64 (100.00)	
Gender* **	**Female**	44 (57.14)	29 (45.31)	0.162
	Male	33 (42.86)	35 (54.69)	
Need for active resuscitation at birth* **		0 (0.00)	5 (7.81)	**0.018**
Neonatal respiratory support* **		2 (2.60)	38 (59.38)	**<0.001**
Neonatal mortality* **		0 (0.00)	1 (1.64)	0.445

* Median (Min-Max) **Mean ± SD ***n (%)
BPD: Biparietal diameter, HC: Head circumference, AC: Abdominal circumference, FL: Femur length, EFW: Estimated fetal weight, FGR: Fetal growth restriction, S/D: Systole/diastole, PI: Pulsatility index, RI: Resistive index, MCA-PSV: Middle cerebral artery-Peak Systolic Velocity, CPR: Cerebroplacental ratio, UCR: Umbilicocerebral ratio, CPUR: Cerebroplacentouterine ratio, pO_2: Partial oxygen pressure, pCO_2: Partial pressure of carbon dioxide, NICU: Neonatal Intensive Care Unit. Statistically significant p values are indicated in bold

ROC analysis was performed to determine the performance of the measured obstetric Doppler parameters in predicting the development of adverse neonatal outcomes. While umbilical venous blood flow showed the best performance in predicting adverse neonatal outcomes (AUC 0.952, 95% CI 0.902-0.981, p<0.001), ductus venosus PI and renal artery PI had no predictive value for adverse neonatal outcomes (AUC 0.542, 95% CI 0.456-0.627, p=0.398 and AUC 0.551, 95% CI 0.464-0.635, p=0.303 respectively) (Figures 1 and 2). Additionally, UCR, CPUR, and aortic isthmus PI values, which were evaluated for predicting the development of adverse neonatal outcomes, were also found to have predictive values (AUC 0.853, 95% CI 0.783-0.907, p<0.001; AUC 0.912, 95% CI 0.853-0.953, p<0.001, and AUC 0.829, 95% CI 0.756-0.887, p<0.001, respectively). ROC analysis of Doppler parameters in predicting adverse neonatal outcomes is summarized in Table 5. The ROC curves of Doppler parameters evaluated for prediction of adverse neonatal outcomes are shown in Figures 1 and 2.

Discussion

This study demonstrated that UA PI, MCA PI, mean Ut A PI and the CPR, UCR and CPUR ratios derived from the ratio of these parameters to each other were effective in predicting composite adverse neonatal outcomes, such as Apgar score less than 7 at 5 min, cord blood pH less than 7.10, or the need for neonatal intensive care in fetuses with late-onset FGR. Additionally, when all obstetric Doppler parameters were compared, umbilical venous blood flow showed the best performance in predicting adverse neonatal outcomes, whereas aortic isthmus PI, which is not commonly used in routine clinical practice, proved effective in predicting adverse neonatal outcomes in fetuses with late-onset FGR. The study found that Doppler assessment of the ductus venosus, a critical aspect of the management of fetuses with early-onset FGR, did not predict adverse neonatal outcomes in fetuses with late-onset FGR. Additionally, renal artery PI was ineffective in predicting composite adverse neonatal outcomes. Late-onset FGR occurs when the fetus fails to attain its growth potential and is typically identified after the 32nd week of gestation[4]. Although there are fewer perinatal complications

Table 5. ROC analysis of Doppler parameters in the prediction of adverse neonatal outcomes

Variables	Cut-off	Sensitivitity	Specificity	LR+	LR-	PPV	NPV	AUC (95%CI)	p-value
UmbAS/D	3.45	78.12 (66.0-87.5)	92.21 (83.8-97.1)	10.03	0.24	89.3	83.5	0.843 (0.772-0.944)	**<0.001**
UmbA PI	1.23	75.00 (62.6-85.0)	97.4 (90.9-99.7)	28.87	0.26	96.0	82.4	0.853 (0.783-0.907)	**<0.001**
UmbA RI	0.72	70.31 (57.6-81.1)	94.81 (87.2-98.6)	13.54	0.31	91.8	79.3	0.815 (0.741-0.875)	**<0.001**
MCA PI	1.33	59.38 (46.4-71.5)	85.71 (75.9-92.6)	4.16	0.47	77.6	71.7	0.715 (0.633-0.788)	**<0.001**
CPR	1.13	68.75 (55.9-79.8)	94.81 (87.2-98.6)	13.23	0.33	91.7	78.5	0.852 (0.783-0.906)	**<0.001**
UCR	0.82	71.87 (59.2-82.4)	93.51 (85.5-97.9)	11.07	0.30	90.2	80.0	0.853 (0.783-0.907)	**<0.001**
Ut A PI	1.09	67.19 (54.3-78.4)	93.51 (85.5-97.9)	10.35	0.35	89.6	77.4	0.860 (0.792-0.913)	**<0.001**
DV PI	0.74	53.97 (40.9-66.6)	59.74 (47.9-70.8)	1.34	0.77	52.3	61.3	0.542 (0.456-0.627)	0.398
CPUR	1.02	76.56 (64.3-86.2)	97.4 (90.9-99.7)	29.48	0.24	96.1	83.3	0.912 (0.853-0.953)	**<0.001**
UmbV flow	118.9	92.19 (82.7-97.4)	92.21 (83.8-97.1)	11.83	0.08	90.8	93.4	0.952 (0.902-0.981)	**<0.001**
AoI PI	2.5	71.87 (59.2-82.4)	83.12 (72.9-90.7)	4.26	0.34	78.0	78.0	0.829 (0.756-0.887)	**<0.001**
RenAPI	1.99	69.84 (57.0-80.8)	44.16 (32.8-55.9)	1.25	0.68	50.6	64.2	0.551 (0.464-0.635)	0.303
MCA-PSV	52.0	53.97 (40.9-66.6)	58.44 (46.6-69.6)	1.30	0.79	51.5	60.8	0.541 (0.455-0.626)	0.401

LR: Likelihood ratio, PPV: Positive predictive value, NPV: Negative predictive value, AUC: Area under curve, UmbA: Umbilical artery, S/D: Systole/diastole, PI: Pulsatility index, RI: Resistive index, MCA: Middle cerebral artery, PSV: Peak systolic velocity, CPR: Cerebroplacental ratio, UCR: Umbilicocerebral ratio, CPUR: Cerebroplacentouterine ratio, DV: Ductus venosus, Umb V: Umbilical vein, AoI: Aortic isthmus, RenA: Renal Artery. Statistically significant p values are indicated in bold

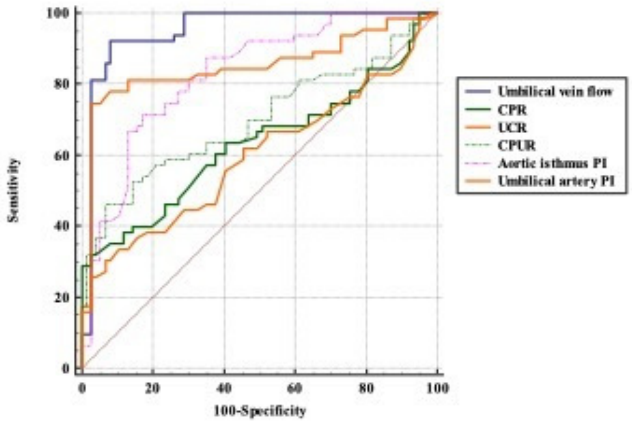

Figure 1. ROC curves showing predictive value of umbilical vein flow, CPR, UCR, CPUR, aortic isthmus PI, and UA PI for adverse neonatal outcomes

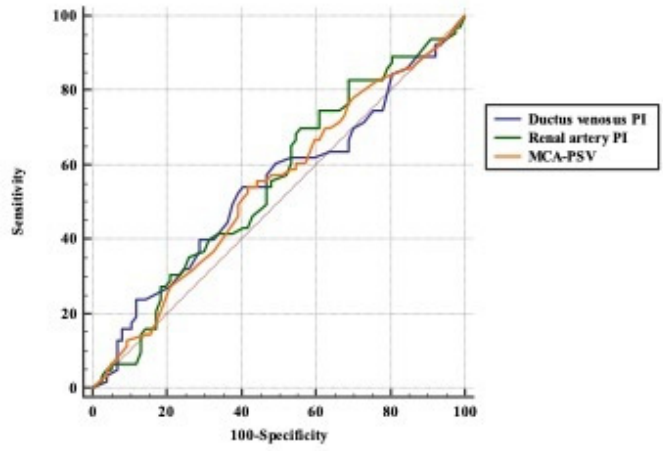

Figure 2. ROC curves showing the predictive value of ductus venosus PI, renal artery PI, and middle cerebral artery PSV for adverse neonatal outcomes

in late-onset FGR compared with early-onset FGR, there is still a greater risk of adverse short- and long-term outcomes, such as hypoxemic episodes and minor neurodevelopmental delays, compared with fetuses that are growing normally[7]. Identification of fetuses at a higher risk of adverse perinatal outcomes is critical to improving the outcome of pregnancies with late-onset FGR. Several Doppler indices have been associated with adverse outcomes in pregnancies affected by late-onset FGR. A recent study showed that assessing umbilical venous blood flow may be a more effective way of identifying fetuses with late FGR who are at a greater risk of adverse perinatal outcomes. However, the precise role of Doppler ultrasound in predicting outcomes in pregnancies affected by late FGR remains to be determined. The results of this study suggest that umbilical venous blood flow is the most effective obstetric Doppler parameter for predicting adverse neonatal outcomes in pregnancies that are complicated by late-onset FGR. Rizzo et al.[12] recently conducted a study that evaluated 243 consecutive singleton fetuses that were affected by the late FGR. The study found that the composite rate of adverse perinatal outcomes was 32.5%. To adjust for fetal

size, the ratio of umbilical venous blood flow to the abdominal circumference (UVBF/AC) was used to measure umbilical venous blood flow. Using a multivariable logistic regression analysis, the study found that Ut A PI, CPR, and UVBF/AC were all independently associated with adverse perinatal outcomes. They found that UVBF/AC was more accurate than the mean Ut A PI and CPR in predicting composite adverse outcomes[13]. Triunfo and colleagues conducted a study on how effective various fetal Doppler parameters are in predicting perinatal outcomes in late-onset FGR and reported that umbilical venous blood flow had poor diagnostic performance for adverse perinatal outcomes. According to their report, combining all the Doppler parameters under study increased the accuracy of diagnosing adverse perinatal outcomes, but it remained considerably lower[14]. This discrepancy may be explained by variations in the study sample, which consisted of only 40 cases of FGR, inconsistencies in the definition of late FGR, and differences in reported outcomes. The measurement of blood flow in the umbilical vein using obstetric Doppler ultrasound is a challenging task, and the consistency of the reproducibility of assessment of this vessel in terms of intra- and interobserver variability is not well-documented in the existing literature. Even minor errors in the factors involved in the calculation of umbilical venous blood flow can lead to significant errors in the estimation of the absolute flow. This is particularly evident in the case of the umbilical vein diameter, as its values are divided by two and squared in the calculation of vessel area, as mentioned in the reference[15]. Despite these limitations, the clinical role of umbilical venous blood flow deserves further investigation.

Insufficient blood flow between the uterus and placenta is associated with changes in blood flow resistance throughout the fetal vasculature, uterus, and placenta. As a result, the uterine and umbilical arteries demonstrate higher resistance to placental flow, caused by uteroplacental insufficiency. In response to fetal hypoxia, the fetus adapted by redirecting blood flow to essential organs. A decrease in MCA PI indicates vasodilation of the fetal cerebral vessels[16]. The ratio of MCA PI to UA PI is known as CPR. In the third trimester, having a significantly low CPR is significantly correlated with unfavorable perinatal outcomes in small for gestational age and appropriate for gestational age fetuses[17]. Recently, our study group conducted a study that found that CPR values below the 5th percentile were better predictors of adverse neonatal outcomes than UA PI and CPR <1 in late-onset FGR pregnancies[18]. At present, CPR appears to be the most promising method for identifying high-risk fetuses in the late stages of pregnancy, but its clinical application is not uniform. Low CPR values have been associated with stillbirth, even after adjustment for fetal size, and cerebral vasodilation has been shown to be a more sensitive indicator of placental insufficiency than elevated UA-PI[19]. This study revealed that in cases of late-onset FGR pregnancies, the CPR, UCR, and CPUR ratios, calculated from the UA PI, MCA PI, and mean Ut A PI values, were all able to predict composite adverse neonatal outcomes, such as low Apgar scores at 5 min, umbilical cord blood pH less than 7.10, and the need for neonatal intensive care. Additionally, fetuses with an abnormal CPUR were found to have a 4.5 times higher risk of adverse neonatal outcomes. Recent research aligns with our study's findings, indicating that reversing CPR to obtain UCR is a more sensitive indicator of adverse perinatal outcomes[20]. Although using inverse ratios derived from the same Doppler values, the TRUFFLE study showed a stronger association between UCR and neonatal neurodevelopmental disability[21]. Although a high UCR PI at 36 weeks' gestation is linked with perinatal mortality near term, it is not routinely measured in clinical practice. Recently, a new Doppler parameter known as CPUR has been proposed by MacDonald et al.[22], which is calculated by dividing CPR by the mean Ut A PI. This combination of Doppler measurements was found to have the strongest correlation with placental insufficiency. The researchers showed that CPUR was a more effective predictor of FGR than either mean Ut A PI or CPR alone in a prospective study that analyzed fetal growth in 347 women who had not previously given birth and were at 36 weeks' gestation. They showed that a low CPUR ratio with ~90% specificity had a sensitivity of 50% for birth weight <10th percentile, 68% for <5th percentile and 89% for <3rd percentile[22]. Although the use of these ratios is not always an optimal approach, their use in clinical practice does not preclude the clinical evaluation of a single Doppler parameter. Several of the ratios examined in this study, including UCR and CPUR, represent innovative Doppler ratios that demonstrate a significant correlation with placental insufficiency and greater sensitivity for identifying FGR than traditional Doppler parameters. If these findings can be replicated in future studies in separate subject groups, these parameters could be used to more accurately identify fetuses that are at an increased risk of stillbirth during the antenatal period, potentially leading to more effective obstetric interventions.

This investigation aimed to assess whether the Doppler indices of aortic isthmus PI and renal artery PI, in conjunction with other Doppler parameters commonly used in clinical practice, could be used to predict adverse neonatal outcomes in late-onset FGR pregnancies. The results of this analysis suggest that the PI of the aortic isthmus is a reliable predictor of adverse neonatal outcomes in pregnancies affected by late-onset FGR, whereas the PI of the renal artery provides no predictive information. The section of blood vessels between the origin of the left subclavian artery and the point where the ductus arteriosus meets the aorta is known as the aortic isthmus. Some experts have suggested that the aortic isthmus is the only arterial link between the right and left fetal circulatory systems. In summary, the circulatory pattern of the blood reflects the balance between the output of the ventricles and the impedance of the placental or cerebral vasculature. Recent experimental and clinical studies have shown that the Doppler flow pattern

observed in the aortic isthmus can indicate disturbances in fetoplacental hemodynamics and provide valuable insights into the overall circulatory dynamics of the fetal heart[23]. A prospective observational cohort study consisting of 70 singleton pregnancies with early-onset FGR by Choudhary et al.[24] found that all aortic isthmus Doppler indices, including aortic isthmus PI, were associated with adverse perinatal outcomes in early FGR. Del Río et al.[25] reported a higher incidence (41%) of aortic isthmus PI >95th percentile in severe FGR. However, the PORTO study, a large-scale investigation that included a substantial number of late-onset FGR cases, did not demonstrate any clinical benefit of aortic isthmus PI[26]. The usage of color Doppler flow imaging has simplified the examination of the renal arteries and other small fetal vessels. Typically, the fetal kidneys receive approximately 2-3% of the total cardiac output under normal circumstances. The renal arteries, along the rest of the peripheral circulation, respond to neurohumoral stimuli triggered by factors such as arterial wall constriction, acidosis, and hypoxia. The increased reactivity of renal arteries in response to changes in fetal oxygenation compared with umbilical arteries argues for their use in detecting fetuses at a risk of adverse consequences of hypoxia. Fetal renal artery Doppler blood flow velocities have been assessed in several clinical scenarios, such as FGR. Stigter et al.[27] carried out an observational study using a prospective design to investigate changes in the renal circulation in preterm infants with severe FGR using Doppler ultrasound during a period of gradual deterioration in prenatal fetal well-being. The study's findings revealed that there was no association between renal artery PI and birthweight, cord blood pH, or AFI corrected for gestational age. Contag et al.[28] conducted a retrospective analysis of 9.700 ultrasound data from 2.852 pregnant women aged 20-40 weeks, but they found that renal artery Doppler indices did not improve the detection of fetuses that would develop any component of adverse neonatal outcomes. These findings suggest that, while studies have indicated that altered renal blood flow is associated with hemodynamic changes in fetuses with complicated FGR, renal artery Doppler PI is unlikely to improve the prediction of fetuses at a high risk of adverse neonatal outcomes in late-onset FGR pregnancies.

Study Limitations

While our investigation provides valuable insights, it is important to acknowledge some limitations, such as the restricted sample size and the focus on a single center population. Nevertheless, the number of participants was sufficient to evaluate the efficacy of these Doppler parameters in predicting adverse neonatal outcomes. Additionally, we could not analyse placental biomarkers, so we could not build multiparametric predictive models combining these new Doppler parameters with biomarkers; however, our study was not designed to do this. A major strength is that multiple Doppler parameters in the same cohort were evaluated together by a single experienced clinician, which minimizes the bias in the results. Another strength is the prospective cohort design.

Conclusion

Although late-onset FGR is associated with lower rates of perinatal morbidity and mortality compared with early-onset FGR, the incidence of adverse outcomes such as hypoxemic events and long-term neurodevelopmental abnormalities is still higher in fetuses with late-onset FGR than in normal fetuses. To effectively manage pregnancies affected by late-onset FGR, it is crucial to identify fetuses at an increased risk of adverse perinatal outcomes. Our study evaluated the effectiveness of various Doppler blood flow parameters in predicting adverse neonatal outcomes in fetuses with late-onset FGR. In addition to MCA PI and CPR Doppler parameters, this study found that umbilical venous blood flow and aortic isthmus PI Doppler parameters, which are not commonly used in clinical settings, as well as the recently introduced UCR and CPUR ratios, were effective in predicting adverse neonatal outcomes in this group of patients. The clinical application of these Doppler parameters in fetuses with late-onset FGR appears to be of critical value in the management of these patients. Therefore, further investigation is required to develop multiparametric predictive models that integrate these novel Doppler parameters with maternal characteristics to accurately identify pregnancies affected by late-onset FGR that have an increased likelihood of short- and long-term morbidity.

Ethics

Ethics Committee Approval: The study protocol was approval of the Clinical Research Ethics Committee of Malatya University (Ethics Committee approval number: 2020/04).
Informed Consent: Informed consent was obtained from all participants before enrollment.
Peer-review: Internally peer-reviewed.

Authorship Contributions

Design: R.M., Data Collection or Processing: R.M., C.Y., Analysis or Interpretation: R.M., H.Ö., Ş.Y., Literature Search: C.Y., H.Ö., Writing: R.M., C.Y., H.Ö., Ş.Y.
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Reproductive and oncologic outcomes in women with non-epithelial ovarian cancer: Single center experience over 25 years

Non-epitelyal over kanserli kadınlarda reprodüktif ve onkolojik sonuçlar: 25 yıllık tek merkez deneyimi

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Abstract

Objective: This study aimed to present our single-center clinical experience regarding tumor clinicopathologic features, treatment modalities, and reproductive and oncologic outcomes in patients with non-epithelial ovarian cancer (NEOC) over 25 years.

Materials and Methods: A total of 100 patients with clinicopathological diagnosis of NEOC who were treated at our tertiary care center between 1996 and 2022 were included in this retrospective cohort analysis study. Data on demographic, clinical and obstetric characteristics of patients at the time of initial diagnosis as well as tumor clinicopathologic features, treatment modalities, and oncological and reproductive outcomes were recorded.

Results: NEOCs involved germ cell tumors (GCTs) in 46 (46%) patients and sex cordstromal tumors (SCSTs) in 54 (54%) patients. Thirty patients with GCTs and thirty-four patients with SCSTs possessed histological subtypes with malignant features. Most patients with GCTs (37%) and SCSTs (55.6%) had FIGO Stage 1 disease at the time of initial diagnosis. Overall, 76.6% of patients in the GCT group (n=23) underwent fertility-sparing surgery (FSS), while 76.5% of the patients in the SCST group (n=26) were treated with non-fertility-sparing surgical procedures. All patients who underwent FSS and had a recurrence in their follow-up (n=4) was stage 3 patients. Seven out of 10 patients (2 patients at stage 3 and 5 patients at stage 1) who desired pregnancy delivered between 38 and 40 gestational weeks without any congenital anomaly. The prognosis was excellent in both groups, with 5-year overall survival (OS) rates of 93.5% in GCTs and 96.3% in SCST groups. The 5-year disease-free survival was 89.1% in GCTs and 94.4% in SCSTs. FSS was not associated with worse oncologic outcomes.

Conclusion: NEOCs usually have a good prognosis because they are detected at an early stage. FSS may be indicated for women of reproductive age with early-stage NEOCs.

Keywords: Disease-free survival, fertility, non-epithelial ovarian tumor, prognosis

Öz

Amaç: Bu çalışma, non-epitelyal over kanserli (NEOC) hastalarda, tümörün klinikopatolojik özellikleri, tedavi modaliteleri ve reprodüktif ve onkolojik sonuçlarına ilişkin merkezimizin 25 yıllık klinik deneyinimi sunmayı amaçlamıştır.

Gereç ve Yöntemler: Bu retrospektif kohort analizi çalışmasına, non-epitelyal over tümörü klinikopatolojik tanısı ile 1996 ile 2022 yılları arasında üçüncü basamak bir merkezde tedavi edilen 100 hasta dahil edildi. Hastaların ilk tanı anındaki demografik, klinik ve obstetric özellikleri ile tümörün klinikopatolojik özellikleri, tedavi yöntemleri, onkolojik ve reprodüktif sonuçları kaydedildi.

Bulgular: Non-epitelyal over tümörü hastalarının 46'sında germ hücreli tümör (GHT) ve 54'ünde ise sees kord-stromal tümörü (SKST) mevcuttu. GHT'lerde otus ve SKST'lerde otuz dört hasta, malign özelliklere sahip histolojik alt tipler sahipti. GHT (%37) ve SCST (%55,6) hastalarının çoğu ilk tanı anında FIGO ever 1 hastalığa sahipti. GHT hastalarının %76,6'sına (n=23) fertility koruyucu tümör rezeksiyonu (FSS) ve SKST'li hastaların yaklaşık %76,4'üne (n=26) fertility koruyucu olmayan cerrahi işlemler uygulandı. FSS uygulanan ve takiplerinde nüks gelişen hastaların tamamı (n=4) ever 3'teki hastalardı. Gebelik elde etmek isteyen 10 hastadan 7'si (2 hasta ever 3 ve 5 hasta ever 1) 38-40 hafta arasında doğum yaptı, konjenital anomali saptanmadı. Her iki grupta da

PRECIS: We aimed to report a single-center experience in non-epithelial malignant ovarian tumors by presenting different clinical and pathological characteristics, management, and reproductive and oncologic outcomes.

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prognoz mükemmel olup, 5 yıllık genel sağkalım GHT'de %93,5 ve SKST'de %96,3 idi. Beş yıllık hastalıksız sağkalım ise GHT'lerde %89,1 ve SKST'lerde %94,4 idi. FSS daha kötü onkolojik sonuçlarla ilişkili değildi.

Sonuç: NEOC, genellikle erken evrede tespit edildikleri için iyi bir prognoza sahiptir. Erken evre non-epitelyal over tümörleri plan fertil yaştaki kadınlarda FSS yapılabilir.

Anahtar Kelimeler: Hastalıksız sağkalım, fertilite, non-epitelyal over tümörü, prognoz

Introduction

Ovarian cancer is considered the gynecologic cancer with the highest associated mortality because most patients are already at an advanced disease stage at diagnosis[1]. Epithelial ovarian cancers are the most common type, while non-epithelial primary tumors are very rare entities accounting for 10% of all ovarian malignancies (0.25/100.000)[2,3]. Non-epithelial ovarian cancers (NEOCs) include germ cell tumors (GCTs), sex cord-stromal tumors (SCSTs), sarcomas, and small cell carcinoma of hypercalcemic type[4]. Malignant GCTs represent 5% of all ovarian cancers and SCST account for approximately 3-5% of ovarian malignancies with endocrine manifestations[5]. Both GCTs and SCSTs include a wide variety of sub-histological types along with similarities in their presentation, evaluation, management, and prognosis[6]. For GCTs, dysgerminomas and immature teratomas are the most common histological subtypes (70%), while the rarer subtypes include yolk sac tumor, embryonal carcinomas, non-gestational choriocarcinomas, and mixed germ cell tumors[4]. For SCSTs, subtypes include granulosa cell tumors (juvenile and adult type), Sertoli cell tumors and Sertoli Leydig cell tumors, fibromas, and thecomas[4]. Although each histological subtype has its own characteristics, they may resemble each other in terms of initial clinical presentation, radiological findings, and tumor markers. While SCSTs are a heterogeneous group presenting over various ages, GCTs are primarily diagnosed in adolescents and younger women[5]. Given that these tumors occur mostly in young women, maintenance of fertility is an important consideration and each patient should be evaluated individually.

NEOCs have a better prognosis than epithelial ovarian tumors because approximately 60-70% of both SCSTs and GCTs are diagnosed at a localized stage[2]. Surgery for young patients with GCTs and early-stage SCSTs should consider a fertility-sparing approach (unilateral salpingo-oophorectomy with preservation of the contralateral ovary and the uterus) without compromising the oncological management[5]. GCTs are very sensitive to platinum-based regimens, which makes patients with GCTs to be considered as proper candidates for fertility-sparing surgery (FSS) even at the advanced stage. However, the value of adjuvant chemotherapy in the setting of SCSTs remains inconclusive due to the lack of randomized trials and definitive prognostic factors[2]. Unilateral salpingo-oophorectomy can be performed in patients with stage 1 disease-deserving of fertility. Hysterectomy and bilateral salpingo-oophorectomy should be performed in postmenopausal women and in patients with advanced-stage disease[5].

Little is known about the management of women with NEOCs, possibly due to the infrequent presentation of these cancers. Some proposed treatment policies are not widely accepted[5]. Treatment should be performed depending on the patients age and histopathological type. For Stage Ia pure dysgerminoma, surgery is recommended because of the relatively low recurrence rate in these patients (15-25%)[7]. Moreover, some studies revealed that close surveillance after FSS can be used in the management of all grades of immature teratoma and all stage I dysgerminomas with reserving chemotherapy only for the relapsed cases[7,8]. All patients with stage I yolk sac tumors are treated with adjuvant treatment after surgery[9], while publications suggest close and active surveillance after the surgery[8]. The most commonly used regimen in patients with NEOCs is the bleomycin/etoposide/cisplatin (BEP) combination[10]. Stage Ia granulosa cell tumors do not require adjuvant therapy[5]. Adjuvant therapy has been administered to stage 1c patients in some studies, but its benefit remains controversial[11]. Debulking surgery followed by adjuvant chemotherapy is the most effective treatment for advanced-stage SCSTs[5].

This study aimed to evaluate clinical characteristics, tumor clinicopathological features, treatment modalities, and oncological and reproductive outcomes in NEOC patients according to histological subtypes.

Materials and Methods

A total of 100 patients with clinicopathological diagnosis of NEOC who were treated at our tertiary care center (Department of Gynecological Oncology, Akdeniz University Faculty of Medicine, Antalya, Turkey) between 1996 and 2022 were included in this retrospective cohort analysis study.

The study was conducted in accordance with the ethical principles stated in the "Declaration of Helsinki" and approved by the institutional ethics committee (Akdeniz University Clinical Research Ethics Committee - KAEK-657; date: 09.11.2022). Informed consent was obtained from each subject or their first-degree relatives (for the deceased ones).

Demographic, clinical and obstetric characteristics of patients at the time of initial diagnosis and tumor clinicopathologic features were retrieved from paper- and electronic medical records. Data on age, body mass index (BMI), clinical manifestations at the time of diagnosis, reproductive history, presence of pregnancy at the time of diagnosis, menopausal status, tumor characteristics (histopathological subtype and stage according to the International Federation of Gynecology and Obstetrics (FIGO 2014) staging classification[12], tumor size

and histological grade, serum tumor markers when available, treatment characteristics regarding the primary treatment modality, type of surgical interventions, chemotherapy (regimen, setting and the number of cycles), treatment protocols in case of recurrence, oncological outcome recurrence status, overall survival (OS), disease-free survival (DFS), reproductive outcome, congenital anomaly of offspring, and secondary malignancy were recorded. Tumors were classified according to the World Health Organization (WHO 2014) classification. Information that could not be accessed through medical reports (i.e., obstetric results and menstrual pattern) was obtained by a phone call. Patients with sarcoma and small cell carcinoma of hypercalcemic type, those with insufficient data or lack of attendance to follow-up, and those with ovarian metastasis originating from non-gynecologic primary sites were excluded from the study.

Follow-up visits for recurrence assessment were performed at 3-month intervals and 6-month intervals for the first 2 years and following years. Data on symptoms, tumor markers, and pelvic examination findings were recorded at each visit. Imaging modalities used in relapse detection were chest X-ray, pelvic ultrasound, and computed tomography (CT) or positron emission tomography CT (PET/CT). OS was defined as the time from initial diagnosis to death. DFS was defined as the interval between the date of remission and the date of the first recurrence detected. FSS was defined as the preservation of the uterus and at least part of one ovary.

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows version 23.0 (IBM Corp., Armonk, NY). For descriptive statistics, the mean, standard deviation, median, minimum-maximum values, and frequencies were used, depending on the normality of the data. Data were expressed as mean ± standard deviation (SD), median and n (%) where appropriate. Survival analysis was performed via Kaplan-Meier analysis.

Results

Overall, GCTs and SCSTs were noted in 46 (46%) and 54 (54%) patients with NEOC, respectively. Baseline demographic, clinical, and obstetric characteristics and tumor clinicopathologic features of patients are shown in Tables 1 and 2.

The mean age at diagnosis was 31.7 years (range, 11 to 63 years) in patients with GCT, while it was 52.8 years (range, 13 to 77 years) in those with SCST. Thirty patients in the GCT group and 34 patients in the SCST group possessed histological subtypes with malignant features. The most common subtypes of GCTs were mature teratoma (32.6%) and dysgerminoma (23.9%). Among SCSTs, the most common subtype was adult granulosa cell tumor (53.7%), followed by fibroma (27.8%). Acute abdominal pain was the key clinical presentation in 65.2% of patients with GCTs and in 40.7% of patients with SCSTs. The majority of patients with GCTs were premenopausal (78.3%), while the majority of patients with SCSTs were postmenopausal (66.7%). Most patients with GCTs (37%) and SCSTs (55.6%) had FIGO Stage 1 disease at the time of initial diagnosis. None of the patients presented with FIGO Stage 4 disease. The mean value of Ca-125 was 95.6 IU/mL in GCT patients (n=37) and 57.6 IU/mL in SCST patients (n=43).

Treatment modalities and oncological outcome in patients with malignant NEOCs are demonstrated in Table 3. Overall, 76.6% of patients in the GCT group (n=23) underwent fertility-sparing tumor resection (FSS), while 76.5% of the patients in the SCST group (n=26) were treated with non-fertility-sparing

Table 1. Baseline demographic, clinical and obstetric characteristics and tumor clinicopathologic features in patients with germ cell tumor (n=46)

	Germ cell tumors (n=46)
Age (years), mean ± SD	31.7±14.6
BMI (kg/m²), mean ± SD	24.2±5.9
Gravidity/Parity, mean	1.1/0.9
Menopausal status, n (%)	
Premenopausal	36 (78.3)
Postmenopausal	10 (21.7)
Initial complaint (abdominal pain), n (%)	30 (65.2)
Tumor type, n (%)	
Malignant	30 (65.3)
Benign	16 (34.7)
Histology subtype, n (%)	
Dysgerminoma	11 (23.9)
Immature teratoma	6 (13)
Yolk sac tumor	2 (4.3)
Mixed GTCs	3 (6.5)
Mature teratoma	15 (32.6)
Somatic-type tumors associated with teratoma*	4 (8.7)
Monodermal, teratoma**	3 (6.5)
Gonadoblastoma	2 (4.3)
FIGO stage, n (%)	
I	17 (37)
II	2 (4.4)
III	11 (23.9)
IV	0 (0)

*Somatic-type tumors associated with teratoma include three cases of squamous-cell carcinoma arising from mature cystic teratoma, one case of carcinoid tumor. **Monodermal teratomas included two cases of PNET and one cases of benign struma ovary, SD: Standard deviation, BMI: Body mass index, GTC: Germ cell tumor

Table 2. Baseline demographic, clinical and obstetric characteristics and tumor clinicopathologic features in patients with sex cord stromal tumor (n=54)

	Sex cord-stromal tumors (n=54)
Age (years), mean ± SD	52.8±13.9
BMI (kg/m²), mean ± SD	27.2±6.2
Gravidity/Parity, mean	3.9/2.7
Menopausal status, n (%)	
Premenopausal	18 (33.3)
Postmenopausal	36 (66.7)
Initial complaint (abdominal pain), n (%)	22 (40.7)
Tumor type, n (%)	
Malignant	34 (62.9)
Benign	20 (37.1)
Histology subtype, n (%)	
Adult granulosa cell tumor	29 (53.7)
Juvenile granulosa cell tumor	1 (1.8)
Sertoli-Leydig cell tumors	3 (5.6)
Sex cord tumor with annular tubules (SCTAT)	1 (1.9)
Fibroma	15 (27.8)
Thecoma	5 (9.3)
FIGO stage	
I	30 (55.6)
II	2 (3.7)
III	2 (3.7)
IV	0 (0)

SD: Standard deviation, BMI: Body mass index

surgical procedures. FSS was applied in 29 (45.3%) patients overall, including unilateral salpingo-ovariectomy in 25 (39%) patients, cystectomy in 2 (3.1%) patients, and bilateral salpingo-ovariectomy in 2 (3.1%) patients. Adjuvant therapy was indicated in 8 patients with SCST and 24 patients with GCT. Most patients in the GCT group received bleomycin, etoposide, and cisplatin (BEP) combination chemotherapy for median 2.7 cycle. Other rarely administered chemotherapeutics were paclitaxel, carboplatin, vincristine, doxorubicin and cyclophosphamide, followed by ifosfamide and etoposide, 5-FU and prednisolone.

The median duration of follow-up was 90 months (range, 3 to 324 months) and 83.5 months (range, 8 to 252 months) for malignant GCTs and SCSTs, respectively. Overall, 11 of 64 (17.1%) patients with malignant NEOC developed recurrence, including 6 cases with GCTs and 5 cases with SCSTs. Most of the recurrences were detected in the abdomen (8 of 11 patients)

Table 3. Treatment modalities and oncological outcome in patients with malignant non-epithelial ovarian cancers

	Germ cell tumors	Sex cord-stromal tumors
Surgery type, n (%)		
Fertility-sparing	23 (76.6)	8 (23.5)
Non-fertility-sparing	7 (23.4)	26 (76.5)
Adjuvant chemotherapy		
BEP regimen, n (%)	19 (41.3)	2 (3.7)
Other regimens, n (%)	5 (10.9)	6 (11.2)
Number of cycles, median	2.7	1
Recurrence treatment, n (%)		
Exclusive surgery	2	0
Exclusive chemotherapy	1	0
Surgery and chemotherapy	1	1
Median follow-up (months)	90	83.5
Oncological outcome		
Recurrence, n (%)	6 (20)	5 (14.7)
5-year DFS rate (%)	89.1	94.4
5-year OS rate (%)	93.5	96.5

BEP: Bleomycin-etoposide-cisplatin, DFS: Disease-free survival, OS: Overall survival

and most patients underwent a second surgery followed by chemotherapy (7 of 11 patients). GCT was the diagnosis in three out of five patients with mortality. The 5-year OS rates were 93.5% and 96.3% in the GCTs and SCSTs groups, respectively (Figure 1). The 5-year DFS rate was 89.1% in patients with GCTs and 94.4% in those with SCSTs (Figure 2).

There were thirty-one patients younger than 40 years who had a final pathology result reported as malignant. Of these 31 patients, 17 were nulliparous, 18 were married, and 13 were single. The chemotherapy regimens included BEP in 16 patients, VIP (etoposide, ifosfamide, cisplatin) in 2 patients, and a combination of cyclophosphamide, 5-FU, and prednisolone in one patient with a Sertoli-Leydig cell tumor, while 12 patients did not receive any chemotherapy as they were diagnosed at stage 1. FSS was not performed only for 2 patients in this group because they did not have a desire for pregnancy. All patients who underwent FSS and had a recurrence in their follow-up (n=4) were stage 3b or 3c, and unfortunately one of them died due to disseminated disease. Seven out of 10 patients (two patients at stage 3 and five patients at stage 1) who desired pregnancy delivered full-term babies (n=9) between 38 and 40 gestational weeks with no congenital anomalies. The pregnancy rate was 70%, and none of the pregnancies were with assisted reproductive technology (ART) (Table 4). The median interval between surgery and delivery was 24 months (range, 9 to 156 months). No recurrence occurred in these patients. None of the

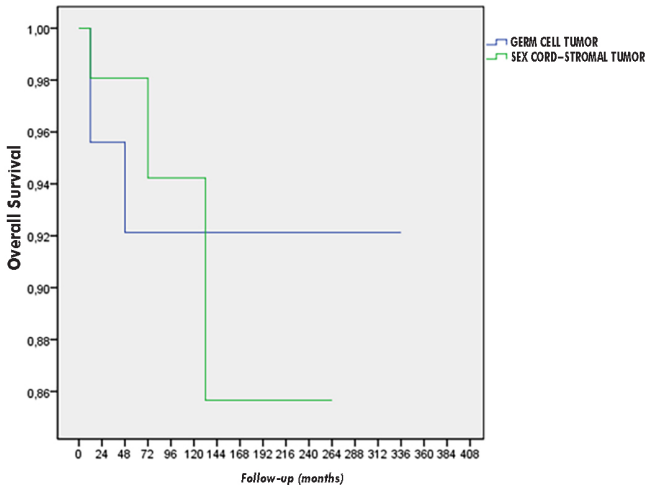

Figure 1. Kaplan-Meier curves for five-year overall survival (OS) in women with GCTs and SCSTs

GCTs: Germ cell tumors, SCSTs: Sex cordstromal tumors

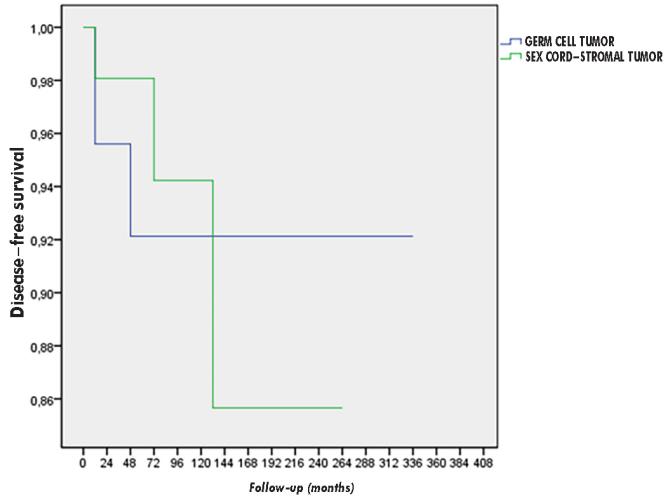

Figure 2. Kaplan-Meier curves for five-year disease-free survival (DFS) in women with GCTs and SCSTs

GCTs: Germ cell tumors, SCSTs: Sex cordstromal tumors

patients had undergone completion surgery after childbearing. Twenty patients did not try to get pregnant after fertility-preserving procedures.

Three women were pregnant at the time of diagnosis; the histological types in these patients were Sertoli-Leydig cell tumor (stage 1a), dysgerminoma (stage 1c2), and immature teratoma (stage 1c1). Fertility-preserving surgery including unilateral salpingo-oophorectomy and complete surgical staging was performed for treating these patients.

Discussion

NEOCs are considered to be diagnosed at an early age and to have a good prognosis in relation to the excellent chemotherapy response[13]. NEOCs include ovarian GCTs and SCSTs, and both groups have benign and malignant forms[13]. In this study, clinical and treatment characteristics and oncological and reproductive outcomes of GCTs and SCSTs were assessed in our series of NEOC patients. Moreover, the oncologic outcomes were also evaluated specifically among women undergoing FSS, which has been addressed only by a few studies to date[14-27].

NEOCs are relatively rare forms of ovarian cancer that occur mostly in women of childbearing age, except for granulosa cell tumors, which have a wide age spectrum including both premenopausal and postmenopausal women. Our findings support the data from previous studies with NEOC patients indicating overall good obstetric and survival outcomes along with no recurrences in women undergoing FSS even at the advanced stage[28]. Studies on fertility preservation surgery are mainly conducted in the setting of GCTs[13]. FSS did not adversely affect recurrence rates in all reviewed studies, and therefore, it is recommended as the gold standard surgical management of patients with early-stage GCTs[29]. Johansen et al.[15] indicated that the ability to conceive was preserved by using FSS since all conceptions were natural and all deliveries occurred at full term in their study. The pregnancy rate varies from 50% to 93%, and the live birth rate ranges from 65% to 95%[19,20,25,30-35].

The pregnancy rate (70%) in our study was similar to that in previous studies. Literature data on FSS outcomes in women

Table 4. Pregnancies achieved after fertility-sparing operation according to the tumor type

Patient no	Type of tumor	Stage	CT	Time to pregnancy (month)	Mode of delivery	Gestational week	Congenital anomaly	Recurrence
1	Dysgerminoma	1a	BEP	50	NVD	40	None	No
2	Dysgerminoma	3a1	BEP	71	CS	40	None	No
3	Granulosa cell tumor	1c1	None	11	NVD	40	None	No
4	Sertoli-Leydig	1a	None	9	CS	40	None	No
5	Dysgerminoma	1c2	BEP	24	CS	39	None	No
6	Immature teratoma	3c	BEP	156	CS	39	None	No
7	Dysgerminoma	1c2	BEP	23	CS	38	None	No

BEP: Bleomycin-etoposide-cisplatin, NVD: Normal vaginal delivery, CS: Cesarean section, CT: Chemotherapy

with SCSTs are scarce and mainly based on case reports or short series[36-40]. In a systematic review by Bercow et al.[14], FSS was considered not to be associated with worse DFS or OS compared to conventional surgery. There is a scarce amount of data regarding the fertility and pregnancy outcomes of granulosa cell tumors because these tumors are very rare and their peak incidence is in the perimenopausal period. In a review of a few retrospective studies on fertility-sparing management and pregnancy in patients with granulosa cell tumor by Iavazzo et al.[38], the authors recommended FSS to be performed only in well-selected patients after their informed consent. Some authors also reported no significant difference between FSS and radical surgery in terms of survival outcome[41]. In our study, most of the women who delivered were in the GCTs group, in accordance with consideration of GCTs rather than SCSTs to be more common in the reproductive age. Notably, chemotherapy was not considered to have a negative effect on fertility in NEOC patients[42]. Various combined regimens including vincristine, dactinomycin, cyclophosphamide, bleomycin, etoposide, cisplatin, doxorubicin, and vinblastine have been used after FSS, revealing satisfactory results on conception and pregnancy rates after chemotherapy exposure[18,35,42-49]. Most of our patients who delivered also received chemotherapy. Meanwhile, pregnancy or even delivery after completing chemotherapy may not affect recurrence or mortality[34].

Supporting the previously reported series, the survival outcome in our study confirms the overall good prognosis of ovarian non-epithelial tumors. Park et al.[30] found the 5-year DFS and OS rates for GCTs to be 86% and 97%, respectively. Malignant SCSTs carry a favorable prognosis with a 5-year OS of 97.2%[34]. Due to related high rates of recurrence and mortality, OS of advanced-stage disease, especially in SCSTs, is poor[34].

The type of surgery, patient age at the time of investigation, patient desire to conceive, fear of recurrence, and tumor histologic subtype are considered amongst the factors with considerable impact on fertility rates. Bilateral salpingo-oophorectomy and uterine conservation enable pregnancy by egg donation for women with gonadoblastoma. However since the oocyte donation is illegal in our country, preservation of the uterus does not increase fertility rates. Unfortunately, two patients with gonadoblastoma in our series could not have children due to this restriction, despite their desire for pregnancy.

Nonetheless, the conception rate may increase in the longer term. Some patients in our series did not try to conceive despite having FSS, possibly due to reasons such as prediction of good outcomes after fertility preservation and high chemotherapy response in case of recurrence[13]. Although these reasons appear to be highly acceptable for GCTs, they should be discussed in detail with patients have SCSTs.

Patients of reproductive age with NEOC should have access to professional family planning and infertility counseling to discuss fertility outcomes and treatment options[50]. Although the exact numbers of our patients who received presurgical family planning counseling and visited a reproductive medicine specialist are unknown, obstetric outcomes may be better if adequate counseling is given to these patients[13].

To reduce the risk of recurrence, completion surgery should be discussed with women who no longer intend to conceive. However, due to high curability rates, completion surgery after childbearing may not be necessary for GCTs. The use of completion surgery after childbearing remains debatable in SCSTs[51]. This decision may be personalized because there are still uncertainties regarding the long-term outcomes after this type of surgery[50]. The patients must be fully informed about oncological and obstetrical outcomes.

Study Limitations

The major limitations of this study seem to be retrospective single center design and small sample size of the cohort in relation to the rarity of these tumors, which prevented the conduction of reliable subgroup analyses with respect to different tumor histological subtypes. Also, our results regarding the obstetric outcomes after FSS should be interpreted with caution given the likelihood of a large sample to provide more reliable results. Furthermore, reproductive potential, which is a multifactorial phenomenon with considerable interindividual differences, was not detailed in our study.

Conclusion

In conclusion, FSS seems to be a potentially favorable surgical modality in the setting of NEOC for young women who intend to conceive. It can be offered to patients even at advanced disease stages, particularly in those with GCTs, depending on tumor histopathology and prognostic factors. Recurrence is considered to be rare in general, while it develops more frequently at advanced disease stages. Adjuvant chemotherapy does not seem to affect fertility outcomes. Larger prospective studies are needed to better evaluate long-term oncologic and reproductive outcomes in women with ovarian cancer undergoing FSS.

Ethics

Ethics Committee Approval: The study was conducted in accordance with the ethical principles stated in the "Declaration of Helsinki" and approved by the institutional ethics committee (Akdeniz University Clinical Research Ethics Committee - KAEK-657; date: 09.11.2022).

Informed Consent: Informed consent was obtained from each subject or their first-degree relatives (for the deceased ones).

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: S.S., C.K., H.A.T., S.D., T.Ş., Concept: S.S., C.K., H.A.T., S.D., T.Ş., Design: S.S., C.K., H.A.T., S.D., T.Ş., Data Collection or Processing: S.S., C.K., H.A.T., S.D., T.Ş., Analysis or Interpretation: S.S., C.K., H.A.T.,

S.D., T.Ş., Literature Search: S.S., C.K., H.A.T., S.D., T.Ş., Writing: S.S., C.K., H.A.T., S.D., T.Ş.

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Gestational trophoblastic neoplasia of intermediate trophoblasts: Epithelioid trophoblastic tumor and placental site trophoblastic tumor, a study of morphologic, immunohistochemical, and next generation sequencing

İntermediate trofoblastların gestasyonel trofoblastik neoplazisi: Epitelioid trofoblastik tümör ve plasental site trofoblastik tümörlerin morfolojik, immünohistokimyasal ve yeni nesil dizileme çalışması

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Abstract

Objective: Gestational trophoblastic tumors are very rare neoplasms. We determined the distinctive morphological, immunohistochemical, and clinical features of placental site trophoblastic tumors (PSTT) and epithelioid trophoblastic tumors (ETT) in our cohort.

Materials and Methods: Nine cases of PSTT and four cases of ETT were retrieved from the archives. Histomorphologic, immunohistochemical, and clinical features were noted. A molecular study was performed on one PSTT and one ETT case using next-generation sequencing.

Results: While the nodular pattern, geographic necrosis, and extracellular eosinophilic globules were peculiar to ETTs, vessel wall affinity, marked pleomorphism, intranuclear pseudoinclusion, spindle tumor cell, and vacuolar degeneration were more specific for PSTTs in our series. An immunohistochemical panel of p63, hPL, and CD146 were helpful for the exact typing of the tumor. p63 positivity supports the ETT and diffuse staining of hPL and CD146 supports the PSTT diagnosis. Three of the patients with metastatic disease (lung and brain metastasis) except one have a high mitotic count (12 and 8) and a long interval between (8 and 10 years) antecedent pregnancy and diagnosis. While KIT and TP53 mutations were observed only in PSTT, amino acid changes in KDR, APC, and SMAD4 genes were detected both in the ETT and PSTT cases.

Conclusion: In the prediction of metastasis, the long intervals between antecedent pregnancy and diagnosis, deep myometrial invasion, mitotic count, and Ki67 proliferation index were involved rather than other histomorphological parameters, but none of the parameters is an absolute predictor of the metastasis.

Keywords: Epithelioid trophoblastic tumor, gestational trophoblastic neoplasia, immunohistochemistry, placental site trophoblastic tumor, prognosis

PRECIS: In this cohort, we evaluate the distinctive morphological, immunohistochemical, and clinical features of PSTT and ETT which are quite rare gestational trophoblastic neoplasms.

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Öz

Amaç: Bu çalışmanın amacı nadir görülen gestasyonel trofoblastik neoplaziler olan Plasental Site Trofoblastik Tümör (PSTT) ve Epiteloid trofoblastik tümör (ETT) için ayırt edici klinik, morfolojik, immünohistokimyasal ve moleküler genetik özellikleri belirlemektir.

Gereç ve Yöntemler: Hacettepe Üniversitesi'nde 2001-2020 yılları arasında tanı alan 9 PSTT ve 4 ETT olgusu incelenmiş olup karakteristik histomorfolojik, immünhistokimyasal ve klinik özellikler kaydedilmiştir. Bir PSTT ve bir ETT olgusuna uygulanmış olan yeni nesil dizileme çalışmasının verileri değerlendirilmiştir.

Bulgular: Morfolojik olarak nodüler patern, coğrafi nekroz ve ekstraselüler eozinofilik globüller ETT'ye özgü iken; damar duvarı afinitesi, belirgin pleomorfizm, intranükleer psödoinklüzyonlar, iğsi tümör hücreleri ve vakuolar dejenerasyon PSTT'ye daha spesifiktir. İmmünohistokimyasal olarak p63 pozitifliği ETT'yi, hPL ve CD146'nın yaygın boyaması PSTT'yi desteklemektedir. p63, hPL ve CD146'dan oluşan bir panelin kullanılmasının tümörün tiplendirilmesi için oldukça yardımcı olabileceği görülmüştür. KIT ve TP53 mutasyonları sadece PSTT'de gözlenirken, hem ETT hem de PSTT olgusunda KDR, APC ve SMAD4 genlerinde amino asit değişiklikleri tespit edilmiştir. İzleminde metastatik hastalığı olan üç hastanın biri hariç diğerlerinde yüksek mitoz sayısı (12 ve 8) ve bununla korele olarak yüksek Ki67 proliferasyon indeksi (%28 ve %30), derin myometrial invazyon ve önceki gebelik ile tanı arasında uzun bir zaman aralığı (8 ve 10 yıl) olduğu kaydedilmiştir.

Sonuç: ETT ve PSTT'lerde kötü prognoz ve metastaz tahmininde, histomorfolojik parametrelerden ziyade, önceki gebelik ile tanı arasında uzun bir zaman aralığı olması, derin myometriyal invazyon, mitotik aktivite ve Ki67 proliferasyon indeksi önem taşımaktadır; ancak hiçbir parametre metastaz için mutlak bir belirleyici değildir.

Anahtar Kelimeler: Epiteloid trofoblastik tümör, gestasyonel trofoblastik neoplazi, immünohistokimya, plasental site trofoblastik tümör, prognoz

Introduction

The World Health Organization classification of gestational trophoblastic disease (GTD) includes complete and partial hydatidiform mole, invasive hydatidiform mole, choriocarcinoma (CC), placental site trophoblastic tumor (PSTT), epithelioid trophoblastic tumor (ETT), exaggerated placental site, and placental site nodule. Neoplastic forms of GTDs are called gestational trophoblastic neoplasms (GTNs) [1]. This group includes CC, PSTT, and ETT[2]. Among them, PSTT and ETT arising from intermediate trophoblasts (IT) are much rarer and a limited number of reported cases are present in the literature, most of them are case reports and their exact incidence is unknown[3-6].

PSTT is formed by the neoplastic transformation of the implantation site IT, whereas ETT occurs by the neoplastic transformation of chorionic type IT. Although the histopathological examination shows that they have similar morphology with the type of IT they originate, these morphological findings may not always be recognizable in curettage specimens, which contain a limited amount of tumor samples. Accurate recognition of these tumors, especially differentiating them from choriocarcinoma and the benign mimics of GTN, is critical for the patient to receive the appropriate treatment. Although most of the PSTT and ETT behave benignly, in some series, approximately 15-25% of patients had recurrence and metastasis, and half of them died from tumor, but prognostic morphologic and immunohistochemical parameters predictive of these tumors could not be established[7,8]. Because of having difficulty making an accurate diagnosis for these rare tumors, in most countries, GTDs are diagnosed and treated in certain centers by gynecologic pathologists and oncologists who are particularly interested in GTDs[9].

In our study, we determined the distinctive morphological, immunohistochemical, and clinical features of PSTT and ETT in our cohort. Furthermore, we assessed common mutations in GTNs arising from ITs by using next-generation sequencing (NGS) in two cases.

Materials and Methods

The Hacettepe University Non-interventional Research Ethics Committee approved this study (the Ethics board approval number is 2022/12-13). All patients consented to the research and publications.

Patient selection: Nine PSTT and four ETT cases were retrieved from the archives of the department of pathology, Hacettepe University diagnosed between 2001 and 2020. Seven of 13 cases were sent to our department for a second opinion. Therefore, we have limited clinical information about these patients. Nevertheless, some patients' survival information was learned by telephone recall.

Histomorphological evaluation: Only archive materials were used and no new study was performed. All hematoxylin-eosin (H&E) and immunohistochemically stained slides were reviewed. While three of the nine PSTT cases have both curettage and hysterectomy samples, four PSTT cases have only hysterectomy samples and two PSTT cases have only curettage samples. Three hysterectomy specimens and one curettage specimen of four ETT patients were reevaluated.

H&E-stained tumor slides were examined for the architecture of the tumor (discohesive cell layers, cohesive islands, nests, and single cells), characteristic invasion patterns (myometrial invasion pattern, vessel wall infiltration), ratio, and characteristics of necrosis (geographic, fibrinoid), presence of extracellular deposits (fibrinoid substance, eosinophilic globules), presence of peri-/intra-tumor lymphocyte infiltration, nuclear features (pleomorphism, nucleoli, hyperchromasia, multinucleation, bizarre nucleus, intranuclear pseudoinclusion), cytoplasmic features (round, spindle-like, polygonal cells, vacuolar degeneration, hemosiderin pigment), the mitotic activity [average mitoses that were counted in 10 consecutive high power fields (HPF)].

Immunohistochemical evaluation: Immunohistochemical stains that were performed at the time of diagnosis (CK7, inhibin, hPL, CD146, p63, beta-hCG, Ki-67) were re-examined. No additional immunohistochemical study was performed because of the absence of paraffin blocks. Cytoplasmic or membranous staining of the tumor cells was accepted to be positive for CK7, inhibin, hPL, CD146, and beta-hCG, and nuclear staining was considered positive for p63 and Ki67. The percentage of stained tumor cells was recorded by counting at least 500 cells in the sections of hysterectomy, and at least 100 cells in curettage. Additionally, the heterogeneity of staining (when the percentage of positive staining cells between the highest and least stained tumor areas differed by less than 20% it was referred to homogenous staining; whereas the difference was >20%, referred heterogeneous staining) was also evaluated.

Molecular study: A molecular study was performed on one PSTT and one ETT case. DNA was extracted from FFPE tissue samples using a QIAamp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. DNA concentration was measured using a Qubit 3.0 fluorometer and Qubit® dsDNA HS Assay kit (Thermo Fisher Scientific, Waltham, MA, USA). Ten nanograms of DNA were used for preparing amplicon libraries using IonAmpliSeq TM Library kit 2.0 (Thermo Fisher Scientific). To amplify the template, Ion AmpliSeq Cancer Hotspot Panel v2 (Life Technologies, California, USA) was used. The panel consisted of a primer pool of 207 amplicons covering 2800 hotspot mutations in 50 genes. The amplified libraries were purified using Agencourt AMPure XP beads (Beckman Coulter Genomics, High Wycombe, UK). Library concentrations were measured using Qubit® dsDNA HS Assay kit and Qubit® 3.0 Fluorometer (Thermo Fisher Scientific). The libraries were stored at -20 °C until the sequencing step was performed. The purified libraries were diluted to 100 pM and then amplified on Ion SphereTM particles (Life Technologies). The templates were prepared and enriched using the Ion OneTouch TM 2 System (Life Technologies), an automated emulsion polymerase chain reaction system. Sequencing was performed on an Ion Personal Genome Machine System (PGM TM; Life Technologies) using Ion 318 TM chips and Ion PGM TM Sequencing Hi-Q view kit v2.

Results

Clinical and laboratory findings: Clinical and laboratory findings are summarized in Table 1. The mean age of PSTT patients was 29 while it was 37 for ETT patients. All except one PSTT patient had a previous normal or molar pregnancy or abortus history. Clinical symptoms were vaginal bleeding, vaginal discharge, amenorrhea, and inguinal pain. Eleven of the 13 patients underwent hysterectomy. We don't have further clinical information about the remaining 2 patients.

Macroscopic and histomorphologic findings: Six of the 11 patients' hysterectomy specimens were examined at our center. Available macroscopic findings and mitotic counts are shown in Table 2.

Histologically, PSTTs are mostly hemorrhagic, nodular, exophytic, and/or invade the uterus wall. Nodularity was not obvious in the curettage specimens. In all PSTT cases but one, mostly discohesive, and sometimes both discohesive and cohesive cells formed cell layers/sheets. Accompanying this pattern, individual scattered cells, nests, and cell cords/trabeculae were seen. All but one case revealed smooth muscle infiltration of tumor and vascular wall involvement (Figure 1a). There was geographic type necrosis in up to 75% of the tumor. Focal or diffuse fibrinoid material accumulation and peri-intra-tumor lymphocyte infiltration were present. Spindle cells were seen in 3 cases.

Table 1. Immunohistochemical findings, number of positive staining cases, ratio, heterogeneity[a], and intensity of staining

	Positive cases (n/n)		Positive tumor cell ratio (%)		Heterogeneity of staining[a]		Staining intensity (weak-strong)	
	PSTT	ETT	PSTT	ETT	PSTT	ETT	PSTT	ETT
CK7	4/4	4/4	100	100	Homogenous	Homogenous	Strong	Strong
Inhibin	6/6	3/3	1-100	5-80	Homogenous/ heterogeneous	Homogenous/ heterogeneous	Weak/strong	Strong
hPL	8/8	4/4	60-100	1-20	Homogeneous except one	Heterogeneous	Strong	Strong
βHCG	6/7	1/4	5-30	20	Homogenous/ heterogeneous	Heterogeneous	Strong	Strong
P63	0/7	3/3	-	20-75	Negative	Homogenous/ heterogeneous	-	Strong
CD146	5/5	2/2	50-100	25-30	Homogeneous except one	Heterogeneous	Strong	Strong
Ki-67	8/8	4/4	1-30	5-80	Homogeneous except two	Homogenous	Strong	Strong

[a]: If the percentage of positive staining cells between the highest and least stained tumor areas differed less than 20% it was referred "homogenous staining"; if the difference was >20%, referred "heterogeneous staining"

Table 2. Clinical and morphological prognostic parameters, follow-up times, and metastasis status (disease-free survival: DFS, loss to follow up: LTFU)

	Diagnosis	Age	Previous pregnancy	Time since antecedent pregnancy (months)	Clinical symptoms	Beta-HCG (mIU/mL)	Tumor size (cm)	Myometrial invasion	FIGO stage	Mitotic count (10 HPF)	ki67 index	Necrosis	Follow-up time	Metastasis	Survival status
1	PSTT	26	-	-	Vaginal bleeding	-	-	-	-	2*	10%	no	7 years	no	DFS
2	PSTT	27	Abortion	3 months	Vaginal bleeding	87	2.5	-	-	2	15-20%	no	-	-	LTFU
3	PSTT	23	Molar pregnancy	12 months	Vaginal bleeding	330	5	-	-	3*	10%	yes	6 years	lung (in 4 month)	DFS
4	PSTT	35	Healthy pregnancy	30 months	Amenorrhea	17	0.5	Superficial	I	1 in curettage and hysterectomy	10%	no	-	-	LTFU
5	PSTT	33	Molar pregnancy	120 months	Vaginal discharge	78	2.4	Deep	I	3 in curettage/8 in hysterectomy	30%	no	2 years	Lung and brain (in 2 years)	LTFU
6	PSTT	34	Healthy pregnancy	12 months	Vaginal bleeding	210	5.5	Superficial	I	0 in curettage/1-2 in hysterectomy	1%	yes	-	-	LTFU
7	PSTT	30	Healthy pregnancy	36 months	Vaginal bleeding	4	3.5	Deep	I	0-1	<5%	no	10 years	no	DFS
8	PSTT	24	Healthy pregnancy	6 months	Vaginal bleeding	27	-	-	-	1	-	yes	-	-	LTFU
9	PSTT	32	Molar pregnancy	18 months	Vaginal bleeding and inguinal pain	13	3	Superficial	I	1	15%	yes	2 years	no	DFS
10	ETT	41	Healthy pregnancy	48 months	Vaginal bleeding	-	1	Superficial	I	1	10%	no	9 years	no	DFS
11	ETT	44	Healthy pregnancy	96 months	Vaginal bleeding	-	5	Deep		12	28%	no	8 years	Lung (in 2 years)	DFS
12	ETT	40	Abortion	36 months	Inguinal pain	-	4	Deep		2	15%	yes	-	-	LTFU
13	ETT	24	Molar and healthy pregnancy	12 months	Vaginal bleeding	151	-	-		10*	80%	yes	3 year	no	DFS

*In these cases, mitotic count was evaluated on the curettage specimens

Whether focal or diffuse, significant nuclear pleomorphism (Figure 1b) was seen in all PSTT cases. In three cases, there was a vesicular nucleus and prominent nucleoli. In most cases, nuclear hyperchromasia, bi/multinucleation, bizarre nucleus, and intranuclear pseudoinclusion were observed (Figure 1b, c). Cytoplasms were mostly both clear and eosinophilic with fine granulation. Prominent cell membranes and vacuolar degeneration (Figure 1e), intracytoplasmic hemosiderin pigment, and signet ring-like cells in 2 cases were seen.

In most ETT cases, cohesive cell layers and some cases accompanying single scattered cells and nests were seen. In hysterectomy specimens, nodular, (Figure 2a) expansive myometrial invasion patterns were noted. The vascular wall involvement was not observed. Geographic necrosis (Figure 2b) was seen in all cases and ranged between 10-60% of the tumor. Eosinophilic globules (Figure 2c) and intra-peritumoral lymphocyte infiltration and hyaline-band-like material surrounding the cell groups were noticed. Spindle cells were not observed. There were varying degrees of moderate to severe nuclear pleomorphism and multinucleated giant cells, vesicular nucleus, and prominent nucleoli. Intranuclear pseudo-inclusion was seen only in the case that had focal pleomorphism. Clear and eosinophilic cells with finely granular cytoplasm were observed together in all cases. Koilocytosis-like sub-membranous cytoplasmic condensation and intracytoplasmic hemosiderin pigments were observed. Vacuolar degeneration was not observed.

Immunohistochemical findings: Immunohistochemical findings of the tumors are summarized in Table 1. CD146 and hPL are usually positive in PSTT and showed homogenous staining (Figure 1f-g). In contrast, these markers showed heterogeneous staining in ETT (Figure 2d-e). All ETTs were positive with p63 (Figure 2f; in contrast, all PSTTs were negative with p63 (Figure 1h).

Follow-up findings and treatment: Follow-up time and metastasis status are shown in Table 2. Five of the PSTT patients' follow-up information was available. Only one of them got a medical treatment that an EMA/CO (etoposide, methotrexate, actinomycin D, cyclophosphamide, vincristine/oncovine) regimen. Two of the patients had metastatic disease. The first patient (case 3) didn't receive any treatment after diagnosis, had a lung metastasis, and underwent a lung wedge resection in another hospital. The second patient (case 5) had both lung and brain metastases and was treated with EMA/CO regimen and cranial radiotherapy.

Three ETT patients' follow-up information was available. A patient (case 11) had lung metastasis two years after the diagnosis. A metastasectomy procedure was performed without adjuvant treatment. A patient (case 13) was treated with EMA/CO regimen.

Molecular analysis: We applied NGS for one of the ETT and PSTT cases, which were both operated and followed up at our institution without recurrence or metastasis. KIT, KDR, APC, TP53, and SMAD4 somatic variants were identified. While KIT and TP53 mutations were observed only in PSTT, amino acid changes in KDR, APC, and SMAD4 genes were detected both in the ETT and PSTT cases. Furthermore, ETT showed missense mutations in PIK3CA, RB1, and SMARCHB1.

Discussion

PSTT and ETT are GTNs that usually occur years after a normal or molar gestation, and both arise from IT trophoblasts; implantation site, and chorionic type respectively. Although their characteristic morphologic features are defined in textbooks, they fall short of enabling even gynecologic pathologists to recognize and differentiate these tumors, particularly in curettage specimens, due to their low frequency and difficulty in detecting their characteristics in a limited

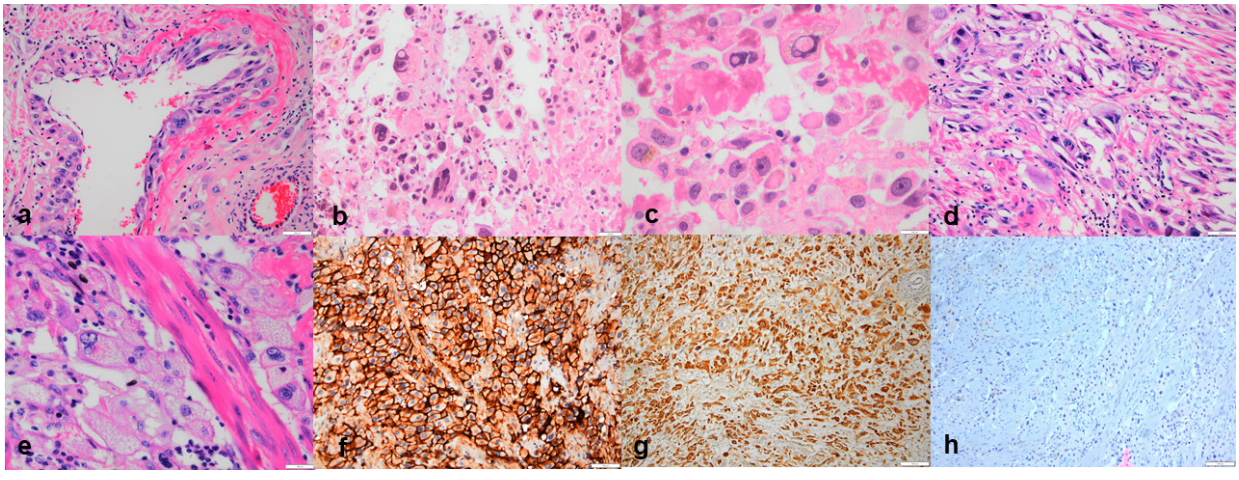

Figure 1. In addition to classical histological features in PSTT, a) vessel wall affinity (H&E 200x), b) marked pleomorphism (H&E 100x) and c) intranuclear pseudo inclusion (H&E 200x), d) spindle tumor cell (H&E 200x), e) vacuolar degeneration of tumor cell (H&E 200x) are observed as specific findings for PSTT in our cases. Immunohistochemically f) CD146 (100x) and g) HPL (100x) showed homogenous strong positivity h) while p63 (100x) was negative

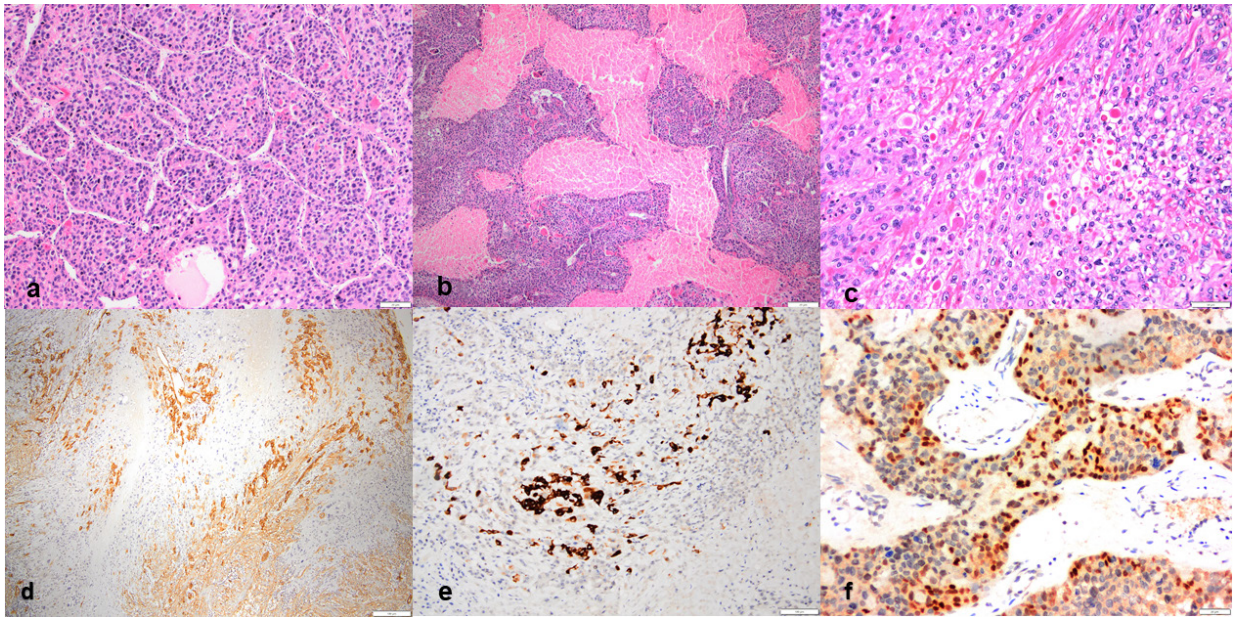

Figure 2. In our series a) nodular pattern (H&E 200x), b) geographic necrosis (H&E 100x) and c) extracellular eosinophilic globules (H&E 200x) were peculiar in ETT cases. Immunohistochemically d) CD146 (40x) and e) HPL (100x) showed heterogeneous strong positivity f) while p63 (200x) was positive (200x).

tissue fragment. Accurate recognition of these tumors is critical for the patient to receive appropriate treatment. Hysterectomy is recommended for ETT and PSTT because of their relative resistance to chemotherapy, which is used just for metastatic patients or patients with adverse prognostic factors[7,8,10]. Histomorphologic features of PSTTs typically consist of sheets of cells and ETTs exhibit nests and cords[11,12]; in our cohort the most commonly encountered architectural pattern was sheets/layers of cells in both tumor groups. The cells-forming these sheets were often discohesive in PSTTs, and cohesive in ETTs. However, in both groups' sheets of cohesive and discohesive cells were coexisting. The nested pattern also appeared in both the groups.

Differentiating PSTT and ETT in biopsy specimens is also hard, especially for non-gynecologic pathologists. It is stated that one of the main distinctive features is the growth pattern of the tumor[11,13,14]. In PSTTs, tumor cells infiltrate the myometrium in the form of single cells, cords, or nests in the periphery of the tumor, whereas ETTs are nodular and generally well-circumscribed tumors, but they may have focal infiltrative areas[13]. We have a similar observation in hysterectomy specimens. However, in curettage specimens, it is not usually possible to distinguish whether the tumor has a predominantly infiltrative or an expansive border.

It is already known that PSTT shows vessel wall affinity[13]. Similar to normal implantation sites, tumor cells migrate through vessel walls and replace them while maintaining the vascular architecture[14]. In our cohort, we also demonstrated vessel wall affinity as a specific feature of PSTT. The frequent presence of such findings, even in curettage specimens, can be considered as a diagnostic for PSTT. However, while deciding on a truly transformed vessel, it should be well distinguished from perivascular tumor infiltration that may also be seen in ETT[11,14].

In this study, necrosis was a common finding with varying degrees in both groups. One of the remarkable findings in our series was a demonstration of geographic necrosis in ETTs, in addition to fibrinoid necrosis seen in both tumor groups. Tumor involvement of the vessel wall leads to fibrinoid necrosis and extracellular fibrinoid or hyaline material accumulation, which is accepted as the characteristic feature of ETT[11,14,15]. This morphologic appearance was present in both groups of ETT and PSTT in our series; therefore, it should not be considered a feature directly in favor of ETT. In our study, we also noticed the presence of extracellular hyaline globular bodies in ETTs, and this finding has not been previously reported in the literature so far.

It has been reported that generally, a monomorphic cell population is dominant in both tumor groups[11,14]. Also, there are publications indicating that nuclear atypia is modest in ETTs, whereas there are varying degrees of pleomorphism in PSTTs[12,16]. In our cohort, cells with marked nuclear pleomorphism were seen in the cases of PSTT, but in only one of the ETT cases. Therefore, nuclear pleomorphism favors PSTT when accompanying other specific morphologic features.

The presence of intranuclear pseudo-inclusion has not been considered a conspicuous finding in these tumors heretofore. However, it was a striking feature in all cases of the PSTT in our study. In ETTs, it was seen only in very few cells where marked nuclear pleomorphism was observed. In addition to that finding, bizarre nuclei, bi/multinucleation, cytoplasmic vacuolization, and spindle cells, which are thought to be secondary to the

degeneration of tumor cells, were often determined in PSTTs in our series.

When we evaluated the immunohistochemical findings, there was no contradictory result with the previously reported data[17-19]. CK7 demonstrated the tumor architecture better than inhibin. While hPL and CD146 were highly positive in PSTTs, p63 was immunoreactive in most of the ETT cells. However, it is noteworthy to be aware of the misleading immunostaining patterns to evaluate the immunohistochemical studies correctly, particularly in curettage specimens. Immunomarkers weren't positive in 100% of cells, and heterogeneous patterns can be seen. Thus, to agree on the positivity of the tumor, a threshold should be determined. According to our data, in ETT, there may be low heterogeneous staining with hPL and CD146 (heterogeneous staining patterns up to 20% with hPL and up to 30% with CD146 should be considered against PSTT). p63 is a quite reliable immunomarker for the diagnosis of ETT because of it's negative in PSTTs. In PSTTs when 60% or more tumor cells are positive with hPL and 75% or more with CD146, you may establish the diagnosis reliably.

The mean age was 29 for PSTT, and 37 for ETT similar to the literature. As antecedent pregnancy history in PSTT patients was mostly term and molar pregnancies may be present[16], also in our cohort, the patients had either molar or normal pregnancies before the diagnosis.

Metastasis rates reported for GTN are 25-30% and the most common metastasis sites are the lungs and brain[20]. The prognostic factors for PSTT and ETT are quite similar. In the literature, age >40 years, >48 months since previous pregnancy, FIGO stage, the presence of metastases, necrosis, increased mitotic count (>2.5/mm^2 or >5/10 HPF), deep myometrial invasion, and increased beta-HCG levels have been reported as possible poor prognostic factors[12,16,20,21].

In our series, 2 of the 5 PSTT patients with follow-up information had metastatic disease. The first patient (case 3) had no prominent worse prognostic features but had lung metastasis. The second patient (case 5) had a history of molar pregnancy 10 years before the diagnosis, had a deep myometrial invasion, with a mitotic count of 8/10 HPF in the hysterectomy specimen, and 3/10 HPF in the curettage specimen had both lung and brain metastases occurred during follow-up. Metastatic disease was detected in one of the three ETT patients. She had a normal pregnancy 8 years before the diagnosis, the tumor had a deep myometrial invasion, with a mitotic count of 12/10 HPF had lung metastases three years after diagnosis. The second patient who had a high mitotic count (10/10 HPF) and a high Ki-67 proliferation index (80%) was treated with EMA/CO regimen and three months after that, hysterectomy was performed in another hospital the tumor was reported as CC. We don't have the clinical follow-up.

In our study, mitotic activity was significantly increased in patients with metastasis in both tumor groups, and this was correlated with an increased Ki-67 proliferation index. Also, two of the three patients with metastasis in the tumor groups had a time interval of more than 48 months since antecedent pregnancy and deep myometrial invasion. These findings showed that mitotic count, Ki-67 proliferation index, the long interval between antecedent pregnancy and diagnosis, and deep myometrial invasion may have was central to predicting tumors with a potential for metastasis. Be aware that the mitotic count in curettage specimens may be less than in hysterectomy specimens, as seen in case 3.

To our knowledge hitherto, there have been some molecular studies related to PSTT and ETT, particularly focused on the Y chromosome[22,23]. Most of the cytogenetic analyses were performed by comparative genomic hybridization and concluded the absence of the Y chromosome in these tumors[24,25]. In NGS studies, we identified some similar point mutations in both the PSTT and ETT cases, besides several distinct mutations. KIT and TP53 mutations were detected only in PSTT, whereas ETT revealed missense mutations in PIK3CA, RB1, and SMARCHB1. However, as we performed NGS in just 2 cases, the specificity and importance of these observations must await further studies with larger series.

Study Limitations

The strengths of this article are that it is a different cohort of GTN of IT, which are rare in the literature, that it contains immunohistochemical and morphological supporting data that can help in the differential diagnosis, and that it contains molecular data on the molecular mechanisms involved in the pathogenesis of these tumors, although it has been done in two cases. The weaknesses are the lack of follow-up information in some patients and the fact that molecular studies can be performed in few cases.

Conclusion

As a result, nodular pattern, geographic necrosis, and extracellular eosinophilic globules were peculiar to ETTs, vessel wall affinity, marked pleomorphism, intranuclear pseudo-inclusion, spindle tumor cell, and vacuolar degeneration were seen more commonly for PSTTs in our series. An immunohistochemical panel of p63, hPL, and CD146 was helpful for the exact typing of the tumor. However, while interpreting the immunoreactivity of tumor cells, the percentage and heterogeneity of the staining should be considered cautiously. In the prediction of metastasis, the long interval between antecedent pregnancy and diagnosis, deep myometrial invasion, mitotic count, and ki67 proliferation index were involved rather than other histomorphological parameters, but none of the parameters seems to be an absolute predictor of the metastasis.

Ethics

Ethics Committee Approval: The Hacettepe University Non-interventional Research Ethics Committee approved this study (the ethics board approval number is 2022/12-13).

Informed Consent: All patients consented to the research and publications.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: F.Ö.A., F.G., G.E.T.K., A.C.G., Y.G.G., A.U., Concept: F.Ö.A., F.G., G.E.T.K., A.C.G., Y.G.G., A.U., Design: F.Ö.A., F.G., G.E.T.K., A.C.G., Y.G.G., A.U., Data Collection or Processing: F.Ö.A., F.G., G.E.T.K., A.C.G., Y.G.G., A.U., Analysis or Interpretation: F.Ö.A., F.G., G.E.T.K., A.C.G., Y.G.G., A.U., Literature Search: F.Ö.A., F.G., G.E.T.K., A.C.G., Y.G.G., A.U., Writing: F.Ö.A., F.G., G.E.T.K., A.C.G., Y.G.G., A.U.

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Effect of Fetuin-A and oxidative stress on the occurrence of unexplained infertility

Açıklanamayan infertilite oluşumunda Fetuin-A ve oksidatif stresin etkisi

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Abstract

Objective: Unexplained infertility refers to a diagnosis in which all standard examinations are usually normal and is statistically seen in approximately 30-40% of infertile couples and endometriosis encountered in 25-50% of patients with unexplained infertility. Unexplained infertility is thought to be closely associated with endometriosis and serum and peritoneal fluid levels of Fetuin-A is increased in patients with endometriosis. Fetuin-A is proposed as a diagnostic marker for endometriosis and has anti-inflammatory effects on several diseases. Oxidative stress also is central to the etiopathogenesis of infertility in women. The aim of this study was to evaluate serum Fetuin-A and oxidative stress parameter concentrations impact on unexplained infertility.

Materials and Methods: In the study, serum Fetuin-A, IL-1β, CA I, TAS, TOS levels, and PON and ARE enzyme activities were measured using the Enzyme-Linked ImmunoSorbent Assay in the sera of diagnosed unexplained infertility females (n=44) and controls (n=41).

Results: There was no statistically significant difference between unexplained infertile patients and control groups in terms of serum IL-1β, CA I, OSI, and PON levels ($p>0.05$). Serum Fetuin-A and ARE levels were significantly higher in unexplained infertility compared with the control, whereas serum TAS and TOS levels were lower ($p<0.05$, $p<0.01$, $p<0.05$, $p<0.05$, respectively).

Conclusion: It is thought that increased Fetuin-A levels may be a response to the inflammatory process and increased ARE activity may be a sign of the impaired oxidant-antioxidant balance in unexplained infertility. This may contribute to the pathogenesis of infertility, and the data obtained will make a significant contribution to new works to be done in this sense.

Keywords: Unexplained infertility, Fetuin-A, oxidative stress, carbonic anhydrase I, ARE

Öz

Amaç: Açıklanamayan infertilite, tüm standart muayenelerin genellikle normal olduğu ve istatistiksel olarak kısır çiftlerin yaklaşık %30-40'ında görülen bir tanıyı ifade eder. Açıklanamayan infertilite hastalarının %25-50'sinde endometriozise rastlanmaktadır. Açıklanamayan infertilitenin endometriozis ile yakından ilişkili olduğu düşünülmektedir. Endometriozisli hastalarda serum ve peritoneal sıvıda Fetuin-A düzeyleri artmaktadır. Fetuin-A, endometriozis için tanısal bir belirteç olarak önerilmiştir ve çeşitli hastalıklar üzerinde anti-enflamatuvar etkilere sahip olduğu bilinmektedir. Oksidatif stres de kadınlarda infertilite etiyopatogenezinde önemli bir rol oynamaktadır. Bu çalışmanın amacı, serum Fetuin-A ve oksidatif stres parametreleri konsantrasyonlarının açıklanamayan infertilite üzerindeki etkisini değerlendirmektir.

Gereç ve Yöntemler: Bu çalışmada, açıklanamayan infertilite tanısı konmuş kadınlar (n=44) ve kontrollerin (n=41) serumlarında Fetuin-A, IL-1β, CA I, TAS, TOS seviyeleri ve PON ile ARE enzim aktiviteleri Enzyme-Linked ImmunoSorbent Assay kullanılarak ölçüldü.

Bulgular: Açıklanamayan infertil hasta ve kontrol grubu arasında serum IL-1β, CA I, OSI ve PON düzeyleri açısından istatistiksel olarak anlamlı fark yoktu ($p>0,05$). Açıklanamayan infertilitede kontrole göre serum Fetuin-A ve ARE düzeyleri anlamlı olarak yüksek, serum TAS ve TOS düzeyleri ise daha düşüktü (sırasıyla $p<0,05$, $p<0,01$, $p<0,05$, $p<0,05$).

PRECIS: We have investigated in women with unexplained infertility serum Fetuin-A, IL-1β, CA I, TAS, TOS levels, and PON, ARE enzymes activities' possible effects and relationships among these parameters on disease.

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Sonuç: Açıklanamayan infertilitede artmış Fetuin-A düzeylerinin inflamatuvar sürece bir yanıt olabileceği ve artmış ARE aktivitesinin bozulmuş oksidan-antioksidan dengenin bir işareti olabileceği düşünülmektedir. Elde edilen bu veriler açıklanamayan infertilitenin patogenezine katkı sağlayacak ve bu anlamda yapılacak yeni çalışmalara ışık tutacaktır.

Anahtar Kelimeler: Açıklanamayan infetilite, Fetuin-A, oksidatif stres, karbonik anhidraz I, ARE

Introduction

Unexplained infertility (UI), which is a major problem of the reproductive system, refers to a failure in achieving a successful pregnancy after at least one year of regular unprotected sexual intercourse despite ovulation, tubal patency, and semen parameters are normal[1]. Many studies have accepted that infertility and endometriosis are associated clinically[2]. Fetuin-A is a multifunctional glycoprotein that is secreted exclusively by the liver parenchymal cells and it is proposed as a diagnostic marker for endometriosis[3]. Fetuin-A is the acute phase protein response to various inflammatory situations and has a protective effect on systemic inflammation[4]. IL-1β is localized in endometrial macrophages and endothelial cells as an inflammatory cytokine. Serum and peritoneal fluid levels of IL-1β have increased in endometriosis[5]. Carbonic anhydrases (CAs, EC 4.2.1.1) are a family of zinc enzymes with 16 isoenzymes that catalyze the reversible hydration reaction of carbon dioxide to bicarbonate. It has critical physiological roles or pathological processes, including the reproductive system. CA has reported that ovarian autoantibodies are associated with UI and premature ovarian failure[6]. Antibodies against autoantibodies including CAs have been shown in endometriosis, in which autoimmune mechanisms may be involved[7].

The resulting oxidative stress is central to the etiopathogenesis of infertility in women and causes health problems in the female reproductivity system, such as UI, endometriosis, PCOS, and recurrent pregnancy loss[8]. Excessive production of ROS affects the body's natural antioxidant defense system and disrupts the environment in which normal physiological reactions occur[9]. Total antioxidant status (TAS), total oxidant status (TOS), and OSI are critical parameters that can be used to assess redox and the degree of oxidative stress status[10]. Paraoxonase-1 (PON1) is synthesized in the liver as a high-density lipoprotein-associated enzyme having both paraoxonase (PON) and arylesterase (ARE) activities. PON and ARE have an antioxidant effect against lipid peroxidation and play a significant role in the anti-inflammatory process. Although there are several reports evaluating the possible link between serum PON activity and oxidative stress in PCOS in literature,[11,12] no study has been found associated with the cases of UI. In this study, it was aimed to evaluate serum Fetuin-A, IL-1β, and CA I levels, antioxidant molecules, and enzyme activities in UI.

Materials and Methods

The study group consisted of 44 female patients diagnosed with UI and 41 controls (women whose cause of infertility is male factor). Patients and controls were chosen from people without systemic diseases. The study protocol has been performed in accordance with the ethical standards described in an appropriate version of the 1975 Declaration of Helsinki, as revised in 2000, and it was approved by the Gazi University Clinical Research Ethics Committee with decision number 512 (Date: 26.02.2018). All participants gave written informed consent.

Peripheral venous blood samples were collected from the study group. The blood samples were dissociated to their serum by centrifugation at 1000x g for 15 min. at 4 °C and immediately placed into sterile Eppendorf tubes. Following this procedure, all serum samples were frozen at -80 °C. The human ELISA kits were used for serum Fetuin-A, IL-1β, and CA I according to the manufacturer's instructions. Serum TAS and TOS values, PON and ARE enzyme activities were measured by spectrophotometer by manual methods.

Serum Fetuin-A, IL-1β, and CA I (Elabscience, USA) were measured according to the ELISA kit procedure. TAS and TOS measurements were applied in serum (Rel Assay Diagnostics, Turkey). TAS reduces the antioxidants in the sample the dark blue-green ABTS radical to the colorless reduced ABTS form and changes in absorbance at 660 nm is related to the total antioxidant level of the sample. The TOS kit principle is oxidant present in the sample oxidizing the ferrous ion-o-dianisidine complex to the ferric ion and the detected color intensity is related to the total amount of oxidant molecules. The oxidative Stress Index (OSI) is a proportional index obtained by dividing total peroxides by total antioxidants with the formulas below.

OSİ (Arbitrary Unit) = TOS (μmol H_2O_2 Equiv./L) / TAS (mmol Trolox Equiv. /L)

Serum PON levels were determined using paraoxon (o,o-diethyl-o-p-nitrophenyl phosphate) as the substrate, which is the active catabolic metabolite of parathion from organophosphate compounds. PON serum activity was measured spectrophotometrically according to the definition of Gan et al.[13]. Based on this method, to prepare a PON reactive mixture solution, freshly prepared 2 mM of paraoxon substrate solution was dissolved in the presence of 2 mM $CaCl_2$ in a total volume of 90 mL 100 mM Tris-HCl buffer (pH 8.0). The assay and blank tubes contained 700 μL of PON reactive mixture solution, and just assay tubes included 20-μL serum, while blank tubes had 20 μL distilled water instead of serum. After incubation for 5 min at 37 °C in a water bath, the changes in absorbance at 3 min were continuously monitored at 412 nm. The amount of p-nitrophenol was calculated from the molar extinction coefficient (18.000 $M^{-1}cm^{-1}$), and the results were expressed as U/mL. Validation was performed by measuring the

same sample at least three times for consistency. Serum ARE levels were measured using phenyl acetate as the substrate, one of the aromatic carboxylic acid esters. ARE serum activity was measured spectrophotometrically according to the definition of Naderi et al.[14]. Briefly, 20 µL of a 1:50 dilution of serum and 20 µL distilled water were added to 700 µL of ARE reactive mixture solution containing freshly prepared 2 mM phenylacetate in 100 mM Tris-HCl pH 8.0 and 2 mM $CaCl_2$ for assay and blank tubes, respectively. After incubation for 5 min at 25 °C in a water bath, the changes in absorbance at 3 min were continuously recorded at 270 nm. The amount of phenol was calculated from the molar extinction coefficient (1310 $M^{-1}cm^{-1}$), and the results were expressed as U/mL. Validation was implemented by evaluating the same sample at least three times for consistency.

Statistical Analysis

Statistical analysis of the data was performed using the SPSS version 22.0 for Windows (SPSS Inc, Chicago, IL, USA). Descriptive statistics are given with mean ± standard deviation. The distribution of the data was examined using the Kolmogrov-Smirnov normality test. "Independent Samples t-test" was applied in the analysis between the two independent groups. The confidence interval was accepted as 95%, and the p-value less than 0.05 was considered statistically significant. The pearson correlation coefficient was used for the relationship among the measured parameters.

Results

The clinical characteristics and biochemical parameters of the study group are given in Table 1. No statistically significant difference was found between the UI patient group and the control group in terms of age, BMI, FSH, TSH, LH, progesterone, estradiol (E_2), number of oocytes, LH/FSH ratio, ALT, and AST levels (p>0.05).

Table 1. Clinical features and biochemical parameters in the study groups

	Control group (n=41)	UI group (n=44)
Age (X ± SH)	30.41±0.75	32.14±0.69
BMI (kg/m^2)	24.85±0.52	25.58±1.56
FSH (mIU/mL)	6.32±0.31	7.20±0.44
TSH (uIU/mL)	2.07±0.14	2.23±0.17
LH (mIU/mL)	3.28±0.46	4.78±0.64
Progesterone (ng/mL)	0.64±0.05	0.60±0.04
E_2 (pg/mL)	34.97±2.40	41.23±2.39
Number of Oocyte	11.32±1.02	12.73±0.89
LH/FSH Ratio	0.55±0.07	0.68±0.08
ALT (U/L)	16.41±1.08	16.20±1.84
AST (U/L)	16.07±0.48	18.41±1.56

Serum levels of Fetuin-A and IL-1β of both groups and statistical comparison between the two groups are given in Figure 1. Serum levels of Fetuin-A were higher in the UI group compared to controls (21.24±0.64 µg/mL, 19.65±0.46 µg/mL, p<0.05). Additionally, IL-1β (2.18±0.46 pg/mL, 1.78±0.40 pg/mL, p>0.05) and CA I (784.86±56.61 pg/mL, 752.13±13.10 pg/mL, p>0.05) concentrations were not found statistically significant between the study groups (Figure 1).

Serum TAS (1.72±0.02 mmol/L, 1.77±0.02 mmol/L, p<0.05) and TOS (6.00±0.16 µmol/L, 6.70±0.31 µmol/L, p<0.05) levels were found to be significantly lower in the UI patient group compared to the control group. No significant difference was found between UI patients and controls in terms of serum OSI levels (3.51±0.12 arbitrary unit, 3.79±0.17 arbitrary unit, p>0.05) (Figure 2).

PON serum concentrations were not statistically different among the study groups (46.76±4.45 U/mL, 41.02±3.37 U/mL, p>0.05). However, ARE levels are higher in UI patients than in controls (98.77±9.26 U/mL, 39.40±3.61 U/mL, p<0.01) (Figure 3).

In the UI group, using bivariate correlation analysis, positive correlations were found between LH/FSH ratio and PON (r=0.325, p<0.05), TOS and OSI, TOS, and CA I (r=0.856, p<0.01; r=1.000, p<0.01 respectively), and OSI and CA I (r=0.856, p<0.01); however, negative correlations between

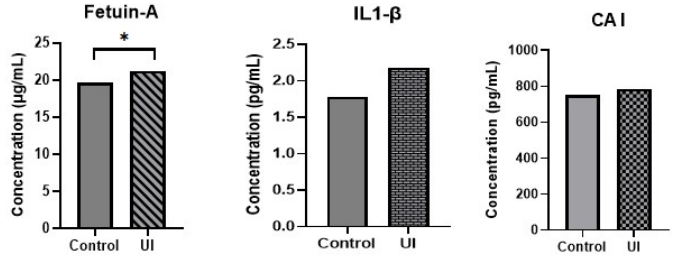

Figure 1. Serum Fetuin-A, IL-1β, and CA I levels in the study groups

** Significantly increased in unexplained infertility patients compared to controls (p<0.05)*

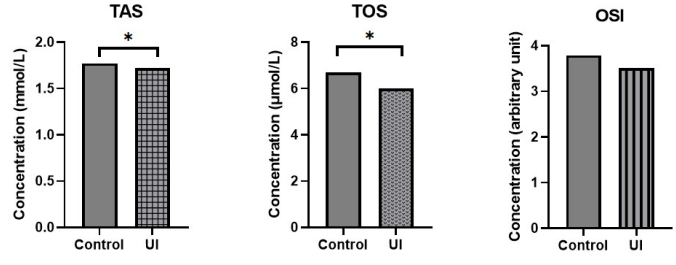

Figure 2. Serum TAS, TOS, and OSI levels in the study groups

** Significantly decreased in unexplained infertility patients compared to controls (p<0.05)*

serum TOS and ARE, TAS and OSI, OSI and ARE, CA I and ARE were found (r=-0.379, p<0.05; r=-0.492, p<0.01; r=-0.339, p<0.05; r=-0.379, p<0.05, respectively) (Table 2).

In the control group, positive correlations were found between age and mean levels of serum TOS, age and OSI, age and CA I (r=0.413, p<0.01; r=0.468, p<0.01; r=0.413, p<0.01, respectively), BMI and TOS, BMI and OSI, BMI, and CA I (r=0.391, p<0.05; r=0.355, p<0.05; r=0.391, p<0.05, respectively), LH and TAS, LH and Fetuin-A (r=0.353, p<0.05; r=0.327, p<0.05, respectively), LH/FSH ratio and TAS (r=0.316, p<0.05), TOS and OSI, TOS and CA I, OSI and CA I (r=0.978, p<0.01; r=1.000, p<0.01; r=0.978, p<0.01, respectively). On the other hand, there were negative correlations between PON and LH/FSH ratio, ARE and TSH (r=-0.361, p<0.05; r=-0.319, p<0.05, respectively) (Table 3).

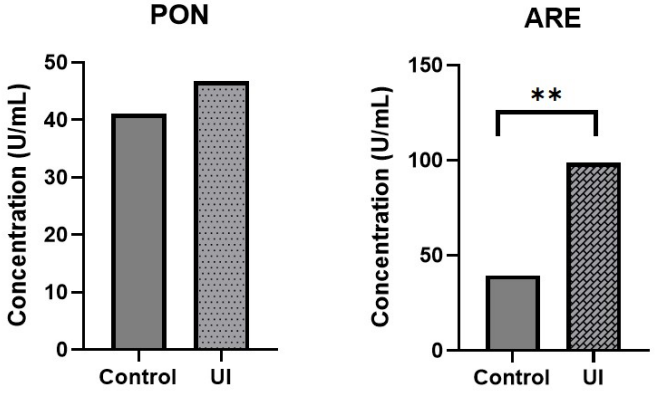

Figure 3. Serum PON and ARE levels in the study groups

*** Significantly increased in unexplained infertility patients compared to controls (p<0.01)*

Table 2. Correlations between the parameters measured in the patient group

UI group (n=44)	CA I	TAS	TOS	OSI	ARE
CA I	-	-	1.000**	0.856**	-0.379*
TAS	-	-	-	-0.492**	-
TOS	1.000**	-	-	0.856**	-0.379*
OSI	0.856**	-0.492**	0.856**	-	-0.339*
ARE	-0.379*	-	-0.379*	-0.339*	-

*Difference according to the control group p<0.05,
**Difference according to the control group p<0.01

Table 3. Correlations between the parameters measured in the control group

Control group (n=41)	CA I	TOS	OSI
CA I	-	1.000**	0.978**
TOS	1.000**	-	0.978**
OSI	0.978**	0.978**	-

*Difference according to the control group p<0.05,
**Difference according to the control group p<0.01

Discussion

In cases of UI, fertility does not occur, although all standards-regarding infertility are normal. Potential reasons for this include endocrinological, immunological, genetic factors, and reproductive physiology diseases[15]. Although it is known that UI is linked to metabolic diseases, it is clear that new biomarkers representing this condition are strongly needed in clinical practice. Various molecules related to the inflammatory pathways in UI include adipokines, inflammatory cytokines, oxidative stress molecules, and transcription factors, which are candidates for new molecular targets for the prognosis and treatment of UI. There is no study in the literature evaluating UI in terms of Fetuin-A, IL-1β, CA I, TAS, TOS, OSI levels, and PON and ARE activities. We mainly investigated these parameters in this study.

Fetuin-A is an important adipokine that is expressed and secreted by the adipose tissue. It has an anti-inflammatory effect in the acute phase response[4]. Although there are some controversial data on the role of Fetuin-A in infertility, it has not been evaluated in UI. In one of these studies at issue, higher circulating Fetuin-A levels were observed in patients with PCOS compared with healthy women by Liu et al.[16] Moreover, they suggested that serum Fetuin-A was associated with the occurrence of PCOS according to binary logistic regression analysis and concluded the results as Fetuin-A may be a useful biomarker for screening PCOS. Obviously, higher levels of serum Fetuin-A in patients with PCOS has been asserted to be connected with PCOS[17]. Similarly, a significant increase in both women undergoing IVF treatment and women with recurrent implantation failure was denoted compared with healthy controls in terms of serum Fetuin-A levels[18,19]. On the other hand, Gurbuz et al.[20] did not find any statistical differences between PCOS and the control group. In our study, serum Fetuin-A levels were found to be statistically higher in UI patients compared with the control group. This result obtained in our study is consistent with the studies conducted by Liu et al.[16], and Sak et al.[17]. We think that increased levels of Fetuin-A may contribute to the etiopathogenesis of UI.

Interleukin-1 beta (IL-1β) exerts various biological functions as a proinflammatory cytokine. To investigate the possible effect of inflammation on the risk of in vitro fertilization and embryo transfer (IVF-ET) failure, serum samples of infertile and pregnant women were collected on the second day of menstruation, and the inflammatory cytokine IL-1β, IL-6, and IL-8 levels were evaluated by Xie et al.[21]. Among these inflammatory cytokines, serum IL-8 levels were found to be higher compared with controls who became pregnant after treatment, while no significant difference was found between the groups between IL-1β and IL-6 levels. In another study by Sequeira et al.[22], IL-1β was measured in the serum and embryo

culture medium of infertile female patients who received IVF treatment, and there was no significant difference between the groups of pregnant and non-pregnant women. Our results showed that serum IL-1β levels were similar in both the patient and control groups. It is seen that the results obtained from our study are compatible with the studies of Xie et al.[21] and Sequeira et al.[22].

Carbonic anhydrase enzymes are common in many tissues and organs in the human body. The ovary is the target of autoimmune attacks in various pathologies that progress toward dysfunction. The presence of Anti CA I antibodies has been shown in recurrent pregnancy losses, endometriosis, and PCOS, and it is thought to be a reliable diagnostic indicator[7,23,24]. In a study by Menteşe et al.[24] with patients with PCOS, it was reported that serum Anti-CA I autoantibodies were higher levels compared with healthy controls. Anti-CA I has not been reported so far in sera obtained from infertility patients with unexplained presence. In our study, although serum CA I levels were higher in the UI patient compared with the healthy control group, no significant difference was found. There is no study in the literature that evaluated the levels of CA I enzyme in UI.

Although it is known that numerous factors that cause oxidative stress in infertility play a role in its etiopathogenesis, the uncertainty regarding its effect on UI continues[25]. It has been reported that oxidative stress plays a role in various etiological factors that may contribute to infertility, such as impaired endometrial receptivity, impaired oocyte quality, premature ovarian failure, endometriosis, tubal disease, pelvic adhesions, and immunological and endocrinological abnormalities. Isbilen et al.[26] found higher levels of TOS and lower levels of OSI in patients with UI, although it was not significant compared with healthy women. Additionally, significantly lower TAS values were reported in healthy fertile women compared with the patient group. Şentürk et al.[27] found significantly higher TOS and OSI levels in women with UI than in fertile women but did not find any difference in TAS values. In our study, decreased TOS and TAS levels were found in patients with UI patients. The data we obtained contradicted the previous results in the literature. Additionally, it was observed that there was no difference between the groups in OSI values, similar to Isbilen et al.[26] and these results contradict the results of Şentürk et al.[27] The significant decrease in our study serum TAS, TOS, and OSI values in UI and the positive correlations between TOS and OSI, TOS and CA I, and OSI and CA I in both the control and UI groups show us the impaired oxidant-antioxidant balance in UI.

In this study, we hypothesized a possible relationship between serum PON/ARE levels and UI development. There are controversial results in the literature. Carlioglu et al.[28] showed lower PON and ARE levels in untreated patients with PCOS than the controls, while these enzyme levels were significantly increased after treatment. Accordingly, it has been reported that PON enzyme activity was decreased in women with endometriosis[29]. Verit et al.[30] suggested that serum PON enzyme level was significantly reduced in women with moderate/severe endometriosis than in women with minimal/mild endometriosis and control groups, and in women with minimal/mild endometriosis compared with controls, correlatively. The authors also found a significant negative correlation between serum PON levels and stage of endometriosis and conclude that serum PON enzyme levels can be used as a diagnostic marker for endometriosis. However, this is inconsistent with the study by San Millan et al.[31], who did not show a significant difference between the PCOS and controls in the serum PON activities. These results were supported by Bragatto et al.[32] for women with endometriosis in minimal/mild and moderate/severe stages for both PON and ARE enzymes, even when the patients were analyzed separately according to the endometriosis stage. In the same way, Younis et al.[33] also demonstrated no differences between the endometriosis, PCOS, and UI groups in baseline and peak serum levels of PON. In our study, serum ARE levels were found to be significantly higher in the UI patient group than in the control group. Similarly, although serum PON levels were increased in patients compared with controls, this increase was not statistically significant. These conflicting results suggest that much is still unknown related to the causes and mechanisms involved in the endogenous production of PON/ARE enzyme. It is thought that increased ARE activity in the body may contribute to the pathogenesis of infertility.

Study Limitations

The findings of this study must be seen considering some limitations. The first is that our study has a small sample size. More precise results can be obtained with a study in which more women with IU participate. The second limitation, in UI, there can be many factors that can also affect oxidative stress, such as lifestyle, medications, malnutrition, obesity, and environmental factors. The third limitation it can be stated that measuring and comparing the parameters measured in the serum also in the follicular fluid may be more helpful in the interpretation.

Conclusion

Considering these data obtained from our study, it is thought that increased Fetuin-A levels may be a response to the inflammatory process and increased ARE activity may be a sign of the impaired oxidant-antioxidant balance in UI and may contribute to the pathogenesis of infertility. It is predicted that Fetuin-A and ARE levels can be considered biomarkers in evaluating the UI status. We believe that more comprehensive studies should be conducted by increasing the number of samples so that the results can be more reliable.

Ethics

Ethics Committee Approval: The study protocol has been performed in accordance with the ethical standards described

in an appropriate version of the 1975 Declaration of Helsinki, as revised in 2000, and it was approved by the Gazi University Clinical Research Ethics Committee with decision number 512 (Date: 26.02.2018).

Informed Consent: All participants gave written informed consent.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: T.T., T.T., Z.C.İ.D., A.G., Concept: T.T., T.T., Z.C.İ.D., A.G., Data Collection or Processing: T.T., T.T., Z.C.İ.D., A.G., Analysis or Interpretation: T.T., T.T., Z.C.İ.D., A.G., Literature Search: T.T., T.T., Z.C.İ.D., A.G., Writing: T.T., T.T., Z.C.İ.D., A.G.

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Zonulin as a potential biomarker for diminished ovarian reserve: A prospective study

Azalan yumurtalık rezervi için potansiyel bir biyobelirteç olarak zonulin: Prospektif bir çalışma

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Abstract

Objective: The purpose of this research is to investigate the relationship between zonulin levels and diminished ovarian reserve (DOR), and to evaluate the potential role of autoimmunity in the development of DOR. The study contributes to the understanding of the pathogenesis of DOR, which can be an unexpected diagnosis often associated with infertility and unpleasant physical symptoms in women.

Materials and Methods: This cross-sectional study was conducted by scanning 224 patients. The demographic characteristics of the patients were recorded. Antral follicle counts of the patients were determined by ultrasound, and Anti-Mullerian hormone (AMH) levels were examined. Follicle-stimulating hormone (FSH), luteinizing hormone, estradiol, AMH measurement, and antral follicle counts were made on the 2nd or 3rd day of menstrual bleeding. The zonulin levels of the participants were measured by the ELISA method. The patients were divided into two groups according to the presence of DOR. The patients' demographic characteristics and hormone levels were compared between these two groups, serum zonulin levels were examined, and the relationship between other hormone parameters and zonulin was investigated.

Results: When the median ages of the patients in both groups were compared, the median age of patients with DOR was 38 years, significantly higher ($p<0.001$) than the median age of those without DOR, which was 27 years. The median zonulin levels of both groups were compare; it was observed that it was 19.71 ng/mL in the group with DOR and 11.03 ng/mL without DOR, and a statistically significant difference was found between the zonulin levels of the patients in both groups ($p<0.001$). A moderate inverse correlation ($p<0.001$) between patients' zonulin and AMH levels, and a moderate correlation between FSH levels ($p<0.001$).

Conclusion: In conclusion, zonulin levels of patients with DOR were higher than women without DOR. Evaluation of zonulin levels may also be considered during the diagnosis of DOR.

Keywords: Diminished ovarian reserve, zonulin levels, autoimmunity, Anti-Mullerian hormone, AMH, infertility

Öz

Amaç: Bu araştırmanın amacı, zonulin düzeyleri ile azalmış over rezervi (DOR) arasındaki ilişkiyi ve otoimmünitenin DOR gelişimindeki potansiyel rolünü değerlendirmektir. Çalışma, kadınlar için sıkıntılı fiziksel semptomlar ve infertilite ile ilişkili beklenmedik bir teşhis olan DOR patogenezinin anlaşılmasına katkıda bulunmayı amaçlamaktadır.

Gereç ve Yöntemler: Bu kesitsel çalışma, 224 hastanın taranmasıyla gerçekleştirildi. Hastaların demografik özellikleri kaydedildi. Hastaların antral folikül sayıları ultrason ile belirlendi ve Anti-Müllerian hormon (AMH) düzeyleri incelendi. Folikül uyarıcı hormon (FSH), luteinleştirici hormon, östradiol, AMH ölçümü ve antral folikül sayıları, menstrüel kanamanın 2. veya 3. gününde yapıldı. Katılımcıların zonulin düzeyleri ELISA yöntemiyle ölçüldü. Hastalar, DOR varlığına göre iki gruba ayrıldı. Bu iki grup arasında hastaların demografik özellikleri ve hormon düzeyleri karşılaştırıldı, serum zonulin düzeyleri incelendi ve diğer hormon parametreleri ile zonulin arasındaki ilişki araştırıldı.

PRECIS: Serum zonulin may be the potential to be used as a valuable biomarker in predicting the diminished ovarian reserve with women.

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Bulgular: Her iki grup hastaların ortanca yaşları karşılaştırıldığında, DOR olan hastaların ortanca yaşı 38 yıl iken, DOR olmayan hastaların ortanca yaşı olan 27 yıldan önemli ölçüde yüksek (p<0,001) olduğu görüldü. Her iki grup hastaların ortanca zonulin düzeyleri karşılaştırıldığında, DOR olan grupta 19,71 ng/mL ve DOR olmayan grupta 11,03 ng/mL olarak bulundu ve her iki grup hastaların zonulin düzeyleri arasında istatistiksel olarak anlamlı bir fark bulundu (p<0,001). Hastaların zonulin düzeyleri ile AMH düzeyleri arasında orta derecede ters bir korelasyon (p<0,001) ve FSH düzeyleri ile orta derecede bir korelasyon bulundu (p<0,001).

Sonuç: DOR olan hastaların zonulin düzeyleri, DOR olmayan kadınlardan daha yüksek bulundu. DOR tanısı sırasında zonulin düzeylerinin değerlendirilmesi de dikkate alınabilir.

Anahtar Kelimeler: Azalmış over rezervi, zonulin, otoimmünite, Anti-Müllerian hormon, AMH, infertilite

Introduction

There is a significant variation in the number of oocytes in each woman, and biologically, the total amount is at its maximum before the woman giving birth. There is limited scientific knowledge about the factors that control the oocyte pool and how to measure them. The decrease in the quantity and quality of oocytes in old age (usually in the mid-40s) is a normal physiological phenomenon called a decrease in ovarian reserve (DOR)[1,2]. Some women suffer from DOR a lot earlier and become infertile early (pathological DOR). According to recent estimates by the Society for Assisted Reproductive Technology's national system in the United States, 32% of in vitro fertilization (IVF) cycles (about 66,000 cycles) are diagnosed with DOR[3]. The definition of DOR is a state of low fertility due to decreased ovarian function based on clinical judgment and is usually denoted by follicular stimulation hormone (FSH) >10 mIU/mL or Anti-Mullerian hormone (AMH) <1.1 ng/mL[3-5].

For most women, the diagnosis of DOR is unexpected, stressful, often overlaps with an infertility diagnosis and is associated with unpleasant physical symptoms[6]. Many environmental and lifestyle considerations have been suggested, such as the natural age of menopause, the use of oral contraceptives, gender parity, and smoking. However, these factors do not consistently explain the changes in the age of menopause. Ovarian aging correlates strongly with the number and quality of the remaining oocytes[5,6]. Although the etiology of DOR is not yet known, it is thought that most genetic disorders may be related to autoimmunity, iatrogenic, or idiopathic reasons.

Recently, many studies have been done on the structure of the intercellular Tight Junctions (TJ) and its functionalization. The discovery of the zonula occludens toxin (Zot), an enterotoxin that affects TJ competence and developed by Vibrio cholerae, has shed light on the complex mechanisms involved in the modulation of the intestinal paracellular pathway[7,8]. The combination of affinity-purified anti-Zot antibodies and the Ussing chamber technique identified the intestinal Zot homolog called zonulin[8,9].

Zonulin is a protein that plays a role in the pathogenesis of autoimmune diseases, opening the intercellular TJs in the intestines and recycling intestinal permeability[10,11]. Regarding the relationship between zonulin and metabolic disorders, it has been shown in a recent studies[11]. A study investigating the relationship between zonulin and obesity and insulin resistance found that zonulin levels increased significantly in obese and glucose-tolerant individuals[12]. Likewise, it has been positively correlated with inflammatory markers, insulin resistance, and interleukin 6 (IL-6).

Elevated levels of zonulin have been associated with increased intestinal permeability and several inflammatory and autoimmune conditions, including celiac disease, rheumatoid arthritis, and type 1 diabetes[13]. DOR, or diminished ovarian reserve, is a condition characterized by a decreased number of eggs in the ovaries, which can lead to infertility[2,3].

There is currently no research investigating the relationship between zonulin levels and diminished ovarian reserve (DOR). However, some evidence suggested that increased intestinal permeability and inflammation may play a role in the pathogenesis of DOR. Studies have shown that women with DOR have higher levels of inflammatory markers than women with normal ovarian reserve[3,6].

This study aimed to evaluate the relationship between zonulin levels and DOR. This study will evaluate the relationship between autoimmunity, which is a factor in the pathogenesis of DOR.

Materials and Methods

This cross-sectional study was conducted by scanning 224 patients who applied to the Obstetrics and Gynecology outpatient clinic of a university hospital. Ethics committee approval was obtained for the study (Ondokuz Mayıs University/KAEK 2021/95). Written informed consent was obtained from each patient participating in the study. This study was conducted in accordance with the Declaration of Helsinki. Female patients under the age of forty who came to the obstetrics and gynecology outpatient clinic were included in the study. Forty-five patients above forty years of age and with a body mass index ≥30 were excluded from the study. Patients were selected from patients who had regular menstruation and did not have clinical and laboratory findings of PCOS. Additionally, patients with endometriosis, known autoimmune diseases, and thyroid patients, patients with previous ovarian surgery, and chronic diseases (such as diabetes mellitus and hypertension) were excluded from the study. The remaining patients were divided into two groups according to the presence or absence of ovarian insufficiency (Figure 1). The definition of DOR is a state of low fertility due to decreased ovarian function based on clinical judgment and is usually denoted by FSH >10 mIU/mL or AMH <1.1 ng/mL[3].

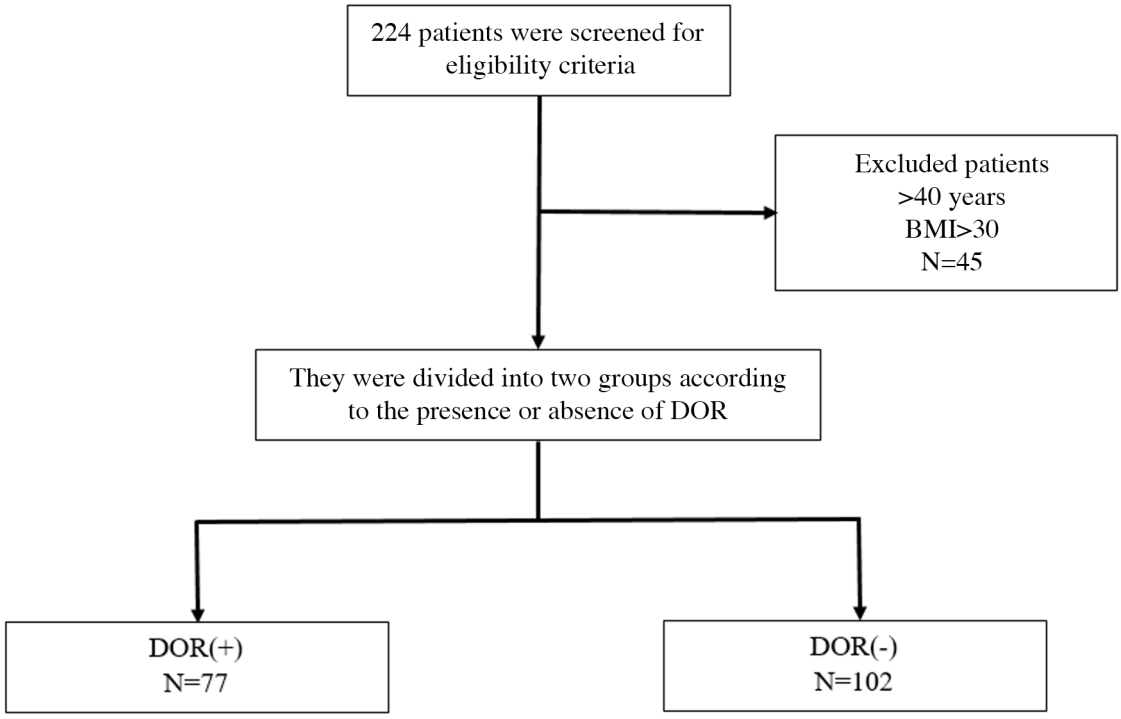

Figure 1. Patient flow diagram

Demographic and laboratory characteristics of the patients [age, body mass index (BMI), FSH, luteinizing hormone (LH), estradiol levels] were recorded. Antral follicle counts of the patients were determined by ultrasound, and AMH levels were examined. FSH, LH, estradiol, AMH measurement, and AFC counts were made on the 2nd or 3rd day of menstrual bleeding. Ultrasound scans (for AFCs) of the patients were performed by C.S.C. and S.C. at Samsun Training and Research Hospital.

Fasting blood samples of all patients were processed in a centrifuge at 3.000 rpm (rounds per minute). Serum was collected and stored at -80 °C until analysis. These stored serums were then used for measuring zonulin values.

Commercially available Enzyme-linked Immunosorbent Assay (ELISA) kits (Sun-Red Bio Company, Cat No. 201-12-5578, Shanghai, China) were used to measure human zonulin concentrations in serum. Enzymatic reactions were measured in an automated microplate photometer. The microtiter plate provided in this kit is pre-coated with a monoclonal antibody specific to human zonulin. Streptavidin-Horseradish Peroxidase is then added to each microplate well and incubated to form an immune complex. Wells containing only Human Zonulin, biotin-conjugated antibody, and enzyme-conjugated avidin will change color after adding chromogen solutions. The addition of the sulfuric acid solution terminates the enzyme-substrate reaction, and the color change is measured spectrophotometrically at a wavelength of 450 nm. The concentration of human zonulin in the samples is then determined by comparing the optical density of the samples with the standard curve. Human zonulin levels were expressed as ng/mL. The coefficients of intra and inter-assay variation were 4.3% (n=20) and 4.9% (n=20), respectively. The sensitivity of this test was 0.223 ng/mL, and the test range is between 0.25 ng/mL and 70 ng/mL.

The patients were divided into two groups according to the presence of DOR. The patients' demographic characteristics and hormone levels were compared between these two groups, and serum zonulin levels were examined. Additionally, the relationship between other hormone parameters and zonulin was investigated. Finally, univariate regression analysis was performed for the presence of DOR, and the zonulin cut-off value to predict the presence of DOR was calculated.

Statistical Analysis

The SPSS 25.0 (IBM, NY, USA) program was used for statistical analysis. Kolmogorov-Smirnov test was performed to evaluate whether the distribution in the groups was parametric. An independent sample t-test was used for comparison between groups. Correlation analysis was performed with the Spearman's test, and logistic regression analysis was applied. $P<0.05$ was determined as statistically significant.

Results

Age, BMI, the number of antral follicles, and hormone levels of the patients are shown in Table 1. When the median ages of the patients in both groups were compared, the median age was 38 (35-39) in the group of patients with DOR and 27 (24-33) in the group without DOR ($p<0.001$). When the mean BMIs of the patients were compared, it was found that they were

Table 1. Selected clinical data for the study groups

	DOR (+) (n=77)	DOR (-) (n=102)	p-value
Age (y)	38 (35-39)	27 (24-33)	**<0.001**
BMI (kg/m^2)	25.4±2.4	24.9±2.3	0.171
AMH (ng/mL)	0.3 (0.03, 0.5)	3.1 (1.9-4.8)	**<0.001**
Antral follicle count (median)	2 (1, 3)	10 (9, 2)	**<0.001**
FSH (mIU/mL)	9.9 (7.9-21.5)	6.2 (5.6-7.3)	**<0.001**
Estradiol (mIU/mL)	55.9 (30.1-80)	41. 6 (37.4-53.1)	0.088
LH (mIU/mL)	9.6 (5.3-11.4)	8.7 (4.9-11.3)	0.121
Zonulin (ng/mL)	19.7 (10.5-53.5)	11.0 (9.2-13.2)	**<0.001**

25.43±2.39 and 24.86±2.29, respectively, and no statistically significant difference was found (p=0.171). When the median antral follicle numbers of the patients were compared, it was seen that the median numbers of the patients in the groups with and without DOR were 2 (1-3) and 10 (9-23), respectively (p<0.001).

The median AMH levels of the patients with and without DOR were 0.3% and 3.1, respectively. When the median FSH hormone levels of the patients were compared, it was observed that it was 9.90 (7.9- 21.5) and 6.22 (5.6- 7.5) in the groups with and without DOR, respectively, and there was a statistically significant difference between the two groups (p<0.001). Similarly, when the median zonulin levels of both groups were compared, it was observed that it was 19.7 (10.5, 53.5) in the group with DOR and 11.03 (9.2, 13.2) without DOR, and a statistically significant difference was found between the zonulin levels of the patients in both groups (p<0.001). There was no statistically significant difference between the median estradiol levels in both groups (p=0.088).

Spearman's test was performed to show the correlation between the patients' AMH, zonulin, FSH, and estradiol levels. A moderate inverse correlation (p<0.001, rs:-0.498) between patients' zonulin and AMH levels (Figure 2a), and a moderate correlation between FSH levels (p<0.001, rs:0.313) (Figure 2b). A strong inverse correlation was also observed between patients' FSH and AMH levels (p<0.001, rs:-0.600) (Table 2). No significant correlation was observed between age and zonulin levels (r=0.225, p=0.07). There was no correlation between estradiol levels and other hormone levels (p>0.05).

Logistic regression analysis was performed to show the effect of hormone levels, age, and BMI on ovarian insufficiency. The regression model was statistically significant: x^2 (4) = 111.8, p<0.001. The model explained 62.3% (Nagelkerke R^2) of the variance in DOR and accurately predicted 82.1% of the cases. Sensitivity was 84.3%, specificity was 79.2%, positive predictive value was 79.2%, and negative predictive value was 84.3%. Age and zonulin levels were statistically significant predictors (Table 3).

Table 2. Correlation analysis of the patients' zonulin levels and hormones

		Zonulin	AMH	FSH
Zonulin	r		-0.498	0.313
	p		<0.001	<0.001
AMH	r			-0.600
	p			<0.001
Antral follicle count	r	-0.452	0.930	-0.368
	p	<0.001	<0.001	<0.001

AMH: Anti-Mullerian hormone, FSH: Follicular stimulation hormone

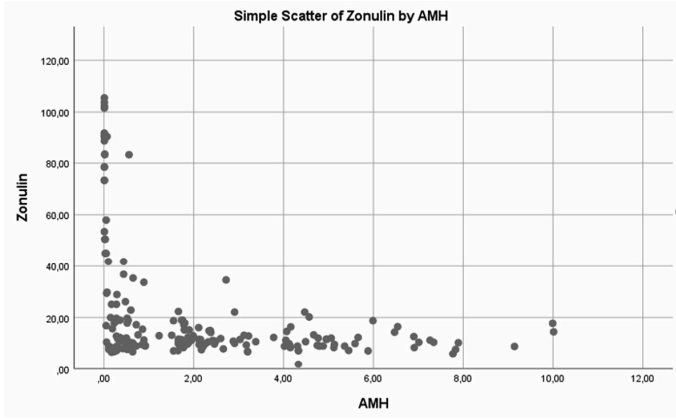

Figure 2a. Correlation analysis of Anti-Mullerian hormone (AMH) and zonulin levels

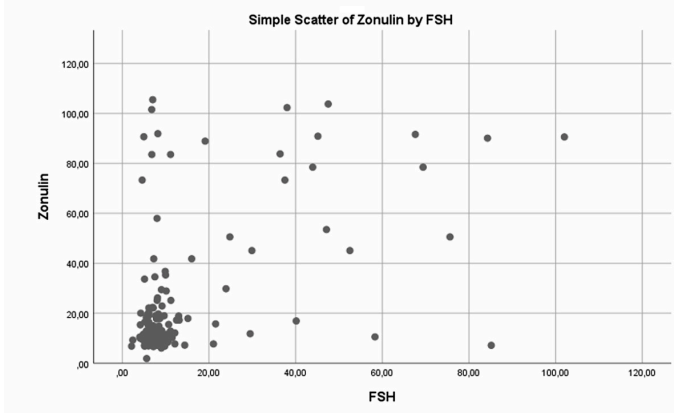

Figure 2b. Correlation analysis of follicular stimulation hormone (FSH) and zonulin levels

Receiver operating characteristic (ROC) curve analysis was performed to predict the distinction of zonulin level from DOR. It was observed that the area under the ROC curve was between 0.734 (95% confidence interval 0.652 and 0.815) and was at an acceptable level of distinction. The sensitivity and specificity of the zonulin value of 22.6 were 46.8% and 99.9% (Figure 3).

Table 3. Logistic regression analysis

	B	S.E.	p-value	HR	%95 CI	
					Lower	Upper
FSH	-0.041	0.031	0.191	0.960	0.903	1.021
Zonulin	-0.105	0.029	<0.001	0.900	0.851	0.953
Age	-0.212	0.039	<0.001	0.809	0.749	0.874
BMI	-0.020	0.145	0.892	0.980	0.737	1.303

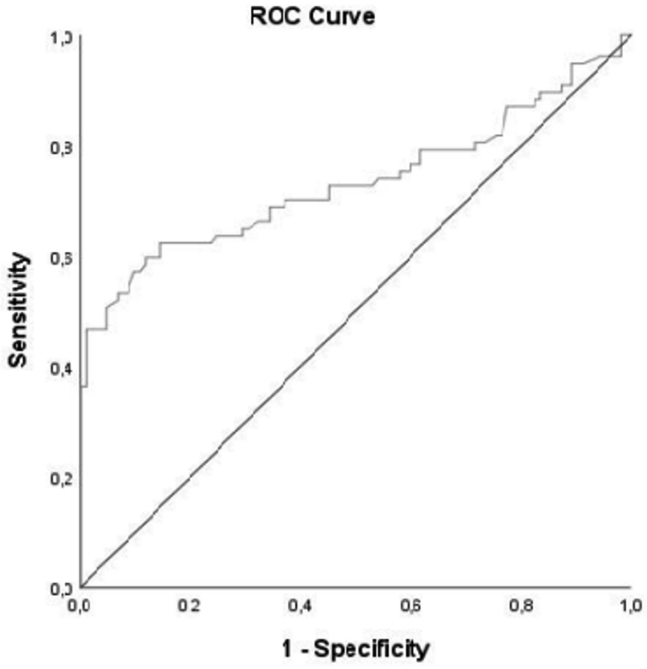

Figure 3. ROC curve analysis of zonulin

ROC: Receiver operating characteristic

Discussion

At the end of the study, it was determined that zonulin levels were significantly higher in the group with ovarian insufficiency and that zonulin levels were moderately negatively correlated with FSH and AMH levels. Additionally, age and zonulin levels were found to be significant as parameters that could predict ovarian insufficiency, and the zonulin cut-off value determined to indicate ovarian insufficiency was recorded as 22.6.

There is no definitive test to assess ovarian reserve, and contradictory results between ovarian reserve tests are commonplace. Therefore, various tests are used to assesment the ovarian reserve. In a worldwide study, 51% of infertility centers chose AMH as the best test to measure the ovarian reserve, 40% chose the ASH as the best test, and only 6% chose the basal FSH[14]. A common clinical challenge is to provide advice to patients with contradictory ovarian reserve test results. With regard to direct diagnoses of DOR, 20% of samples from a large national testing center have inconsistent FSH and AMH[15]. Our study determined that zonulin level could be a significant indicator of ovarian insufficiency and correlated inversely with AMH values and positively with FSH values.

Inflammation of the small intestine leads to changes in bowel patency. It has also been shown that there is a correlation between serum zonulin level and fasting insulin, fasting triglycerides, and IL-6. The zonulin gene overlaps with the haptoglobin 2 gene under the control of IL-6[13,16]. Moreover, in patients with normal glucose tolerance, zonulin is associated with high levels of uric acid, HbA1c, serum IL-6, and low HDL. Ohlsson et al.[16] studied the relationship between zonulin and ovarian function and found that rates of zonulin were associated with ovarian function and were higher in patients with severe menstrual irregularities.

The ovaries are not immunologically protected, so the autoantibodies suggest that the ovaries are detected and that an etiology of autoimmune can produce DOR. The most common autoimmune diseases associated with primary ovarian failure are hypothyroidism, type I diyabetes mellitus, hiperparatiroidizm, sistemik lupus erytematosus, and tipik lupus erythematosus[15-17]. Both early follicular exhaustion and dysfunction may be present in all these conditions.

There are few studies on the relationship between zonulin and metabolic disorders[18,14,19]. One study investigated the relationship between zonulin and obesity and insulin resistance. It has been found that levels of zonulin appear to increase considerably in people who are obese and glucose intolerant and are positively correlated with insulin resistance and inflammatory markers such as IL-6. There are few studies have investigating serum zonulin levels in PCOS patients[4,20]. Zhang et al.[21] reported that zonulin levels have increased significantly in women with disease compared to controls and a strong correlation between insulin resistance, obesity, dyslipidemia, and menstrual disorders. The high levels of zonulin in patients with ovarian insufficiency patients in our study confirm that it has an auto-inflammatory role and causes the development of DOR. Further studies must confirm the role of changes in intestinal permeability and zonulin in insulin resistance and autoimmunity.

Studies have shown that zonulin can be a marker for autoimmune diseases. The pathogenesis of DOR is unclear, and many factors are under investigation. In response to the problem that autoimmunity, which may cause a loss of reserve at an early age, may also be one of these possible factors, we planned to study with the thought that zonulin could be an indicator.

Study Limitations

Our study is the first to evaluate a markers such as zonulin in women with ovarian insufficiency. The information obtained from this study will shed light on future studies. This study had some limitations. The first limitation of the study is that the sample size is small, and it is a single-center study. Due to the study's cross-sectional nature, evaluation as a factor in developing ovarian insufficiency is limited. Another limitation

of the study is that cytokine levels such as IL-1, TNF-alpha, and IL-6 were not evaluated.

Conclusion

As a result, the zonulin levels of patients with DOR were higher than women without DOR. Modeling that can be done with other parameters will provide very convenient data for diagnosing DOR.

Ethics

Ethics Committee Approval: Ethics committee approval was obtained for the study (Ondokuz Mayıs University/KAEK 2021/95).

Informed Consent: Written informed consent was obtained from each patient participating in the study.

Peer-review: Internally peer-reviewed.

Authorship Contributions

Concept: Se.Ç., N.Y., C.S.C., S.Ç., Design: Se.Ç., N.Y., C.S.C., S.Ç., Data Collection or Processing: Se.Ç., C.S.C., Analysis or Interpretation: Se.Ç., N.Y., Literature Search: Se.Ç., N.Y., C.S.C., S.Ç., Writing: N.Y., Se.Ç.

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

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Barbed versus conventional suture in laparoscopic myomectomy: A randomized controlled study

Laparoskopik miyomektomide barbed ve konvansiyonel sütürlerin karşılaştırılması: Randomize kontrollü çalışma

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Abstract

Objective: To compare the surgical and clinical results of traditional absorbable polyglactin 910 and barbed sutures in laparoscopic myomectomy.

Materials and Methods: This single-center randomized study included 75 women who underwent laparoscopic myomectomy. The uterine wall defects were closed with a continuous conventional absorbable polyglactin 910 suture (Vicryl; Ethicon, Somerville, NJ, USA) in 41 women and with a unidirectional barbed suture (V-Loc 180; Covidien, Mansfield, MA, USA) in 34 women.

Results: The time required to suture the uterine wall defect was lower in the V-Loc group than in the Vicryl group (p=0.007). However, no significant difference was observed in the operative time between the two study groups. The intraoperative blood loss and need for postoperative blood transfusion were significantly lower in the barbed group than in the Vicryl group (p=0.018 and p=0.048, respectively).

Conclusion: In laparoscopic myomectomy cases, the unidirectional barbed suture is more effective than the conventional absorbable suture. Barbed sutures facilitate the suturing process and reduce the time required to suture the uterine wall defect, blood loss, and the need for postoperative blood transfusion.

Keywords: Absorbable sutures, barbed suture, laparoscopy, myomectomy

Öz

Amaç: Bu çalışmanın amacı laparoskopik miyomektomide geleneksel emilebilir poliglaktin 910 sütür ile barbed sütürlerin cerrahi ve klinik sonuçlarını karşılaştırmaktır.

Gereç ve Yöntemler: Bu tek merkezli randomize çalışmaya laparoskopik miyomektomi yapılan 75 kadın dahil edildi. Uterus duvar defektleri 41 kadında kontinue konvansiyonel emilebilir poliglaktin 910 sütür (Vicryl; Ethicon, Somerville, NJ, ABD) ile 34 kadında ise tek yönlü barbed sütür (V-Loc 180; Covidien, Mansfield, MA, ABD) ile kapatıldı.

Bulgular: Uterus duvarı defektini sütüre etmek için gereken süre V-Loc grubunda Vicryl grubuna göre daha düşüktü (p=0,007). Ancak iki çalışma grubu arasında ameliyat süresi açısından anlamlı bir fark gözlenmedi. İntraoperatif kan kaybı ve postoperatif kan transfüzyonu ihtiyacı V-Loc grubunda Vicryl grubuna göre anlamlı olarak düşüktü (sırasıyla p=0,018 ve p=0,048).

Sonuç: Laparoskopik miyomektomi olgularında tek yönlü barbed sütür, geleneksel emilebilir sütürden daha efektiftir. Barbed sütürler sütürasyon sürecini kolaylaştırır ve uterus duvarı defektini kapatmak için gereken süreyi, kan kaybını ve postoperatif kan transfüzyonu ihtiyacını azaltır.

Anahtar Kelimeler: Emilebilir sütür, barbed sütür, laparoskopi, miyomektomi

PRECIS: Barbed sutures facilitate the suturing process and reduce the time required to suture the uterine wall defect, blood loss, and the need for postoperative blood transfusion.

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Introduction

Myoma is the most common tumor in the uterus[1]. Although it is usually asymptomatic, it can also present clinical problems such as abnormal levels of uterine bleeding, pelvic pressure/pain symptoms, or infertility[2]. Various medical and surgical methods have been used for treating myoma[3]. However, the most effective and curative treatment today is still surgery[4].

Myomectomy can be performed abdominally, laparoscopically, or hysteroscopically. A comparison between laparoscopic and laparotomic surgery demonstrated that the former resulted in shorter hospital stays, faster recovery, less postoperative pain, and better cosmetic results[4].

As with all myomectomy cases, excess bleeding is one of the most important complications of laparoscopic myomectomy[5,6]. Although many prophylactic methods have been used to reduce bleeding, no single method has had a 100% success rate, and research on new methods should be supported[7-9].

In the case of myomectomy, suturing is the quickest and the most effective procedure to stop bleeding. Therefore, the use of a barbed suture in laparoscopic myomectomy was proposed to simplify suturing, which is the most challenging part of the operation[10].

It is thought that a barbed suture will provide rapid and easy suturing due to its small spines, which hold onto the tissue well, do not loosen, and do not require intracorporeal suturing through the loop design at the other end of the suture[11]. It is foreseen that intraoperative blood loss will be reduced due to this rapid and easy suturing technique[12-15].

Although some previous studies have investigated the efficiency of the barbed suture in laparoscopic myomectomy, these studies were primarily retrospective[10-15]. The main aim of this study was to compare the frequently used Vicryl suture with the barbed suture in terms of intraoperative bleeding and suture time in laparoscopic myomectomy cases. Our secondary objectives are to compare the sutures in terms of the operation time, postoperative hemoglobin drop, and the need for postoperative blood transfusion. Thus, we objectively demonstrate the superiority of the conventional Vicryl suture, which is cheaper and more accessible, versus the newer and more expensive barbed suture.

Materials and Methods

This prospective, randomized clinical trial was conducted between May 2018 and May 2019 at the Antalya Training and Research Hospital. Approval was obtained from the hospital's local ethics committee before any study-related procedures were conducted (approval number: 8-16). The participants provided written informed consent before participation.

Study Population, Patient Sampling, and Randomization

Non-pregnant, reproductive age patients with myoma symptoms and indications for laparoscopic myomectomy were included in the study. The diagnosis of the myoma uteri was classified according to the FIGO Leiomyoma Subclassification System[16]. Because H/S (Hysteroscopy) myomectomy was planned for type 0, 1, and 2 fibroids, these were excluded from the study. The study excluded types 7 and 8 fibroids because their suturation requirements were minimal. Type 3 fibroids were also excluded from the study due to the strong possibility of entering the endometrial cavity aggravating the suturation. Finally, multiple myomas were excluded from the study.

Patients with a single type 4, 5, or 6 fibroids and a uterine size reaching the maximum umbilicus level (20-W gestation size) were included in the study. Laparotomy was planned for larger uteruses.

Patients were randomized by 1:1 simple randomization using sealed envelopes. One day before the operation, the service nurse opened the envelope to determine the method to be used on the patient. During the procedure, the fibroids were repaired using Vicryl suture in the first group and barbed suture in the second group. Patients' demographic data (age, gravida, parity, previous abdominal surgery, and chronic diseases), intraoperative findings (operation time, myomectomy time, suture time, number of suture layers, amount of intraoperative bleeding, and fibroid weight), preoperative and postoperative hemoglobin/hematocrit values, postoperative blood transfusion requirements, and hospital stay duration were recorded.

Operation Procedure

All operations were performed by the same surgeon (BM). The first entry into the abdomen was made by direct trocar entry from the umbilicus or Lee Huang point according to the size of the fibroid, and a 10-mm laparoscope was placed in this port. The 30-degree optic was used in all cases to provide a wide viewing angle. Then, 5-mm accessory trocars were placed in the right and left lower quadrant and suprapubic area. Rumi II was used as the uterine manipulator. A Harmonic scalpel (Ethicon Endo-Surgery, Cincinnati, OH, USA) was used to create the incision in the uterine serosa. A Vicryl (Ethicon, Somerville, NJ, USA) 0 USP 40-mm ½ taper needle suture was used for the Vicryl group patients. A V-Loc 180 (Covidien, Mansfield, MA, USA) 0 USP 37-mm ½ taper needle suture was used for the barbed suture group patients. One, two, or three layers were closed continuously depending on the depth of the fibroid inside the myometrium. All fibroids were morcellated with a Rotocut G1 electronic morcellator device (Karl Storz, Tuttlingen, Germany) and then removed from the abdomen.

Hemoglobin was measured the day before the operation and at the eighth postoperative hour. A cut-off value of <7 g/dL was determined for blood transfusion. Transfusion was performed in symptomatic patients with higher values.

Statistical Analysis

Data were recorded and analyzed using IBM SPSS Statistics for Windows (Armonk, NY: IBM Corp.). The Shapiro–Wilk test was used to determine the suitability of the data to the normal

distribution curve. Normally, distributed data are shown as means ± standard deviations; non-normally distributed data are shown as medians and ranges. For categorical data, n (number/frequency) and percentages (%) are used. Parametric methods were used for the analysis of normally distributed variables, and non-parametric methods were used for the analysis of non-normally distributed variables. To compare two independent groups, the independent samples t-test, and Mann-Whitney U test were used. Categorical data were compared using the Chi-squared test. The data were analyzed at a 95% confidence level, and $p<0.05$ was considered statistically significant.

Results

A total of 108 patients were included in the study; however, only 75 of them completed the study. The study flow diagram is presented in Figure 1. The two study groups were similar with respect to age, body mass index, previous abdominal surgeries, the weight of myoma, diameter of myoma, localization of myoma, and layers of suturation. Patients' demographic and clinical characteristics are shown in Table 1.

The perioperative findings are presented in Table 2. The suture time was significantly lower in the V-Loc suture group compared with the Vicryl suture group (p=0.007). Similarly, blood loss and postoperative blood transfusion requirements were lower in the V-Loc group (p=0.018, and p=0.048 respectively). However, operative time, postoperative hemoglobin and hematocrit change, and hospital stay duration were similar between the two groups (p>0.05 for all) (Table 2).

No organ or vessel injury was observed during the procedures. In three cases, conversion to laparotomy was required because of the restriction of the mobilization of the uterus, and these patients were excluded from the study.

Discussion

Unlike other randomized studies in the literature, this study was carefully planned to investigate the effectiveness of suturing, focusing only on an intraoperative hemorrhage. Therefore, the exclusion criteria were kept wide, and no medical or surgical methods were used to reduce bleeding. Additionally, all operations were performed by the same surgeon for the objective of the study, as the suturation procedure is directly dependent on the hand skills of the surgeon.

In line with other studies in the literature, the present study results indicate that barbed sutures shorten the suturation time in laparoscopic myomectomy cases, thereby reducing intraoperative bleeding and postoperative blood transfusion needs. The use of barbed sutures in laparoscopic myomectomy was first introduced by Greenberg and Einarsson[10] in 2008. There have since been a few studies have investigating the efficiency of the barbed suture in laparoscopic myomectomy cases. Of these, only three were randomized clinical trials, and the rest were retrospective studies[17].

The randomized studies by Alessandri et al.[11] and Ardovino et al.[14] had similar designs. The amount of intraoperative

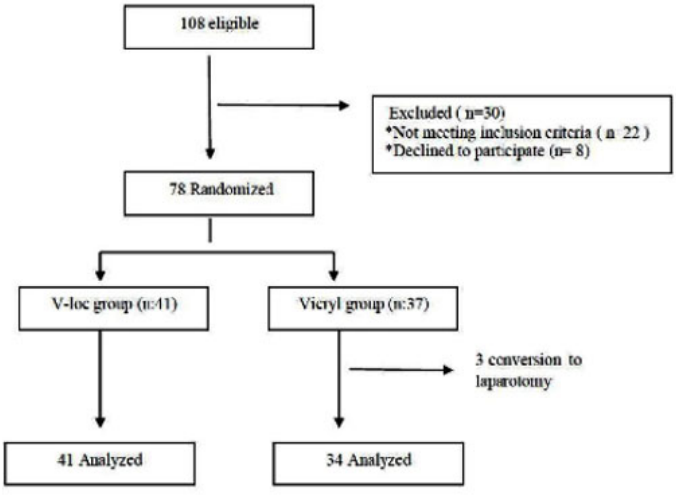

Figure 1. The study flow diagram

Table 1. Demographic values and characteristics

Characteristic	V-Loc suture (n=41)	Vicryl suture (n=34)	p-value
Age (years)	35.98±1.011	36.91±0.991	0.515
Weight (kg)	63 (48-90)	65 (50-97)	0.184
Height (cm)	161.76±0.918	161.09±1.041	0.631
Body mass index (kg/m^2)	23.4 (17.3-37.6)	25.9 (20.0-37.5)	0.160
Previous abdominal surgery (n, %)			
Yes	16 (39%)	11 (32.4%)	0.549
No	25 (61%)	23 (67.6%)	
Diameter of the myoma (cm)	7 (4-15)	7 (4-15)	0.643
Weight of removed myoma (g)	130 (35-435)	120 (37-453)	0.819
Localization of myoma (n, %)			
Corpus anterior	17 (41.5%)	12 (35.3%)	0.696
Corpus posterior	11 (26.8%)	8 (23.5%)	
Fundal	13 (31.7%)	14 (41.2%)	
Type of myoma removed (n, %)			
Type 4	13 (31.7%)	11 (32.4%)	0.696
Type 5	17 (41.5%)	12 (35.3%)	
Type 6	11 (26.8%)	11 (32.4%)	
Layers of suturation (n, %)			
1 layer	3 (7.3%)	4 (11.8%)	0.249
2 layers	36 (87.8%)	25 (73.5%)	
3 layers	2 (4.9%)	5 (14.7%)	

Data are presented as mean ± SD, median (range), or n (%)

Table 2. Intraoperative and postoperative results

Result	V-Loc suture (n=41)	Vicryl suture (n=34)	p-value
Operative time (min)	60 (30-150)	90 (30-150)	0.395
Myomectomy time (min)	25 (6-105)	30 (5-100)	0.454
Suture time (min)	15 (8-50)	23.5 (5.0-60.0)	0.007
Intraoperative blood loss (mL)	80 (10-320)	120 (20-500)	**0.018**
Hemoglobin change (g/dL)	1.9 (0.0-4.7)	2.1 (0.1-4.9)	0.116
Hematocrit change	5.0 (0.4-12.1)	5.8 (0.0-14.0)	0.056
Blood transfusion needed (n, %)	3 (7.3%)	8 (23.5%)	**0.048**
Hospital stay (days)	2 (2-4)	2 (2-4)	0.280

Data are presented as mean ± SD, median (range), or n (%)

hemorrhage was not quantitatively measured in either study; instead, hemoglobin decline was calculated to determine the amount of bleeding. However, the hemoglobin concentration is affected by many factors and does not objectively reflect the amount of bleeding. In both trials, the surgeries were performed by two different surgeons. Since the rate of suturation of each surgeon may be different, this may have been a confounding factor that impacted the suturation times[11,14].

In a randomized study by Giampaolino et al.[15], all operations were performed by the same surgeon. Thus, that study was like the current research. However, for all three of these studies, fibroids were not classified by type[11,14,15]. In one study, only the size of the fibroids was recorded[15]. However, it is known that difficulty in suturing after myomectomy is related to the area of the myoma in the myometrium, regardless of the size of the myoma.

In our study, the duration of the operations was the same in both groups because the surgeon was experienced in conventional intracorporeal suturation. If barbed suturing was performed by surgeons with less experience in intracorporeal suturing, the differences between the two types of sutures may be more evident.

Conclusion

This study revealed that the barbed suture facilitates suturing, shortens the suturing time and reduces the need for a postoperative blood transfusion by decreasing the amount of intraoperative bleeding during laparoscopic myomectomy. Additional studies involving less experienced surgeons with suturing could demonstrate its advantages more clearly.

Ethics

Ethics Committee Approval: Approval was obtained from the hospital's local ethics committee before any study-related procedures were conducted (Antalya Training and Research Hospital - approval number: 8/16, date: 19.04.2018).

Informed Consent: The participants provided written informed consent before participation.

Peer-review: Internally peer-reviewed.

Authorship Contributions

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

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Effect of delivery mode on admission to neonatal intensive care unit in healthy singleton pregnancies

Term sağlıklı tekil gebeliklerde doğum şeklinin yenidoğan yoğun bakım ünitesine kabule etkisi

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Abstract

Objective: The aim of this study was to evaluate the short-term results of perinatal health in vaginal and cesarean deliveries and the indications for admission to the neonatal intensive care unit (NICU) in terms of healthy singleton pregnancies.

Materials and Methods: In this study, 300 pregnant women who gave birth in our tertiary hospital was included. The records of newborns admitted to the NICU of these pregnant women were reviewed between January 1, 2019 and January 1, 2021. Durations of newborn hospitalizations and problems encountered during admission were recorded. The results were statistically evaluated.

Results: There was no significant difference between vaginal delivery and cesarean section groups in terms of the indications for admission to the NICU of term low-risk pregnant women (p=0.91, p=0.17). A higher admission in the NICU was found in the early term group. The early term group required more respiratory support compared to the full term group (p=0.02). When the groups were compared in terms of IV fluid treatment support, hypoglycemia or feeding difficulty, and jaundice requiring phototherapy, no significant difference was found.

Conclusion: Withlimited data available for admission indications to the NICU of newborns born from term pregnancies, we found that the mode of delivery affects hospitalization indications of newborns, need for support, and Apgar scores. Early term delivery is associated with higher rates of neonatal morbidity and admission to the NICU. Better maternal care and prevention of factors that may lead to preterm birth will provide the prevention and management of these problems.

Keywords: Neonatal intensive care unit, neonatal outcome, morbidity

Öz

Amaç: Bu çalışmanın amacı term sağlıklı tekil gebelerde vajinal ve sezaryen doğumlarda yenidoğan yoğun bakım ünitesine yatış endikasyonlarını ve perinatal sağlığın kısa dönem sonuçlarını değerlendirmektir.

Gereç ve Yöntemler: 1 Ocak 2019 - 1 Ocak 2021 tarihleri arasında üçüncü basamak hastanemizde doğum yapan toplam 300 gebe çalışmaya dahil edildi. Bu gebelerden yenidoğan yoğun bakım ünitesine yatırılan bebeklerin kayıtları incelendi. Hastanede yatış endikasyonları, süreleri ve yatış sırasında karşılaşılan sorunlar kayıt altına alındı ve sonuçlar istatistiksel olarak değerlendirildi.

Bulgular: Term düşük riskli gebelerde vajinal doğum ve sezaryen grupları arasında yenidoğan yoğun bakım ünitesine (YYBÜ) yatış endikasyonları açısından anlamlı fark bulunmadı (p=0,91; p=0,17). Erken term grubunda YYBÜ'ye yatış oranı daha yüksek bulundu. Erken term grubu, full term grubuna kıyasla daha yüksek solunum desteği ihtiyacına sahipti (p=0,02). İv sıvı tedavisi ihtiyacı, hipoglisemi veya beslenme güçlüğü ve fototerapi gerektiren sarılık açısından karşılaştırıldığında; gruplar arasında anlamlı fark bulunmadı.

PRECIS: We concluded that the mode of delivery affects hospitalization indications of newborns, need for support, and Apgar scores. Early term delivery is associated with higher rates of neonatal morbidity and admission to the NICU.

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Sonuç: Term gebeliklerden doğan yenidoğanların hastaneye yatış endikasyonlarıyla ilgili mevcut veriler sınırlıdır. Doğum şekli yenidoğanların hastaneye yatış endikasyonlarını, destek ihtiyacını ve Apgar skorlarını etkiler. Erken termde doğum daha yüksek oranda neonatal morbidite ve YDYB yatışı ile ilişkilidir. Daha iyi maternal bakım ve erken doğuma yol açabilecek faktörlerin engellenmesi olası sorunların önlenmesini ve yönetilmesini sağlayacaktır.

Anahtar Kelimeler: Yenidoğan yoğun bakım ünitesi, yenidoğan sonuç, morbidite

Introduction

The definition of term pregnancy is relative. Increasing evidence has shown that neonatal outcomes of early- and full-term infants are different. Early-term newborns have more respiratory distress requiring mechanical ventilation and longer hospital stays. Mortality is increased in these cases. Neonatal intensive care unit (NICU) admission probability compared to term infants is significantly higher[1,2].There appears to be a sustained relationship between neonatal morbidity and gestational age in specific term and preterm labors[3].

For the fetus, the effects of labor on the baby are not clear. Ongoing pregnancies may result in stillbirth or the need for NICU. Some studies indicate that patients who delivered vaginally need NICU at a higher rate than pregnant women who gave birth by cesarean section. In a study comparing cesarean and vaginal deliveries, the number of newborns with an Apgar score below 7 in the first minute was found to be higher in the cesarean section group. In the same study, the 7th minute Apgar score and umbilical cord pH were found to be similar between the two groups[4,5]. Cesarean deliveries can be a life-saving procedure. Adverse events may occur in both mothers and newborns[6,7].

While asphyxia, trauma and meconium aspiration decrease in cesarean delivery, the risk of transient tachypnea of the newborn, surfactant deficiency and respiratory distress secondary to pulmonary hypertension increases. Physiological events in the last weeks of pregnancy are accompanied by changes in the hormonal environment of the fetus and mother with the onset of spontaneous delivery. The fetus is prepared for neonatal transition[8].

The most common neonatal complications in admission to the NICU are respiratory morbidity, hypoglycemia, and sepsis. Newborn pulmonary problems, especially respiratory distress syndrome (RDS), may follow cesarean delivery. Moreover, the admission of the newborn to the NICU has various negative effects on the family[9].

In the literature, there are insufficient evidence evaluating the indications for admission to NICU in term healthy singleton pregnancies according to the mode of delivery. In this study, we evaluated the short-term results of perinatal health in vaginal and cesarean deliveries and the indications for admission to the NICU.

Materials and Methods

The study was planned retrospectively and approved by the ethics committee at the Ankara City Hospital (approval no: E1-20-295).

The hospital records of pregnant women hospitalized for delivery in Ankara City Hospital NICU between January 1, 2019 and January 2021 were analyzed from accumulated data. Women who gave consent for the study were between 37th and 42nd gestational weeks so they are term healthy singleton pregnant women without any additional systemic diseases. Vaginal delivery and cesarean section were included. Due to the need for intensive care, the babies were hospitalized and then discharged as healthy. The data of the patients were obtained retrospectively from computer records and patient files. Deliveries were categorized according to weeks of gestation. The date of the last menstrual period was taken as the basis for determining the gestational age. When pregnant women whose ultrasound measurements were inconsistent with the last menstrual period or in those who did not know or remember the last menstrual period, gestational age was determined according to the earliest ultrasound measurements.

Those who were born under 37 weeks, pre-pregnancy genital and extragenital disease, hypertension, preeclampsia, gestational diabetes, goiter, anxiety and depressive disease, drug addiction, multiple pregnancy, maternal age below 18 and over 45, fetuses with anomalies in detailed ultrasonography, patients with premature rupture of membranes, additional systemic disease, obstetric conditions that may cause premature or emergency delivery, pre-eclampsia and those whose medical records could not be reached were excluded from the study. Indications for NICU admission and maternal demographic and labor characteristics were retrospectively evaluated from the file records. Demographic data collected included; age, gravidity, parity, gestational age at cesarean section, birth weight, hypoglycemia, length of hospitalization, respiratory morbidity, need for NICU admission, mechanical ventilation and phototherapy.

Statistical Analysis

All data related to the disease were recorded in SPSS 22.0 (SPSS for Windows Evaluation, Illinois, Chicago, USA) and statistical analyses were performed. Arithmetic mean ± standard deviation for descriptive parametric data from the vaginal labor group and the cesarean section group, and ratios and percentages were used for nonparametric data. The distribution of the data was evaluated by the Kolmogorov-Smirnov test. The independent t test or Mann-Whitney U test was used to compare parametric values between the two groups. Nonparametric values were compared between the two groups by Pearson's chi-square test or Fisher's exact test. $P<0.05$ was considered statistically significant.

Results

A three hundred pregnant women were included in this study group. One hundred eighty two had vaginal deliveries and 118 had cesarean deliveries. The main demographic characteristics of the patients are shown in Table 1.

There was no significant difference between the distribution of the mean age of women according to the mode of delivery. The mean gestational age of women was 37.6±1.9 weeks and 37.3±1.8 weeks for the vaginal delivery group and the cesarean group respectively (p>0.05, p=0.37). There was no statistically significant difference between the groups in terms of duration of hospital stay, estimated fetal weight, newborn birth weight and birth length (p>0.05, p=0.23, p=0.12, p=0.84, and p=0.74 respectively). The rates of pregnant women with premature rupture of membranes were 18.6% and 11.8% for the vaginal delivery group and the cesarean group, respectively (Table 2). The indications for admission to the NICU were compared according to the mode of delivery. Transient tachypnea of the newborn was 6.4% in the vaginal delivery group and 21.1% in the cesarean section group. Meconium-stained amniotic fluid was 11.5% and 9.3% for the vaginal delivery group and cesarean group respectively. Congenital pneumonia was 3.2% and 3.3% respectively. Early sepsis was 3.8% and 2.5% respectvely. There was no significant difference between vaginal delivery and cesarean section groups in terms of indications for admission to the NICU (p>0.05). The results are summarized in Table 3.

Hundred and forty early - term and 160 full - term pregnant women were hospitalized. A higher admission to the NICU was found in the early-term group. The early term group had a higher need for respiratory support compared to the full term group (p<0.05, p=0.02). When the groups were compared for need iv fluid treatment, hypoglycemia, feeding difficulty, and jaundice requiring phototherapy, there was no significant difference (p>0.05, p=0.36, p=0.32, and p=0.21 respectively) Table 4.

Table 1. Demographic and clinical characteristics

Age, years (Mean ± SD)	27.3±5
Gravidity, (Mean ± SD)	2.2±1.2
Gestational age at delivery, weeks (Mean ± SD)	37.5±1.9
Gestational age at delivery according to Ultrasound (Mean ± SD)	37.4±1.8
Weight gain during pregnancy (kg) (Mean ± SD)	12.4±4.4
Estimated fetal weight (g) (Mean ± SD)	3450±390
Birth weight (g) (Mean ± SD)	3133±485
Birth length (cm) (Mean ± SD)	34.9±1.7
APGAR score min 5, (Mean ± SD)	6.8±1.1
APGAR score min10 (Mean ± SD)	8.5±0.9
SD: Standard deviation	

Discussion

Neonatal morbidity rates vary with the mode of delivery. The infants born by cesarean section are more likely to develop respiratory morbidity. In contrast, infants born vaginally are more likely to develop intracranial hemorrhage, brachial plexus injury, and culture-positive neonatal sepsis[10-11].

Life-threatening maternal outcomes are more common in cesarean deliveries regardless of previous vaginal delivery history. In the literature, the age ranges of pregnant women vary in studies investigating the indications for admission in the NICU according to the mode of delivery[12-13].

Catalano and Sacks[13] found 31.2±5.7 years for pregnant women. Similar to our study, in another study, the mean age was again 29.3±6.6 years. In our study, the mean age of pregnant women was 27.3±5 years. Similar studies have revealed that the age of women giving birth differs between 27 and 38.9 years[14].

Transient tachypnea of the newborn was first described by Avery et al.[15] in 1966. The main cause is the inability to excrete

Table 2. Comparison of neonatal obstetric outcomes according to delivery mode

		Vaginal labor 182	Cesarean section:118	p-value
Age, years (Mean ± SD)[a]		27.1±5.4	27.8±5.5	0.37
Gestational age at delivery, weeks (Mean ± SD)[a]		37.6±1.9	37.3±1.8	0.73
Gestational age at delivery according to ultrasound (Mean ± SD)[a]		37.4±1.9	37.4±1.8	0.16
Weight gain during pregnancy (g) (Mean ± SD)[a]		12.2±4	12.8±4.9	0.23
The duration of hospital stay Stay (day) (mean ± SD)[a]		2.5±1.3	1.7±1.1	0.23
Estimated fetal weight (Mean ± SD)[a]		3445±294	3464±332	0.12
Birth weight (g) (Mean ± SD)[a]		3113±487	3169±481	0.84
Birth length (cm) (Mean ± SD)[a]		34±1.4	34±2.06	0.74
Premature rupture of membranes n (%)[b]		34 (18.6)	14 (11.8)	0.16
Meconium-stained amniotic fluid n (%) [b]		32 (17.5)	20 (16.9)	0.87
Apgar score	1.minute (Mean ± SD)[a]	9.00±0.708	8.00±0.806	0.74
	5.minute (Mean ± SD)[a]	10.00±0.305	9.00±0.417	0.86
A p-value <0.05 is considered statistically significant, A: Independent sample t test was used in statistical analyses, B: Pearson chi-square test was used in statistical analyses. G: Gram, cm: Sentimeter, H: Hour, min: Minutes				

Table 3. Comparison of the indications on admission to NICU according to delivery mode

	Vaginal labor:182	Cesarean section :118	p-value
Transient tachypnea of the newborn, n (%)[b]	12 (6.5)	25 (21.1)	0.91
Respiratuar distress sendrome, n (%)[b]	5 (2.7)	10 (11.8)	0.17
Meconium aspiration (%)[b]	21 (11.5)	11 (9,3)	0.21
Congenital pneumonia, n (%)[b]	6 (3.2)	4 (3.3)	0.15
Early sepsis, n (%)[b]	7 (3.8)	3 (2.5)	0.72
Neonatal asphyxia, n (%)[b]	11 (6.04)	8 (6.7)	0.48
Congenital heart disease, n (%)[b]	5 (2.7)	3 (2.6)	0,35
Pneumothorax, n (%)[b]	2 (1.09)	3 (2.5)	0.40
Cyanotic events n (%)[b]	4 (2.1)	5 (4.2)	0.90
Jaundice requiring phototherapy, n (%)[b]	13 (7.1)	5 (4.2)	0.36
Hypoglycemia, n (%)[b]	8 (4.3)	9 (7.16)	0.87
Hypothermia, n (%)[b]	1 (0.5)	2 (1.6)	0.20
Neonatal death†	0	0	
Days of hospital stay[a]	1.9±1.3	2.7±1.8	0.26

A p-value <0.05 is considered statistically significant, A: Independent sample t-test was used in statistical analyses, B: Pearson chi-square test was used in statistical analyses. G: Gram, cm: Centimeter, H: Hour, min: Minutes, NICU: Neonatal intensive care unit

Table 4. Comparison of morbidities of early- and full-term newborns

Indications on admission to NICU	Early term pregnant:140	Full - term pregnant :160	p-value
Need for respiratory support n (%)	25 (17.8)	12 (7.5)	0.02*
Hypoglycemia, n (%)	12 (8.5)	9 (5.6)	0.36
Iv fluid therapy, n (%)	36 (25.7)	29 (18.1)	0.49
Feeding difficulty n (%)	19 (11.8)	15 (9.3)	0.32
Jaundice requiring phototherapy n (%)	15 (10.7)	12 (7.5)	0.21

*p-value <0.05 is considered statistically significant. A: Independent sample t-test was used in statistical analyses, B: Pearson chi-square test was used in statistical analyses. G: Gram, cm: Centimeter, H: Hour, min: Minutes

fluid. In our study, temporary tachypnea was found in 12 and 25 newborns in the term low-risk vaginally delivery group and newborns in the cesarean section group, respectively. The results were consistent with the literature[16].

In the study of Dani et al.[17], 11% of the newborns were admitted to the intensive care unit due to respiratory problems. RDS were diagnosed in 6% of them. In our study, the RDS rates were 2.7 and 11.8 for the vaginal delivery group and the cesarean group, respectively.

Mothers who had vaginal labor started breastfeeding earlier than those who had cesarean section. Especially women who have general anesthesia during C-sections start breastfeeding later than those who gave labor vaginally. It is thought that there may be a significant decrease in the comfort of mothers compared to those who gave vaginal labor due to reasons such as pain, fatigue, and negative effects of anesthesia after cesarean delivery[16]. In our study, feeding difficulty was seen in 11.8% and 9.8% of early term newborns and full-term newborns, respectively.

In the literature, Apgar scores of newborns were examined according to delivery mode. 1st and 5th minute Apgar scores of newborns with vaginal delivery were found to be higher than cesarean section deliveries[18-20]. In our study, there was no difference between the two groups according to Apgar scores (p>0.05).

In obstetric and pediatric practice, late preterm infants are often considered functionally and developmentally mature and are often managed by protocols developed for full-term infants. It is well known that cesarean delivery is an important factor that causes morbidity in newborns[13]. Cesarean delivery has been reported to cause a longer hospital stay and more respiratory morbidities[11,12]. In our study, the most common cause of admission to the NICU was respiratory morbidities. Transient tachypnea of the newborn is the most common among these morbidities. In line with the literature, the hospital stay was longer in the cesarean delivery group[21,22].

Hypoglycemia and electrolyte disorders are reported to be more common morbidities in early-term newborns. In our study, hypoglycemia was more common in early-term newborns. Our finding was consistent with the literature. However, when compared with term newborns, it was not statistically significant. In our study, more dehydration was observed in premature term newborns. IV hydration was applied to them[23]. The need for IV antibiotic therapy was higher in the early-term group in our study, as indicated in the literature[21]. Antibiotic treatment was given with the indication of maternal urinary tract infection and positive cervical culture.

When the literature was reviewed, Binarbaşı et al.[24] found feeding problems with a rate of 9.1% in a study on late preterm infants. In addition, the admission rate due to nutritional intolerance was found to be 5.3%. In our study, it was found to be 11.8 in early term and 9.3 in late term pregnancies. As stated in the literature, this can be explained by the slower sucking of these babies and the more frequent feeding intolerance. Since a significant number of newborns were hospitalized due to respiratory distress, the delayed onset of feeding can also be considered as a factor[24].

McIntire and Lenevo[25] compared the neonatal outcome of late preterm infants with that of infants born at 39 weeks of gestation. Overall, the rate of neonatal morbidity in their report

seems to be higher than in our study, especially about respiratory complications and clinical jaundice requiring phototherapy. In contrast to our study, cesarean delivery was not associated with an increased risk of neonatal morbidity. In the study of Bastek et al.[26] cesarean delivery was a significant risk factor for neonatal morbidity. The relationship of respiratory complications with parity was not reported in previous studies.

In recent years, the effect of mode of delivery (eg, vaginal delivery or cesarean section) on newborns born from term pregnancies has been examined due to the increasing rates of cesarean section and their association with allergic and autoimmune diseases[27,28].

In our study, in terms of meconium-stained amniotic fluid, there was no significant difference between cesarean section and vaginal delivery groups (p=0.87). Other small-sample studies also showed no significant difference in meconium between newborns delivered by C-section and vaginally[29].

Hu et al. evaluated meconium-stained amniotic fluid microbiota in full-term cesarean section and vaginal birth groups.

Their study reported no significant differences in the meconium microbiota of full-term vaginally and cesarean section-delivered newborns[30].

We could not evaluate the meconium microbiota in our study because we had no data.

Study Limitations

There are several limitations to our study, which are mainly related to its retrospective design and relatively small number of patients. The study was single-center. Late preterm babies may be more likely to be diagnosed with more subjective diagnoses (transient tachypnea of the newborn, sepsis diagnosis) than term babies. However, Another limitation is that we do not have data on long - term neonatal outcomes.

Conclusion

With the limited data available for admission indications on term pregnancies, the results of the study showed that the mode of delivery affects the indications for hospitalization, the need for help and support, and the Apgar scores of newborns. This finding should be considered while deciding on the mode of delivery. However, in cases where vaginal delivery is not possible or delayed delivery is dangerous for the mother or the baby, cesarean section should be performed.

Ethics

Ethics Committee Approval: The study was planned retrospectively and approved by the ethics committee at the Ankara City Hospital (approval no: E1-20-295).

Informed Consent: Informed consent was obtained.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: F.E.C., Concept: H.U., Design: R.B.F., Data Collection or Processing: F.E.A., Analysis or Interpretation: Ö.U., Ö.M.T., Literature Search: H.U., Writing: H.U.

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

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Effects of allium cepa on ovarian torsion-detorsion injury in a rat model

Ratlarda ovaryan torsiyon-detorsiyon modelinde allium cepanın etkilerinin incelenmesi

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Abstract

Objective: Ischemia/reperfusion (I/R) damage following detorsion treatment, tissue fibrosis, and adhesions cause secondary tissue damage in the ovaries. Many studies have been evaluated to minimize antioxidant damage in ovarian reserve loss while minimizing I/R damage. However, no study observed long-term effects on the ovarian torsion model in rats. In this study, we evaluated the profibrotic effects of A. cepa on an ovarian torsion model on rats.

Materials and Methods: Group I (n=7) rats were the sham group. Group II (n=7) rats were the torsion group and Group III (n=7) rats were the torsion + A. cepa group. To observe the long-term effects of allium cepa, rats were fed for 21 days. Cellular damage I/R is evaluated by histopathological damage score, and transforming growth factor-beta 1 (TGF-β1) and alpha-smooth muscle actin (α-SMA) is measured to analyze the profibrotic effect.

Results: A. cepa altered cellular damage due to improvement in the histopathological damage score with A. cepa intake. However, the profibrotic mediators TGF-β1 and α-SMA are non- significantly changed by the A. cepa (p=0.477 and p=0.185 respectively).

Conclusion: A. cepa is a potent protective on cellular tissue, minimizing I/R damage on ovarian tissue histologically. Our study implies that A. cepa does not affect fibrosis-related mediators in the rat ovary.

Keywords: Allium cepa, fibrosis, ovarian torsion, ischemia-reperfusion injury, TGF-β1, α-SMA

Öz

Amaç: Over torsiyonunda tedavi amaçlı uygulanan detorsiyon işlemine sekonder oluşan iskemi/reperfüzyon hasarının (I/R); dokuda oluşan fibrozis ve adezyonlara bağlı olarak over dokusuna sekonder hasar verebilir. Over torsiyonunda literatürde birçok çalışma oxidan hasara bağlı over rezervini minimalize etmeye yönelik birçok çalışma mevcuttur. Ancak, literatürde iskemi hasarının torsiyon sonrası uzun dönemde ovarian yapısal değişikliklerine dair bir çalışma mevcut değildir. Bu çalışmada A. cepa'nın profibrotik mediatörler üzerine etkisinin incelenmesi rat modeli üzerinde amaçlanmıştır.

Gereç ve Yöntemler: Grup I (n=7) ratlar kontrol grubu olarak belirlenmiştir. Grup II (n=7) torsiyon-detorsiyon modeli ve Grup III (n=7) torsion-detorsiyon + A. cepa rejimi uygulanacak gruplar olarak belirlenmiştir. Adezyon ve fibrotik değişimlerin izlenmesi için ratlar prosedur sonrası 21 gün beslendi. Hücresel hasar düzeyi "Histopatolojik Hasar skoru" ile ölçüldü. Fibrotik değişiklikler için dönüşen büyüme faktörü-beta 1 (TGF-β1) ve alfa-düz kas aktini (α-SMA) düzeyleri ölçüldü.

Bulgular: A. cepa ile beslenen ratlarda Histopatolojik Hasar skorunda anlamlı düşüş izlendi. Ancak TGF-β1 (p=0,477) ve α-SMA (p=0,185) düzeylerinde istatistiksel olarak anlamlı değişim izlenmedi.

Sonuç: A. cepa, hücresel düzeyle hasarı minimalize etmede potent bir mediatör olarak öngörülürken, profibrotik mediatörler olan TGF-β1 ve α-SMA düzeylerinde anlamlı bir değişiklik oluşturmamıştır.

Anahtar Kelimeler: Allium cepa, fibrozis, over torsiyonu, iskemi-reperfüzyon hasarı, TGF-β, 1, α-SMA

PRECIS: Whether allium cepa has an improving mediator for fibrosis and ovarian damage on ovarian torsion-detorsion injury in a rat model investigated in this study.

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Introduction

Ovarian torsion is an acute pathology of reproductive age that causes a decreased ovarian reserve[1]. Approximately 2.5% to 7.4% of acute abdominal pain cases are diagnosed with ovarian torsion[2]. The pathophysiology of the disease is stasis in arterial and venous blood flow following the rotation of the ovarian tissue from the pedicle itself. Sudden -onset pelvic pain without relief by analgesics, leukocytosis, vomiting, fever, and nausea are the most common symptoms of the disease[3]. Ultrasound imaging findings are the medialized and increased ovarian size, decreased vascular flow, and diagnostic for ovarian torsion[4]. Benign ovarian masses and cysts, particularly dermoid cysts, predispose ovarian torsion incidence[5].

Laparoscopic or laparotomic surgery is the mainstay of treatment, and early diagnosis is essential for minimizing ischemic damage to the ovarian tissue[6]. Moreover, following the laparoscopic detorsion procedure, the reversibility of ovarian tissue is limited because of the ischemia/reperfusion damage (I/R damage) by oxidative stress[7]. The management of ovarian torsion is also important for fertility preservation at reproductive age. According to the studies, providing detorsion within 24 h following ovarian torsion is related to a better ovarian reserve and minimizing ovarian tissue necrosis[8]. Reactive oxygen species (ROS), cause cellular damage by interaction with biomolecules of cells during reperfusion damage[9]. Increased ROS levels stimulate inflammasome expression [Nucleotide-binding and leucine-rich repeat (NLR) genes] by NF-kB mediators. Increased inflammasome expression stimulates profibrotic macrophages and inflammation together[10].

Transforming growth factor-beta 1 (TGF-β1) and alpha-smooth muscle actin (α-SMA) are potential biomarkers to analyze myofibroblast activity and fibrosis in human tissues[11,12]. Moreover, increased ROS and inflammatory mediators may increase fibrosis. Hepatic fibrosis after CCl_4 intoxication in nicotinamide adenine dinucleotide phosphate oxidase deficient mice models reported with ROS[13]. Moreover, in previous studies silibinin that is an antioxidant molecule, improved hepatic fibrosis and regeneration[14].

After ovarian torsion, antioxidant molecules may help minimize I/R damage mechanisms[15]. Many antioxidant molecules have been studied for minimizing ovarian tissue damage for maintaining ovarian functions. Allium Cepa Liliaceae (A. cepa) is a widely known onion bulb a plant that belongs to the botanical family Amaryllidaceae[16]. Quercetin, flavonoids, saponins, and organosulfur are the main components of A. cepa[17]. With these rich derivatives, A. cepa has plenty of therapeutic benefits. Antibiotic, antidiabetic, anti-teratogenic, and anti-inflammatory effects of A. cepa has been widely studied[17,18]. Moreover, A. cepa is compared with Alfa-Tocopherol and Vitamin-C, which are proven antioxidant molecules, and reported that A. cepa is a potential antioxidant molecule[19].

In this study, we investigated whether A. cepa impacts profibrotic mediator levels and histological improvement. We designed a rat ovarian torsion-detorsion model to measure profibrotic mediator levels and evaluate histological evaluation scores for irreversible cellular damage on ovarian tissue.

Materials and Methods

This study was approved by the institutional review board at Dokuz Eylül University Laboratory Animals Local Ethics Committee (no: 40-2020). Twenty-one adult Sprague-Dawley rats (180-250 grams) were collected from Dokuz Eylul University Experimental Animal Laboratory. Rats were sheltered in standard steel cages at a room temperature of 22 °C±2 °C, with 12 h light/dark cycles. Standard rat chow and tap water were provided to rats with ad libitum. Vaginal smears were performed at 6-12-hour intervals[20]. Rats that are all in the estrus phase are included in the study.

Study Protocol

Twenty-one rats were randomly divided into three groups that consisted of seven animals. Surgical procedures were performed under sterile conditions. Anesthesia conditions were provided with an intraperitoneal injection of 50 mg/kg ketamine hydrochloride (Ketalar, 50 mg/kg, Pfizer) and 7 mg/kg xylazine hydrochloride (Alfazyne; Alfasan International BV, Holland).

Under anesthesia, a 2 cm-midline sterile incision opened in the lower abdomen of the rats. Uterine horns, adnexa, and ovaries were identified. In Group I, only a sterile abdomen incision was made. Both ovaries of rats were rotated to the right 360 degrees and clamped over 2.5 h in Group II and III rats. Tissues were detorsioned after 3 h. A. cepa powder was obtained from fresh onion bulbs. Onion bulbs were peeled and dried on air within one week. Normal feeds of rats fortified with A. cepa powder in specific groups.

Group I (n=7) rats received only saline (0.9% NaCl) with oral gavage daily. Group II (n=7) (torsion/detorsion group) received only saline (0.9% NaCl) with oral gavage daily. Group III (n=7) (torsion/detorsion+ A. cepa group) rats received 20% Allium cepa powder + 80% normal feed with oral gavage per day. Each group was fed for 21 days with planned regimens to observe the long-term effects of allium cepa on ovarian torsion models. Rats were sacrificed on day 21st under anesthesia and the ovaries were collected for histopathological and biochemical examination. During the trial, none of the rats died and any adverse effects (hair loss, fatigue, loss of appetite, etc.) did not observed in rats due to A. cepa intake.

Histopathological Examination

The ovaries were embedded in paraffin blocks after formalin fixation. 5 µm thick tissue sections were obtained, stained in hematoxylin-eosin, and evaluated with light microscopy (CX-41, Olympus). Follicular degeneration, vascular congestion, hemorrhage, inflammatory cell presence, and primordial, primary, secondary, and tertiary follicle count scores were evaluated on histopathological examination.

Follicle counting was performed according to the study by Parlakgumus et al.[21]. The ovarian histopathological damage score was evaluated based on the following parameters: follicle cell degeneration, vascular congestion, hemorrhage, and inflammation (0: None, 1: Mild, 2: Moderate, 3: Severe)[22].

Biochemical Examination

The ovarian tissues were collected to detect TGF-beta1 and alfa-SMA levels in rats. TGF-β1 and α-SMA (BTLAB, catalog numbers E1688Ra and E2330Ra) were quantitatively assessed using commercially available enzyme-linked immunosorbent assay kits according to the manufacturer's instructions.

Statistical Analysis

Statistical analysis of the data obtained in the process of the study was done with SPSS (Statistical Package for Social Sciences) 26.0 computer package program. Mean ± standard deviation was determined in the evaluation. The biochemistry and parametric data were summarized as mean ± standard deviation. The difference between the groups was analyzed using the Kruskal-Wallis test, from which group the difference originated was analyzed with the Mann-Whitney U test. A p-level of <0.05 was accepted to demonstrate statistical significance.

Results

Results of the Histopathological Examination

The mean scores of the ovarian damage scores are shown in Table 1. Follicular degeneration was significantly higher in Group II than in Groups I and III [(1±0) vs. (2.5±0.5) vs. (1.5±0.7), p<0.01 respectively]. Vascular congestion was significantly higher in Group II than in Groups I and III [(1.7±0.7) vs. (2.5±0.5) vs. (1.2±0.7), p<0.05 respectively].

Table 1. The mean scores of ovarian damage scores

	Group I	Group II	Group III	p-value
Follicular degeneration	1±0	2.5±0.5*	1.5±0.7	p<0.01
Vascular congestion	1.7±0.7	2.5±0.5**	1.2±0.7	p<0.05
Hemorrhage	1±0	2.1±0.6*	1.4±0.7	p<0.01
Inflammatory cell	1.4±0.7	2.4±0.5**	1.7±0.4	p<0.05
Primordial follicle	5.7±0.7	3±0.8*	5.2±0.7	p<0.01
Primary follicle	4.8±0.3	2±0.8*	4.4±0.7	p<0.01
Secondary follicle	4±1.2	1.8±0.6*	3.5±0.9	p<0.01
Tertiary follicle	3.1±0.3	1.7±0.7*	2.5±0.5	p<0.01

*: p<0.01, Group 2 compared with Groups I and III, **: p<0.05, Group 2 compared with Groups I and III

Hemorrhage was significantly higher in Group II than in Groups I and III [(1±0) vs. (2.1±0.6) vs. (1.4±0.7), p<0.01 respectively]. Inflammatory cell levels were significantly higher in Group II than in Groups I and III [(1.4±0.7) vs. (2.4±0.5) vs. (1.7±0.4), p<0.05 respectively].

Primordial follicle count was significantly lower in Group II than in Groups I and III [(5.7±0.7) vs. (3±0.8) vs. (5.2±0.7), p<0.01 respectively]. Primary follicle count was significantly lower in Group II than in Groups I and III [(4.8±0.3) vs. (2±0.8) vs. (4.4±0.7), p<0.01 respectively]. Secondary follicle count was significantly lower in Group II than in Groups I and III [(4±1.2) vs. (1.8±0.6) vs. (3.5±0.9), p<0.01 respectively]. Tertiary follicle count was significantly lower in Group II than in Groups I and III [(3.1±0.3) vs. (1.7±0.7) vs. (2.5±0.5), p<0.01 respectively]. Histopathologic images of the ovaries are shown in Figure 1.

Results of the Biochemical Examination

TGF-β1 and α-SMA levels were compared between the groups. Levels of TGF-β1 and α-SMA are shown in Table 2. α- SMA levels did not change statistically significantly with A. cepa among Groups A, B, and C (34.8±14 vs. 43.6±5.2 vs. 52.6±18.9, p=0.185, respectively). Moreover, there was no statistically significant decrease observed in TGF-β1 levels among Groups A, B, and C (506.9±109.5 vs. 472.4±131.9 vs. 537.6±101.7 p=0.477, respectively).

Discussion

Ovarian torsion is a critical emergent situation that causes ovarian damage, followed by decreased fertility potential and follicular reserve. Early diagnosis and detorsion are the primary

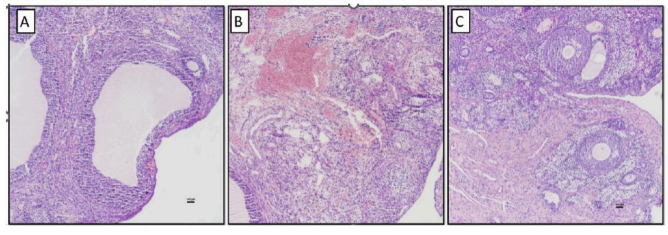

Figure 1. Histopathologic images of the ovaries (H&E staining) (4×). A: Group I: Sham-operated group, B: Group II Torsion/detorsion group, C: Group III Torsion/detorsion + A. cepa group

Table 2. Levels of α-SMA and TGF-β1

	Group I	Group II	Group III	p-value
α-SMA (mean ± standard deviation)	34.8±14	43.6±5.2	52.6±18.9	0.185
TGF-β1 (mean ± standard deviation)	506.9±109.5	472.4±131.9	537.6±101.7	0.477

steps of treatment to minimize this loss. I/R damage with ROS after detorsion is another tissue -damaging factor in ovarian torsion. Excessive ROS production stimulates lipid peroxidase production and SOD, catalase (CAT), and glutathione peroxidase levels for irreversible injury[23,24]. Although the I/R damage mechanism on ovaries with detorsion has not been fully understood, many antioxidants have been used to minimize ovarian tissue loss with reperfusion injury.

Ovarian histopathological evaluation is one of the most decisive methods for estimating I/R tissue damage[25]. We used histopathological tissue damage scores to estimate whether A. cepa has an antioxidant effect. This study demonstrates that A. cepa is a potential alleviating antioxidant in follicular degeneration ($p<0.01$), vascular congestion ($p<0.05$), hemorrhage ($p<0.01$), inflammatory cell presence ($p<0.05$), and primordial, primary, secondary, and tertiary follicle count scores ($p<0.01$). No study in the literature evaluated the antioxidant potential of A. cepa on the ovarian tissue. According to our results, A. cepa improves the histological findings of ovarian I/R damage. Previous studies have reported that A. cepa has an antioxidant effect on the liver, kidney, and brain tissues with decreased malondialdehyde (MDA) levels and increased amino acid levels[26].

Polymorphonuclear leucocytes and macrophages migrate to the ischemia zone because of increased signaling on the damaged tissue[27]. Under ischemic conditions, myofibroblasts may trigger the granulation tissue and fibrosis by secreting platelet-derived growth factors (PDGF), epidermal growth factor (EGF), TGF-β1, and α-SMA[28]. Overexpressed myofibroblast activity may predispose fibrosis and adhesions in damaged tissues[29]. TGF-β1 is the main activating mediator of fibroblast activity with induced myofibroblast migration and activation to inflammation with increasing α-SMA levels[30]. This shows that TGF-β1 is one of the most important profibrogenic mediators of wound tissue remodeling and increases structural stability in damaged tissues[28]. Moreover, TGF-β1 impacts fibrosis and scarring with induced extracellular matrix (ECM) production by myofibroblast activity[31]. Elongated or overexpressed myofibroblast activity may result in fibrosis and organ function abnormalities[29].

Fujishita et al.[32] reported that following laparoscopic ovarian detorsion operation, tubal occlusion, and pelvic adhesions occurred at second-look laparoscopy. Fibrosis negatively affects tissue healing properly and plays a key role in pelvic adhesions. These conditions may cause pelvic structural abnormalities, chronic pelvic pain, and decreased vascular perfusion on I/R damaged ovary.

The TGF-β1 expression has been investigated in many studies. A study reported that atypical prostate hyperplasia decreased TGF-β1 levels and increased IGF levels by A. cepa intake may induce hyperplasia in the gland[33]. Increased hyperplasia levels based on that increased proptosis and inhibited tissue proliferation provided by TGF-β1[34]. However, in our study, there was no statistically significant difference in TGF-β1, and α-SMA levels between Groups I, II, and III ($p=0.477$ and $p=0.185$, respectively). Our results were inconsistent with the previous study. This means that profibrotic mediator levels TGF-β1 and α-SMA might not be hindered by the A. cepa.

Flavonoids such as kaempferol , quercetin, and feruli, cysteine sulfoxides have anti-inflammatory and antioxidant effects on A. cepa[17]. Anticancer, anti-asthmatic, and hepatoprotective features are provided by multiple micronutrients in A. cepa[35]. A. cepa is an important alternative medical nutrient to prevent cell damage based on the histologic evaluation score data. However, there was no correlation observed between the profibrotic mediators TGF-β1 and α-SMA and A. cepa in the ovarian torsion-detorsion model in rats.

Strengths and Limitations of Our Study

This study is an important pilot study observing the long-term effects of ovarian torsion I/R damage and the evaluation of profibrotic mediator levels in the damaged ovarian tissue. These findings should be supported by further studies that will elucidate the fibrotic pathways associated with these mechanisms. However, our study was designed on an experimental rat model. During the administration period, the appropriate dosage may change in the female reproductive system.

Conclusion

The overall results showed that A. cepa may improve the antioxidant cell damage scores histologically. However, the antifibrotic mechanism of A. cepa is still debatable due to non-significant differences in TGF-β1, and α-SMA levels. If our study is supported by further studies, A. cepa is a potential and easily accessible antioxidant nutrition for ovarian detorsion reperfusion injury. Second, a limited number of animals were included in the study due to ethical restrictions.

Ethics

Ethics Committee Approval: This study was approved by the institutional review board at Dokuz Eylül University Laboratory Animals Local Ethics Committee (no: 40-2020).

Informed Consent: Not necessary.

Peer-review: Internally and externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: H.K., O.İ., S.K., Concept: H.K., Design: H.K., Data Collection or Processing: H.K., S.K., F.Y.,Analysis or Interpretation: H.K., Literature Search: H.K., Writing: H.K.

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Review / Derleme

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Efficacy and safety of rectal misoprostol versus intravenous oxytocin on reducing blood loss in cesarean section: A PRISMA-compliant systematic review and meta-analysis of randomized clinical trials

Sezaryen doğumda kan kaybını azaltmada intravenöz oksitosin ile rektal misoprostolün etkinliğinin ve güvenliğinin karşılaştırılması: PRISMA uyumlu sistematik bir inceleme ve randomize klinik çalışmaların meta-analizi

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Abstract

Blood loss is an inevitable complication and a major contributor to maternal morbidity and mortality at cesarean deliveries. We detected a potential preference regarding the efficacy and safety of rectal misoprostol over oxytocin as a uterotonic agent. We searched PubMed, Scopus, Web of Science, Cochrane, and other databases for the relevant trials from inception to September 2022. We included randomized clinical trials (RCTs) that compared rectal misoprostol versus intravenous oxytocin to control bleeding in women undergoing cesarean delivery. Our primary outcomes were the intra- and postoperative blood loss, and hemoglobin drop after delivery. Secondary outcomes included the need for blood transfusion, need for additional uterotonics, difference in operative time, as well as safety outcomes such as the incidence of shivering, pyrexia, nausea, and vomiting. Our search strategy revealed 1007 unique records, of them we retrieved full texts of 19 articles to check their adherence to our eligibility criteria. Seven RCTs with 1,090 participants were included. We found a significant reduction in postoperative blood loss [MD: -27.9; 95% confidence interval (CI): (-53.85, -2.10); p=0.03], and Hb drop after delivery [MD: -11; 95% CI: (-0.19, -0.03); p=0.01]. There is no significant difference regarding intraoperative blood loss, operative time, need for blood transfusion, or need for additional uterotonics. We could not find a significant difference between the two groups regarding safety outcomes, except for a higher shivering incidence in the misoprostol group [RR: 0.33; 95% CI; (0.16, 0.70); p=0.004]. We found a significant reduction in postoperative blood loss with a potentially favorable safety profile in women who administrated rectal misoprostol compared with oxytocin administration. Our findings recommend and prefer rectal misoprostol as a cheaper and effective uterotonic agent over oxytocin, which is expensive and requires an adequate cold chain for transportation and storage.

Keywords: Misoprostol, oxytocin, cesarean section, blood loss, postpartum hemorrhage

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Öz

Kan kaybı sezaryen doğumlarda kaçınılmaz bir komplikasyondur ve maternal morbidite ve mortaliteyi artırır. Bir uterotonik ajan olarak rektal misoprostolün oksitosine kıyasla etkinlik ve güvenliğinin daha iyi olduğuna dair bir yaklaşım bulunmaktadır. Başlangıçtan Eylül 2022'ye kadar ilgili denemeler için PubMed, Scopus, Web of Science, Cochrane ve diğer veri tabanlarını aradık. Sezaryen ile doğum yapan kadınlarda kanamayı kontrol etmek için rektal misoprostol ile intravenöz oksitosini karşılaştıran randomize klinik araştırmaları (RKÇ) dahil ettik. Primer sonlanım ölçütlerimiz intra ve postoperatif kan kaybı ve doğumdan sonra hemoglobin düşüşü idi. İkincil sonlanımlar arasında kan transfüzyonu ihtiyacı, ek uterotonik ihtiyacı, ameliyat süresindeki fark ve ayrıca titreme, ateş, mide bulantısı ve kusma insidansı gibi güvenlik sonuçları yer aldı. Arama stratejimiz 1007 kayıt ortaya çıkardı, uygunluk kriterlerimize uyup uymadıklarını kontrol etmek için bunlardan 19 makalenin tam metnini aldık. Bin doksan katılımcılı 7 RKÇ dahil edildi. Postoperatif kan kaybında [MD: -27,9; %95 güven aralığı (GA): (-53,85, -2,10); p=0,03] ve doğumdan sonra Hb düşüşünde (MD: -11; %95 GA: [-0,19, -0,03; p=0,01) anlamlı bir azalma bulduk. İntraoperatif kan kaybı, operasyon süresi, kan transfüzyonu ihtiyacı veya ek uterotonik ihtiyacı açısından iki grup arasında anlamlı bir fark yoktu. Misoprostol grubunda daha yüksek titreme insidansı dışında güvenlik sonuçları açısından iki grup arasında anlamlı bir fark bulamadık [RR: 0,33; %95 GA; (0,16, 0,70); p=0,004]. Oksitosin uygulamasına kıyasla rektal misoprostol uygulanan kadınlarda potansiyel olarak olumlu bir güvenlik profili ile postoperatif kan kaybında önemli bir azalma bulduk. Bulgularımız, pahalı olan ve taşıma ve depolama için bir soğuk zincir gerektiren oksitosine göre daha ucuz ve etkili bir uterotonik ajan olarak rektal misoprostolün tercih edilmesini desteklemektedir.

Anahtar Kelimeler: Misoprostol, oksitosin, sezaryen, kan kaybı, postpartum kanama

Introduction

Postpartum hemorrhage (PPH) is a serious condition and is considered the main contributor to death in nations that are both developing and developed[1]. PPH is characterized as a blood loss of more than 500 mL during 24 h after a normal vaginal birth or more than 1000 mL following a cesarean section[2]. PPH can complicate up to 5% of births in both developed and developing nations[3]. The World Health Organization (WHO) has reported 100,000 deaths yearly due to PPH.

Cesarean section (CS) is the most frequent major surgical operation done on women in the United States; around one million CS are performed each year, and 15% of births worldwide occur by CS[4]. The rising rate of CS is concerning because blood loss throughout CS is nearly double that of vaginal delivery, and the necessity of blood transfusion, with all its risks, is also greater after CS than after vaginal births[5].

Uterine Atony is the cause of up to 80% of PPH leading to postnatal anemia, and hemorrhagic shock so a rapid transfusion and surgical interventions are needed[6]. Oxytocin is considered as the first line of treatment for PPH[7]. Despite being effective and safe, oxytocin has certain drawbacks: 10-40% of the women who received it were found to require supplementary uterotonics. The use of oxytocin has been linked to tachycardia, hypotension, and antidiuresis[8].

Misoprostol is an analog of prostaglandin E1 that is used to prevent and treat PPH because of its uterotonic effect. Misoprostol is readily accessible, inexpensive, and stable at room temperature. It is easy to administer via multiple routes (oral, sublingual, vaginal, rectal, and intrauterine), has minimal adverse effects and has few contraindications for use. According to certain research, oxytocin and misoprostol both work well to prevent intra and postoperative bleeding[8,9].

Misoprostol may be given orally, or sublingual. Due to the difficulty in use oral or sublingual misoprostol in general or spinal anesthesia and based on pharmacological studies proving that the blood level of rectal misoprostol is the same of oral misoprostol[10], therefore rectal misoprostol is a suitable alternative to oxytocin. We performed a comprehensive review and meta-analysis to evaluate the effectiveness and safety of preoperative rectal misoprostol compared with oxytocin for minimizing blood loss during and after CS.

Materials and Methods

Review Protocol

The current work was carried out in accordance with the principles of the Cochrane Handbook for Systematic Reviews of Interventions[11]. We completely adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement when writing our manuscript[12]. The research protocol was recorded in the International Prospective Register for Systematic Reviews (PROSPERO), ID: CRD42022363622. Because of the context of the study, no ethical approval was necessary.

Search Strategy

The following electronic databases were systematically searched: PubMed, Scopus, Web of Science, and the Cochrane Central Register of Controlled Trials from inception until September 2022. We used the following search terminologies: "misoprostol", "oxytocin", and "cesarean section". Supplemental Table 1 shows the exact literature search strategy for each database. To prevent missing any research and to ensure high-quality screening, all of the listed studies' references were checked. Furthermore, the Clinicaltrials.gov and the WHO Clinical Trials Registry were considered during our search for details of unpublished and ongoing trials.

Eligibility Criteria

We included studies that matched the following criteria: (i) Patients: Women undergoing CS, (ii) Intervention: Rectal misoprostol, (iii) Comparison: IV oxytocin, (iv) Outcomes: Efficacy and safety endpoints, and (v) Study design: Randomized clinical trials (RCTs). On the other hand, we excluded non-human studies, conference abstracts, non-RCTs, cohorts, case-control, case series, and non-English studies.

Table 1. Summary of the included trials

Study ID	Country	Trial duration	Total sample size, n	Study arms	
				Intervention	Control
Adanikin 2013[15]	Nigeria	Between August 2011 and October 2011	n=50	Misoprostol 600 µg (rectal)	Oxytocin 20 IU (IV)
Chaudhuri 2009[16]	India	Between December 2007 and May 2009	n=190	Misoprostol 800 µg (rectal)	Oxytocin 40 IU (IV)
Chaudhuri 2014[17]	India	Between May 2011 and April 2012	n=192	Misoprostol 800 µg (rectal)	Oxytocin 20 IU (IV)
Fazel 2013[18]	Iran	During 2009	n=100	Misoprostol 400 µg (rectal)	Oxytocin 10 IU (IV)
Milhan 2019[19]	Indonesia	Between January 2018 and March 2018	n=84	Misoprostol 800 µg (rectal)	Oxytocin 20 IU (IV)
Ozori 2022[21]	Nigeria	NA	n=140	Misoprostol 600 µg (rectal)	Oxytocin 40 IU (IV)
Shah 2021[20]	Pakistan	Between April 2019 and October 2019	n=334	Misoprostol 800 µg (rectal)	Oxytocin 5 IU (IV)

NA: Not available, IU: International unit, IV: Intravenous

Screening and Study Selection

We used Endnote software to gather the various entries from different databases and eliminate duplicates. The collected references were checked for relevancy. The screening was conducted in two steps: First, title and abstract screening, and then full-text screening for the final selection. Two separate authors finished the screening and resolved these disagreements.

Quality Assessment

To evaluate the quality of each RCT and assess the risk of bias in the included trials, two authors used the second version of the Cochrane Risk of Bias assessment tool[13]. The tool examined domains such as randomization process, deviation from the planned interventions, incomplete outcome data, outcome measurement, and selection of the reported result. The reviewers graded the risk of bias in each category and assessed the overall quality of the studies as "low", "some concerns", or "high". In the case of disagreements, the group discussed until they reached a consensus. As per Egger et al.[14], determining publication bias using Egger's test for funnel plot asymmetry is unreliable when fewer than ten studies are pooled. Therefore, we could not use this test to detect publication bias in our study.

Data Extraction and Outcomes

Two independent authors extracted data from the included studies. Extracting the summary of the included studies included country, trial duration, total sample size, and study arms (intervention group and control group). Baseline characteristics of the enrolled participants included the route of administration, the number of participants, age (years), gestational age (weeks), parity, the type of anesthesia, and type of CS. Efficacy endpoints involve intraoperative blood loss (mL), postoperative blood loss (mL), mean difference in hemoglobin (mg/dL), operative time (min), the need for blood transfusion, and the need for additional uterotonic agents. Safety endpoints involve the incidence rate of shivering, pyrexia, and vomiting. Disagreements were solved later by group discussion.

Statistical Analysis

We used the Review Manager software (RevMan, version 5.4 for Windows) from the Cochrane Collaboration to conduct our meta-analysis. We combined continuous and dichotomous data and calculated a 95% confidence interval (CI). We employed the Mantel-Haenszel and Inverse-Variance methods for analyzing dichotomous and continuous data, respectively. We evaluated the degree of heterogeneity using the chi-square and I-square (I^2) tests and visually assessed forest plots. We considered significant heterogeneity present when the chi-square test yielded a p-value of less than 0.1, and the I2 test indicated more than 50. We used fixed-effects and random-effects models for analyzing homogeneous and heterogeneous results, respectively. We considered p-values of less than 0.05 for the endpoints to be statistically significant. We conducted subgroup analyses based on the various doses of rectal misoprostol (400 µg, 600 µg, and 800 µg).

Results

Literature Search

Following the removal of duplicates, the literature search strategy yielded a total of 1,007 citations. Nineteen articles were trustworthy enough for full-text screening after our title and abstract screening. Finally, seven studies[15-21] were included in the quantitative synthesis (Figure 1) No missing articles were found after examining the included studies' references.

Characteristics of the Included Trials

Seven RCTs with a total number of 1,090 patients; of these, 546 were allocated to the rectal misoprostol group, and 544 were allocated to the oxytocin group. The RCTs were executed in India, Nigeria, Iran, Indonesia, and Pakistan. Rectal misoprostol dosage ranges from 400-800 µg, and oxytocin dose ranges

from 5-40 international units (IU). The summary and baseline characteristics of the included studies are shown in Tables 1 and 2, respectively.

Quality Assessment

Five RCTs[15-17,19,21] were evaluated as having a "low" risk of bias. However, two RCTs[18,20] were evaluated as having "some concerns" and a "high" risk of bias, respectively. Fazel et al.[18] because they provide no specifics on the method of randomization and allocation concealment. Shah et al.[20] provided no information about the randomization process, and there were missing data regarding efficacy and safety endpoints such as the number of patients who needed a blood transfusion and the incidence of nausea (Figure 2).

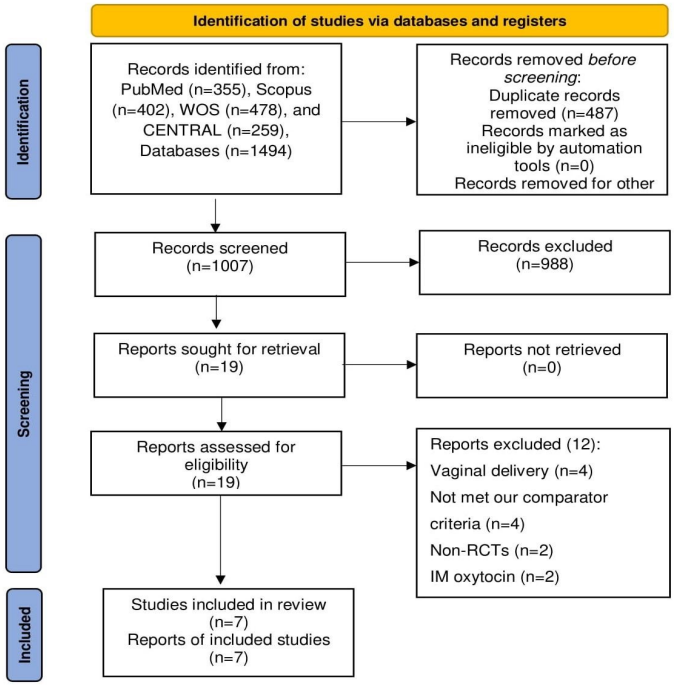

Figure 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram

Meta-analysis of Efficacy Endpoints

A. Intraoperative Blood Loss (mL)

Intraoperative blood loss (mL) has been investigated in four RCTs[15-17,20], including 622 women. No significant difference between rectal misoprostol at all three administrated doses and the oxytocin group in reducing blood loss intraoperatively [n=4 RCTs, MD=-21.05; 95% CI: (-80.29, 38.19); p=0.49]. Subgroup analysis according to the rectal misoprostol dose did not reveal any significant findings (Figure 3).

B. Postoperative Blood Loss (mL)

Postoperative blood loss was reported in four RCTs with a total of 766 patients. Three trials used 800 µg[16,17,20] and one investigated 600 µg[15]. We found a statistically significant reduction in terms of postoperative blood loss in the overall rectal misoprostol group [n=4 RCTs, MD: -27.98; 95% CI: (-53.85, -2.10); p=0.03] and specifically in women who were administrated 800 µg compared to the oxytocin group [n=3 RCTs, MD: -44.05; 95% CI: (-67.34, -20.75); p<0.001] (Figure 4).

Table 2. Baseline characteristics of the included trials

Study ID	Group	Route	Participants, n	Age (years)	Gestational age (weeks)	Parity	Type of anesthesia	Type of CS
Adanikin 2013[15]	Misoprostol	Rectal	n=25	30.6±6.33	39.03±1.62	1.88±1.42	General or spinal	Emergency
	Oxytocin	IV	n=25	30.84±5.69	39.07±1.26	1.76±1.09		
Chaudhuri 2009[16]	Misoprostol	Rectal	n=96	23.95±3.39	39.46±1.69	NA	Spinal	Elective or emergency
	Oxytocin	IV	n=94	24.32±4.98	39.18±1.36	NA		
Chaudhuri 2014[17]	Misoprostol	Rectal	n=96	23.5±4.5	39±1.08	NA	Spinal	Emergency
	Oxytocin	IV	n=96	23.2±3.7	38.8±1.2	NA		
Fazel 2013[18]	Misoprostol	Rectal	n=50	26.6±5.4	38.65±0.58	1.85±0.92	Spinal	Elective
	Oxytocin	IV	n=50	27.1±5.3	38.66±0.85	1.91±0.86		
Milhan 2019[19]	Misoprostol	Rectal	n=42	NA	NA	NA	Spinal	Elective
	Oxytocin	IV	n=42	NA	NA	NA		
Ozori 2022[21]	Misoprostol	Rectal	n=70	32.4±6.2	38.2±1.1	2±0.6	Spinal	Elective or emergency
	Oxytocin	IV	n=70	32.4±5.2	38.3±1.4	2±0.4		
Shah 2021[20]	Misoprostol	Rectal	n=167	30.6±6.5	NA	NA	Spinal	Elective
	Oxytocin	IV	n=167	30.6±6.5	NA	NA		

NA: Not available, IV: Intravenous, CS: Cesarean section

C. Mean Difference in Hemoglobin (mg/dL)

Three RCTs (n=482) reported the mean difference in hemoglobin (mg/dL)[16-18]. Two trials used 800 µg, while Fazel et al.[18] used 400 µg. Administration of 800 µg misoprostol rectally was associated with a significant reduction in hemoglobin drop postoperatively [n= 2 RCTs, MD: -11; 95% CI: (-0.19, -0.03); p=0.01]; however, no significant difference was detected between 400 µg and oxytocin groups (Figure 5).

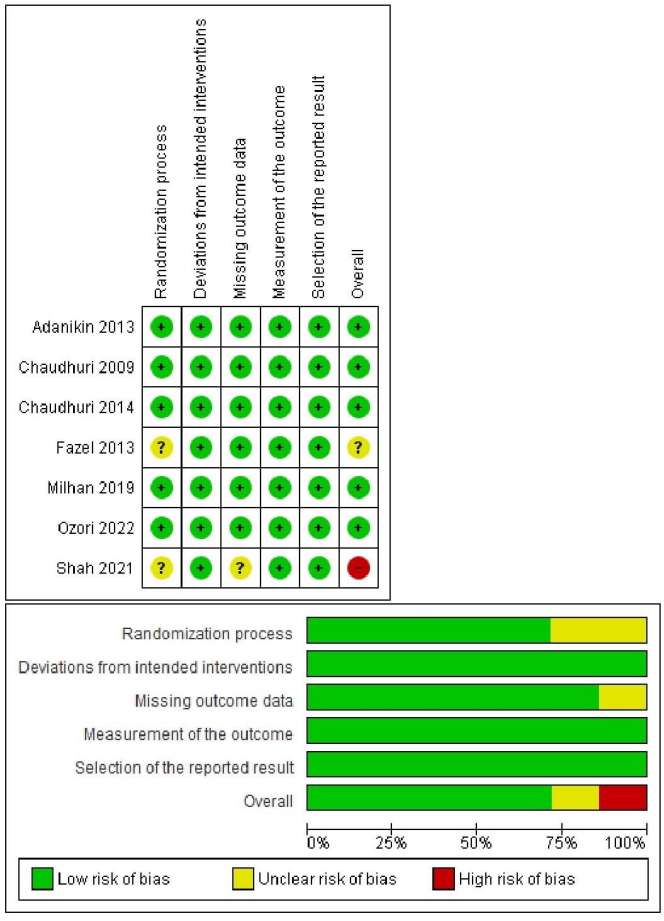

Figure 2. Risk of bias (ROB) summary and graph

D. Operative Time (min)

Operative time was mentioned in three RCTs with a total of 290 patients[15,18,20]. Our results found no significant difference between rectal misoprostol and Oxytocin in reducing the operative time [n= 3 RCTs, MD: -1.36; 95% CI: (-3.32, 0.59); p=0.17] (Figure 6).

E. The Need for Blood Transfusion (%)

Four studies (n=622) reported women who needed a blood transfusion. We could not detect a statistically significant difference between rectal misoprostol and oxytocin in reducing the number of patients who needed blood transfusion [n=4 RCTs, RR: 0.37; 95% CI: (0.10, 1.39); p=0.14] (Supplemental Figure 1).

F. The Need for Additional Uterotonic Agents

The need for an additional uterotonic agent was investigated in three RCTs enrolling 522 women. Our results found no significant difference between different doses of rectal misoprostol and oxytocin in reducing the need for additional uterotonics [n=3 RCTs, RR: 1.06; 95% CI: (0.66, 1.70); p=0.81] (Supplemental Figure 2).

Meta-analysis of Safety Endpoints

Four RCTs compared postoperative safety outcomes in 532 patients[15-18,20]. Postoperative shivering and pyrexia were reported in all four studies. A statistically significant increase was detected in the number of shivering women in the rectal misoprostol group compared with the oxytocin group [n=4 RCTs, RR: 0.33; 95% CI; (0.16, 0.70); p=0.004]. No significant difference was found between the two groups regarding postoperative pyrexia, nausea, and vomiting (Supplemental Figures 3-5).

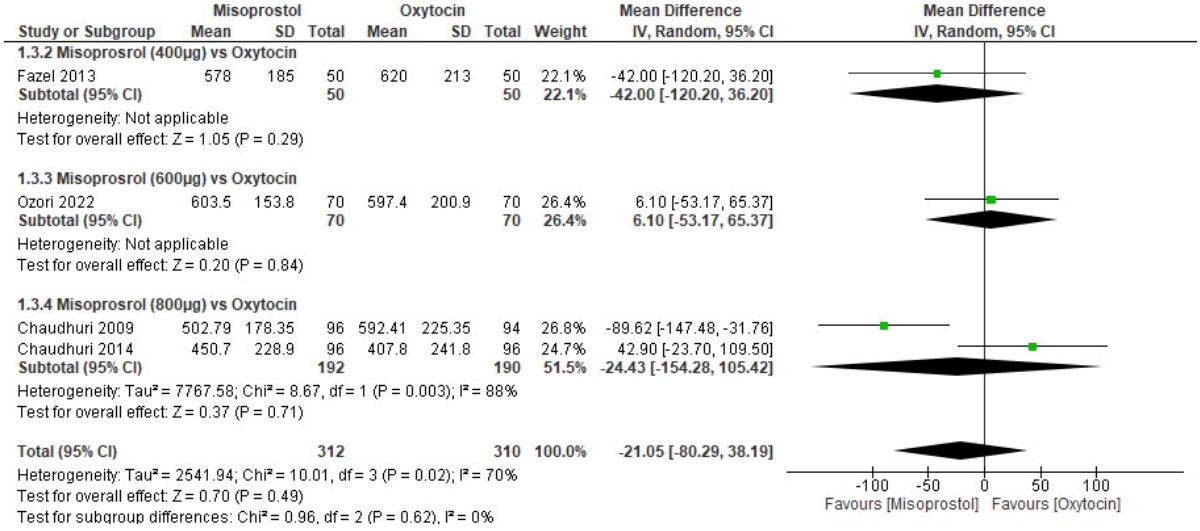

Figure 3. Meta-analysis of intraoperative blood loss (mL)

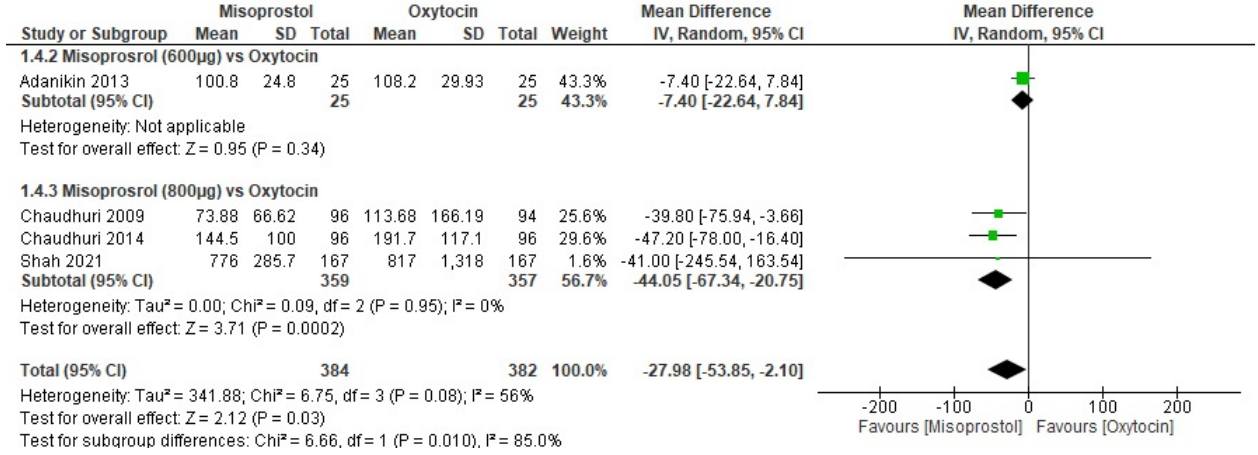

Figure 4. Meta-analysis of postoperative blood loss (mL)

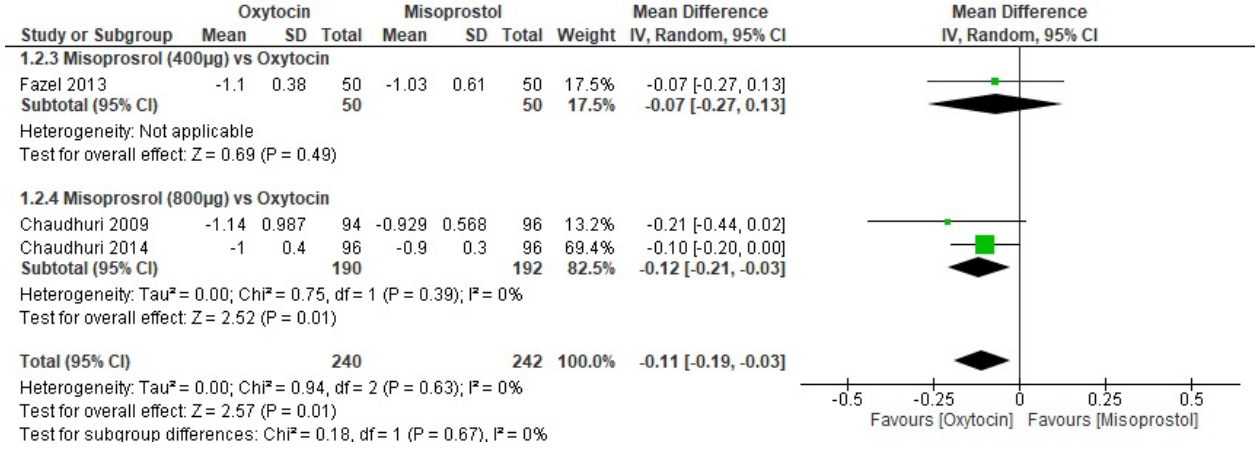

Figure 5. Meta-analysis of the mean difference in hemoglobin (mg/dL)

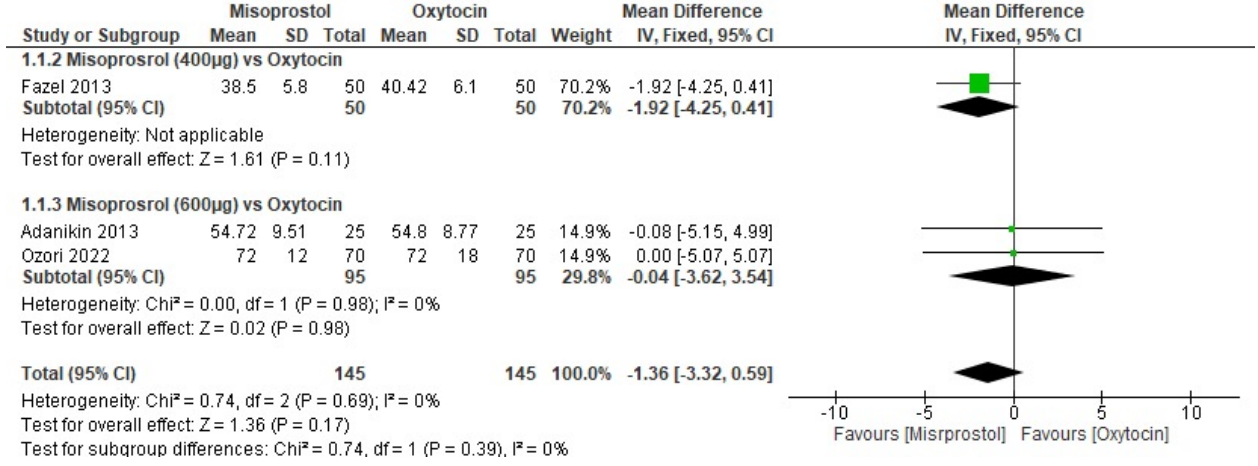

Figure 6. Meta-analysis of operative time (min)

Discussion

Finding Summary

We can summarize our findings in three main points: (I) rectal misoprostol can significantly reduce postoperative blood loss and hemoglobin drop in women undergoing CS compared with oxytocin, (II) no significant difference between the two study groups regarding intraoperative blood loss, need for blood transfusion, need for additional uterotonics, or operative time, and (III) there are some safety concerns on misoprostol in terms of postoperative shivering.

Our Results in the Context of Literature

There are three previous systematic reviews in the literature discussing the efficacy of misoprostol, including the rectal route, compared with oxytocin in reducing blood loss among women who underwent CS[8,9,22]. However, each review included only one RCT; two of them included the same study[16], and one study included Elsedeek's study[23]. We have excluded Elsedeek's study because the study participants had already taken 10 IU intravenous oxytocin, which is incompatible with our criteria. This study is the first to specify on the rectal route of misoprostol and compare it with the routinely administrated oxytocin. Thus, we cannot discuss our results against the results of the previous studies due to the comparison heterogeneit; otherwise, we will stress on the advantage of misoprostol over oxytocin and the feasibility of the rectal route.

Intraoperative Blood Loss

Conde-Agudelo et al.[8] revealed that oral and sublingual misoprostol did not have significant superiority over oxytocin in reducing intraoperative blood loss. Addition, the other two reviews found that the efficacy of sublingual and oral misoprostol had no significant difference compared with oxytocin, which is consistent with our results. However, Maged et al.[22] found that the intrauterine misoprostol group had lower blood loss in comparison with the oxytocin group.

Postoperative Blood Loss

A review reported that there was no significant difference between oxytocin and neither oral nor sublingual misoprostol in terms of postoperative blood loss, which is inconsistent with our findings that showed a significant difference[22]. This disagreement might be referred to the number of included studies, as we included four studies, while the previous review included two studies in each route.

Mean Difference in Hemoglobin

Maged et al.[22] found that sublingual misoprostol did not significantly differ from oxytocin in reducing hemoglobin loss during CS, whereas oxytocin significantly decreased the hemoglobin loss compared with oral misoprostol. Otherwise, intrauterine misoprostol was associated with a significant reduction in hemoglobin drop postoperatively in comparison to oxytocin, which agrees with our findings concerning rectal misoprostol.

Secondary Efficacy Outcomes

Our results on rectal misoprostol were consistent with the study of Conde-Agudelo et al.[8] regarding both the need for blood transfusion and for additional uterotonic agents, they could not find a significant difference between both oral and sublingual misoprostol compared with oxytocin. However, intrauterine misoprostol significantly reduced the need for additional uterotonics compared with oxytocin.

Safety Considerations

In this review, we found that patients who took rectal misoprostol had a higher rate of shivering and pyrexia compared with the oxytocin group. These findings agree with the results of the previous review. Conde-Agudelo et al.[8] reported that sublingual and oral misoprostol groups significantly suffered from shivering in comparison with the oxytocin group. Furthermore, there was a significant difference between the sublingual and oxytocin in terms of pyrexia, but the oral and intrauterine routes of misoprostol did not significantly differ from oxytocin.

Misoprostol is not Inferior to Oxytocin

Despite the slight elevation in the rate of women who experienced shivering and pyrexia after rectal misoprostol administration, the significant reduction in the amount of blood loss postoperatively can demonstrate a non-inferior, even superior, aspect of using misoprostol as a reliable, non-parenteral, and low-cost uterotonic agent in busy postoperative wards of hospitals with limited resources.

The efficacy of the drug is not the only advantage that should be taken into consideration while searching for the drug of choice. The cost, preparation, and storage requirements, time and effort needed for administration, and the availability of the administration routes are critical to be kept in mind. Misoprostol has an upper hand over oxytocin as it has a lower cost (around half of the cost), which is appropriate for low-income countries [24]. Furthermore, the variability of the administration routes allows physicians to deal with different situations avoiding the risk of the parenteral route. However, it is important to mention that we cannot alleviate the need for oxytocin with its rapid action, which could be the choice in difficult situations[25]. Accordingly, we can start with rectal misoprostol, and if there is a need for additional uterotonics, we can use oxytocin. This strategy will save oxytocin in health facilities with limited resources for critically ill patients.

In a comparison of misoprostol with another oxytocic agent such as carbetocin, a recent systematic review and meta-analysis of four RCTs compared carbetocin with misoprostol in women who underwent CS[26]. They found the following: (i) the superiority of carbetocin in preventing and reducing PPH in comparison with misoprostol, (ii) the superiority of carbetocin in reducing the rate of blood transfusion, the need for additional uterotonic agents, and the need for additional surgical intervention in comparison with misoprostol, and (iii) the superiority of carbetocin in terms of safety endpoints (like fever, shivering, and heat sensation) comparison with misoprostol. However, the main limitation of this review was included both rectal and sublingual misoprosto; they could not assess the efficacy and safety outcomes according to the route of administration. Furthermore, similar findings were observed in a published meta-analysis but among women who underwent vaginal delivery[27].

Strength Points and Limitations

This review has many strong points, including that it is the first systematic review assessing the efficacy of rectal misoprostol specifically with a reasonable number of included studies. Moreover, there was no heterogeneity in all the outcomes. However, it has some limitations: First, the variability in the methods used in the included studies to calculate the estimated blood loss; second, the different cut-off times when the blood loss was estimated; third, some studies included patients who already had some additional identifiable risk factors; fourth, the efficacy of the two drugs in reducing the blood loss during CS may be affected by the method of removing the placenta, multiple pregnancy, anatomical variabilities, and surgical skills.

Future Directions and Recommendations

Further RCTs are needed, especially with multicenter and large sample size to compare difference doses and different routes of administration. Also, it is highly recommended to compare the efficacy of the combined regimen of misoprostol and oxytocin during and after CS to obtain the higher efficacy of these uterotonic agents. Finally, carbetocin should be investigated as a new and effective uterotonic agent during the enrollment of future RCTs.

Conclusion

We found a significant reduction in postoperative blood loss with a potentially favorable safety profile in women who administrated rectal misoprostol compared with oxytocin administration. Our findings recommend and prefer rectal misoprostol as a cheaper and effective uterotonic agent over oxytocin, which is expensive and requires an adequate cold chain for transportation and storage.

Ethics

Peer-review: Internally and externally peer-reviewed.

Authorship Contributions

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

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

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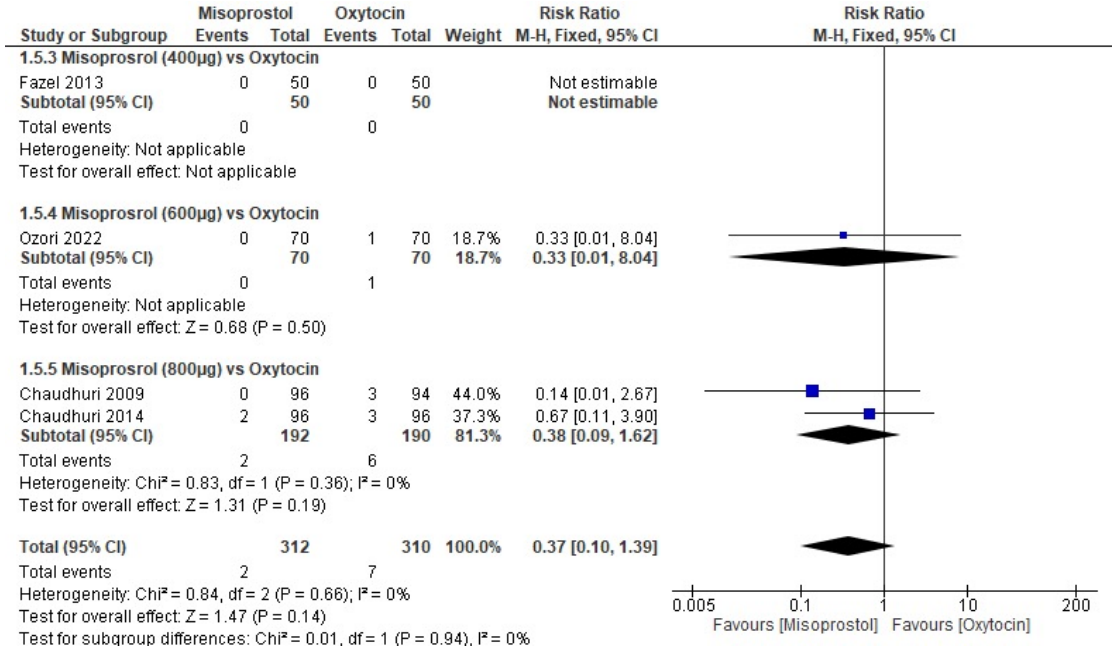
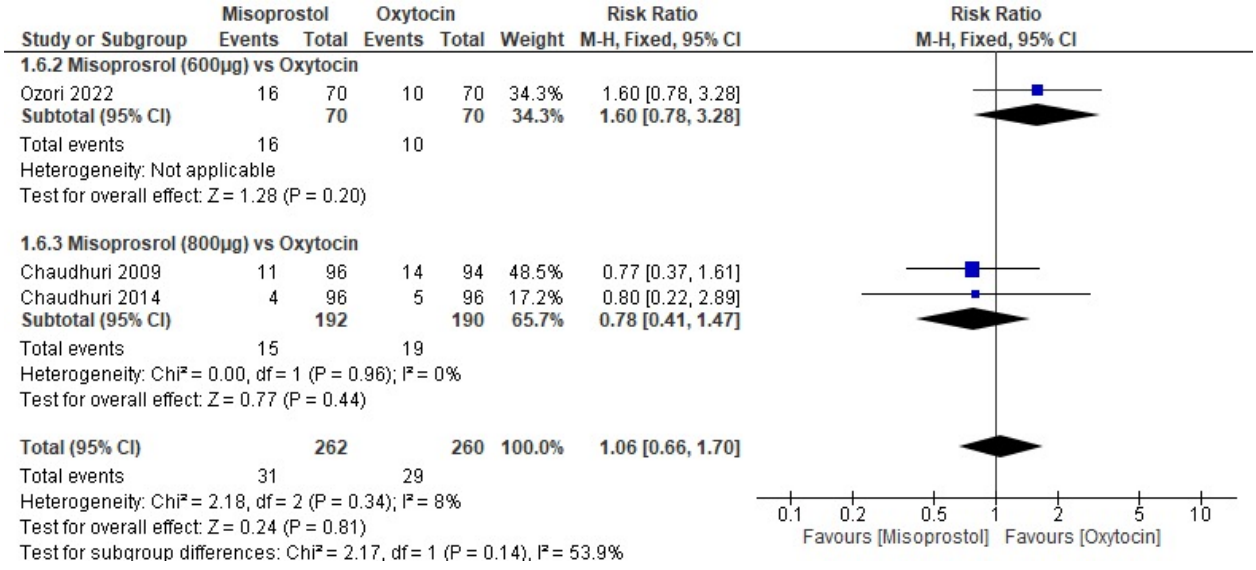
Supplemental Table 1. The exact literature search strategy used in every database

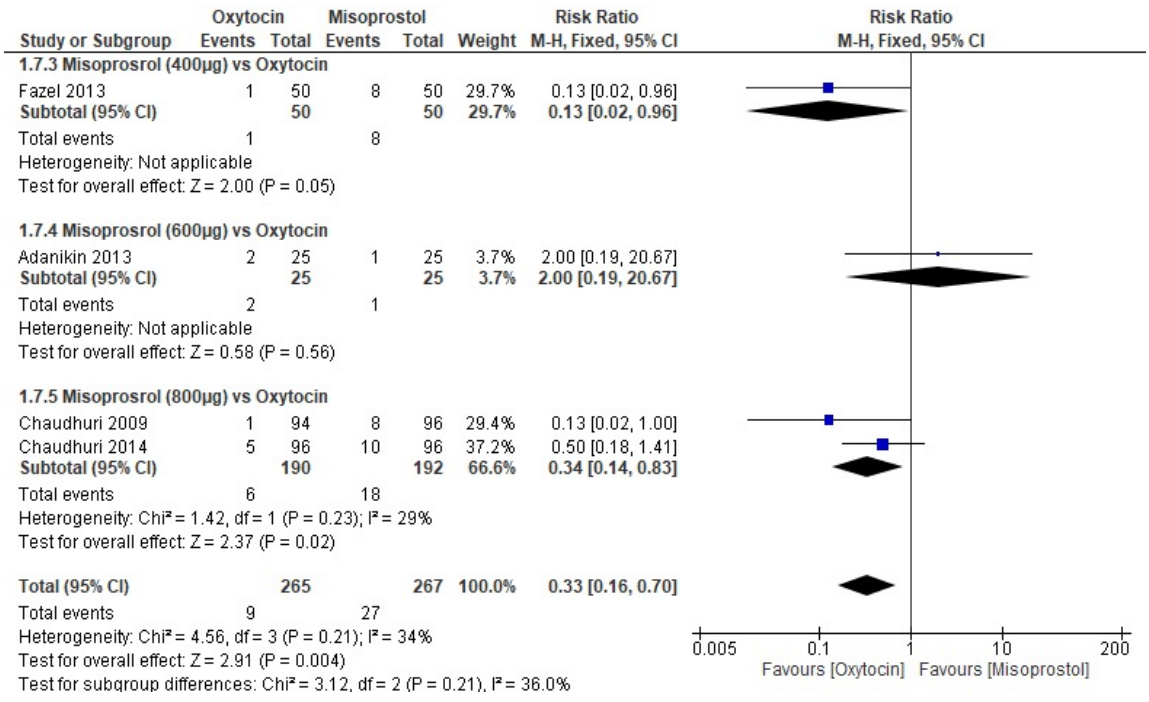
PubMed All Fields: (“cesarean section” OR CS OR “C-section” OR “abdominal delivery”) AND (misoprostol OR “novo misoprostol” OR “apo misoprostol” OR cytotec OR “SC30249” OR “SC29333” OR glefos OR misodel OR mysodelle OR misotac) AND (oxytocin OR pitocin OR syntocin OR ocytocin)
Scopus Article title, Abstract, Keywords: (“cesarean section” OR CS OR “C-section” OR “abdominal delivery”) AND (misoprostol OR “novo misoprostol” OR “apo misoprostol” OR cytotec OR “SC30249” OR “SC29333” OR glefos OR misodel OR mysodelle OR misotac) AND (oxytocin OR pitocin OR syntocin OR ocytocin)
Web of Science All Fields: (“cesarean section” OR CS OR “C-section” OR “abdominal delivery”) AND (misoprostol OR “novo misoprostol” OR “apo misoprostol” OR cytotec OR “SC30249” OR “SC29333” OR glefos OR misodel OR mysodelle OR misotac) AND (oxytocin OR pitocin OR syntocin OR ocytocin)
Cochrane Central Register of Controlled Trials (CENTRAL) Title Abstract Keyword: (“cesarean section” OR CS OR “C-section” OR “abdominal delivery”) AND (misoprostol OR “novo misoprostol” OR “apo misoprostol” OR cytotec OR “SC30249” OR “SC29333” OR glefos OR misodel OR mysodelle OR misotac) AND (oxytocin OR pitocin OR syntocin OR ocytocin)



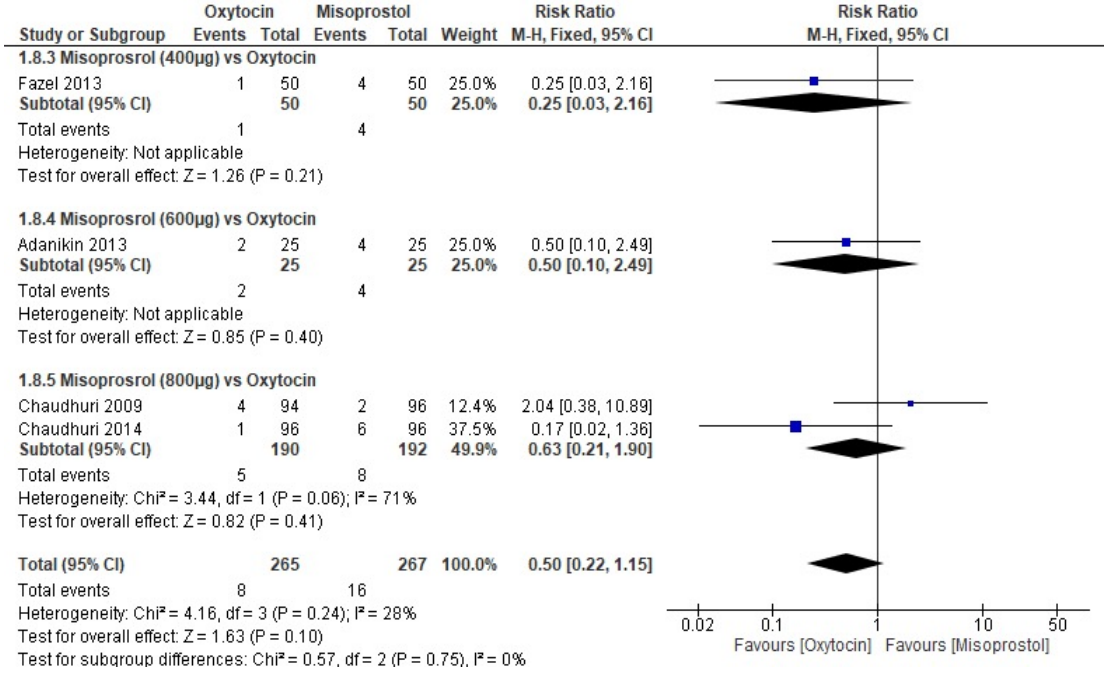
Supplemental Figure 1. Meta-analysis of the rate of patients who needed a blood transfusion



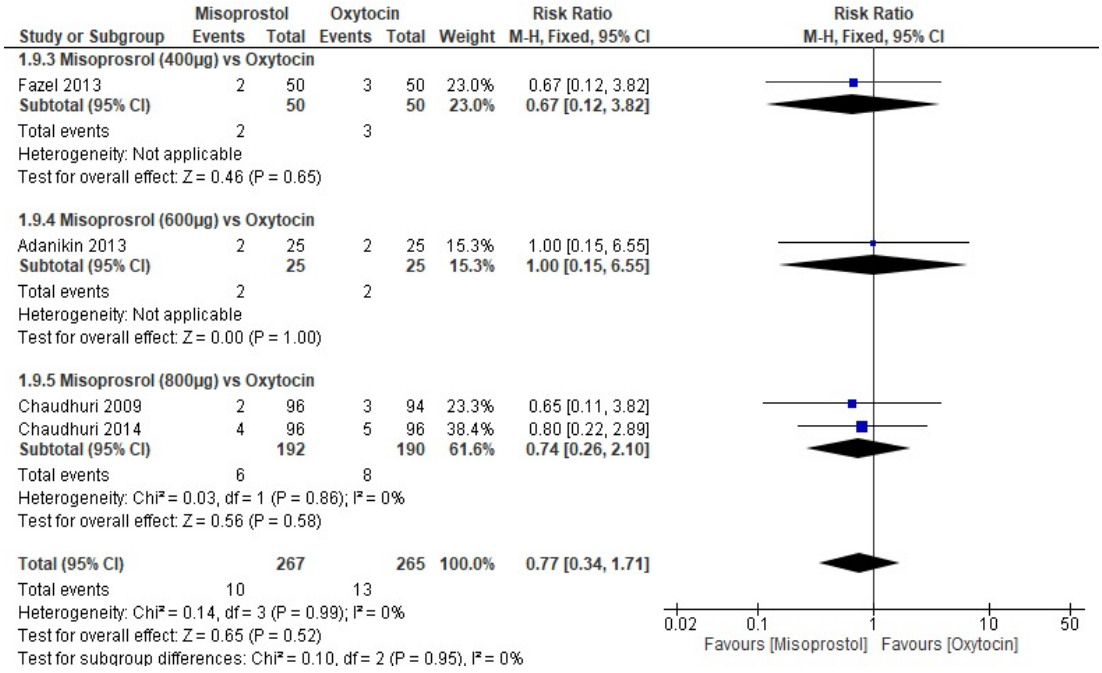
Supplemental Figure 2. Meta-analysis of the rate of patients who needed an additional uterotonic agents



Supplemental Figure 3. Meta-analysis of the rate of shivering



Supplemental Figure 4. Meta-analysis of the rate of pyrexia



Supplemental Figure 5. Meta-analysis of the rate of vomiting

Review / Derleme

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Turk J Obstet Gynecol 2023;20:154-63

How autologous platelet-rich plasma affects pregnancy and birth outcomes in women with repeated embryo implantation failure: A prisma-compliant meta-analysis

Otolog trombosit açısından zengin plazma, tekrarlayan embriyo implantasyon başarısızlığı olan kadınlarda gebelik ve doğum sonuçlarını nasıl etkiler: Prizma uyumlu bir meta-analiz

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Abstract

Repeated implantation failure refer to failure to conceive after three or more embryo transfer attempts. Several interventions were offered to improve maternal and fetal outcomes. Our objective was to investigate the impact of platelet-rich plasma (PRP) as a promising intervention to improve both pregnancy and birth outcomes. We searched PubMed, Scopus, Web of Science, and Cochrane Central, in addition to other relevant resources of grey literature. Only clinical trials were eligible to be included. We performed the meta-analysis using a random effects model. Eight randomized clinical trials, enrolling 1038 women with more than 3 implantation failure attempts, were included. We found a significant increase regarding all our prespecified primary outcomes. Chemical pregnancy rate [relative ratio (RR): 1.96, 95% confidence interval (CI): 1.61, 2.39; p<0.001], clinical pregnancy rate (RR: 4.35, 95% CI: 1.92, 2.88; p<0.001), and live birth rate (RR: 4.03, 95% CI: 1.29, 12.63; p=0.02) were found to be statistically significant and increased in patients who received PRP compared with the control group. Implantation rate (RR: 1.98, 95% CI: 1.34, 2.75; p<0.001), miscarriage rate (RR: 0.44, 95% CI: 0.23, 0.83, p=0.01), and multiple pregnancy rate (RR: 2.56, 95% CI: 1.02, 6.42, p=0.04) were also found to be significantly increased in the PRP group. We provide strong evidence on how intrauterine PRP can improve implantation, pregnancy, and birth outcomes in RIF women, which should direct clinicians to consider this intervention as a very effective tool in assisted reproductive techniques.

Keywords: Platelet-rich plasma, implantation failure, assisted reproductive techniques and in vitro fertilization

Öz

Tekrarlanan implantasyon başarısızlığı, üç veya daha fazla embriyo transferi denemesinden sonra gebe kalamamayı ifade eder. Maternal ve fetal sonuçları iyileştirmek için çeşitli müdahaleler önerildi. Bu çalışmanın amacı, trombosit açısından zengin plazmanın (PRP) hem gebelik hem de doğum sonuçlarını iyileştirmek için umut verici bir müdahale olarak etkisini araştırmaktır. Gri literatürün diğer ilgili kaynaklarına ek olarak PubMed, Scopus, Web of Science ve Cochrane Central'ı araştırdık. Yalnızca klinik araştırmalar dahil edilmeye uygun bulundu. Meta-analizi rastgele etkiler modeli kullanarak gerçekleştirdik. Üçten fazla başarısız implantasyon girişimi olan 1038 kadının dahil edildiği sekiz randomize klinik araştırma dahil edildi. Önceden belirlenmiş tüm birincil sonuçlarımızda önemli bir artış bulundu. Kimyasal gebelik oranı [risk oranı (RO): 1,96, %95 güven aralığı (GA): 1,61, 2,39; p<0,001], klinik gebelik oranı (RO: 4,35, %95 GA: 1,92, 2,88; p<0,001) ve canlı doğum oranı (RO: 4,03, %95 GA: 1,29, 12,63; p=0,02) kontrol grubuna göre PRP uygulanan hastalarda istatistiksel olarak anlamlı ve artmış bulundu. İmplantasyon oranı (RO: 1,98, %95 GA: 1,34, 2,75; p<0,001), düşük oranı (RO: 0,44, %95 GA: 0,23, 0,83, p=0,01) ve çoğul gebelik oranı (RO: 2,56, 95% GA: 1,02, 6,42, p=0,04) de PRP grubunda anlamlı olarak artmış bulundu. Rahim içi PRP'nin RIF kadınlarda

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implantasyonu, hamileliği ve doğum sonuçlarını nasıl iyileştirebileceğine dair güçlü kanıtlar sunuyoruz ve bu da klinisyenleri bu müdahaleyi yardımcı üreme tekniklerinde çok etkili bir araç olarak düşünmeye yönlendirmelidir.

Anahtar Kelimeler: Trombositten zengin plazma, implantasyon başarısızlığı, yardımcı üreme teknikleri ve tüp bebek

Introduction

The process of implantation relies on two crucial factors: a healthy embryo and a well-developed endometrium. A genetically normal zygote must attach to and invade a thoroughly decidualized endometrium, which should ideally measure 7 mm or more for successful implantation[1,2]. Hence, if implantation fails with a genetically normal zygote, it indicates endometrial insufficiency.

Repeated implantation failure (RIF) is defined as nonpregnancy after three high-quality embryo transfers or after ten or more multiple transfers. Deficient endometrial thickness is responsible for nearly 10% of failed intracytoplasmic sperm injection procedures[3]. Various fertility-enhancing modalities have been proposed to counter thin endometrial linings, including estrogen, pentoxifylline, vitamin E for expanding endometrial thickness, aspirin, local sildenafil, tamoxifen, and other factors to enhance endometrial perfusion[4,5]. Despite these multiple approaches, RIF remains a major contributing cause to the failure of assisted reproduction, whose success rate does not exceed 30%.

Platelet-rich plasma (PRP) is extracted from the centrifugation process of whole blood to obtain platelets[6]. PRP contains a high concentration of growth factors that play a significant role in the process of tissue repair and regeneration. These growth factors include transforming growth factor beta (TGF-β), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), and others. These factors accelerate tissue healing, promote angiogenesis, stimulate cell proliferation and differentiation, and modulate inflammation. The efficacy of PRP has been reported in various clinical settings, including orthopaedics, and dentistry. Recently, PRP has also emerged as a promising intervention for improving pregnancy outcomes in patients with RIF, and since the autologous peripheral blood is the source of PRP, it offers a unique, cost-effective, and practical personalized medicine that is also non-immunogenic[7].

PRP has been investigated in various research fields, including ophthalmology, orthopedics, and wound healing, but its role in infertility is yet to be fully explored. Due to its proliferation and nourishment capabilities, PRP has been proposed as a new approach to promote endometrial growth and receptivity. The concept was initially proposed by Chang et al.[8] in 2015, who demonstrated that intrauterine infusion of autologous PRP 48 h before embryo transfer in IVF procedures increased endometrial growth and allowed successful implantation in RIF patients. Other studies have demonstrated that PRP can enhance clinical and chemical pregnancy outcomes in such patients, but conclusive answers to its efficacy are still in question[9]. Therefore, the aim of this systematic review and meta-analysis was to explore the value of autologous intrauterine PRP infusion through clinical trials conducted on IVF patients.

Materials and Methods

We strictly followed the recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) and the guidelines of the Cochrane Handbook[10]. The review protocol was registered on PROSPERO and is available under the registration ID (CRD42022355535).

Search Strategy

We systematically searched Cochrane Central, Embase, MEDLINE (through PubMed), Scopus, and Web of Science (from inception to 31 July 2022). Also, we searched other sources of grey literature such as Google Scholar, Research Gate, clinical trials registries (e.g., clinicaltrials.gov, and WHO Clinical Trials Registry, etc.), and conference proceedings for unpublished and in-press articles. A manual search for citations and reference lists of the relevant published articles was performed to check for additional eligible studies.

To conduct our search, we used various search terms, abbreviations, and synonyms, including "implantation failure", "embryo transfer", "platelet-rich plasma", "PRP", "assisted reproductive technology", and "in vitro fertilization". We have documented our complete search terms and strategy in Supplementary File 1. We limited our search to English articles but did not specify any particular year range for publication.

Eligibility Criteria

The studies that were considered for inclusion had to meet certain criteria, which included (1) the criteria that the study participants were subfertile women who had experienced RIF. RIF was defined as a failure to achieve a clinical pregnancy after at least three attempts of embryo transfer (ET)[11], (2) the intervention consisted of the intrauterine infusion of autologous PRP before ET, (3) the comparison group must not have received any intervention or placebo, but no other active interventions were allowed, and (4) only randomized controlled trials (RCTs) were eligible for inclusion. Studies with missing data or articles that did not provide adequate information on methodology or results were excluded.

Study Selection

Two authors (AS & HMF) independently screened the exported search records to identify the potentially eligible titles and abstracts with exclusion of irrelevant results. Then, full texts of all eligible studies were retrieved to be assessed for their adherence to our inclusion criteria. In the case of an opinion

discrepancy, a third investigator (YS) was involved in the discussion.

Data Extraction

Two authors (YS & SE) independently extracted the following demographics and characteristics from the finally included articles using a standardized data extraction online form. discrepancies were solved by a discussion with a third reviewer (AS). Extracted data were (1) study characteristics (study design, sample size, country, and year of publication), (2) patient demographic criteria (average age, RIF definition, ovarian stimulation protocol), (3) intervention characteristics (preparation method, time, amount, and method to introduce to patients), (4) study endpoints and measured outcomes.

Outcome Measurements

Our main objectives were to determine the chemical pregnancy rate, clinical pregnancy rate (CPR), and live birth rate (LBR) per patient. "Chemical pregnancy" was obtained as a positive detectable serum level of β-hCG two weeks after frozen-thawed ET, while a "clinical pregnancy" was defined based on the definitive clinical signs of pregnancy or the presence of one or more intrauterine gestational sacs on transvaginal ultrasound following six weeks of ET. "Live birth" referred to the delivery of one or more living infants, as stated in reference[12].

Regarding the secondary outcomes, we included the following outcomes: Implantation rate (IR) per embryo, multiple pregnancy rate (MPR) per patient, and miscarriage rate (MR) per clinical pregnancy. We additionally assessed postinterventional endometrial thickness (PIET). The "implantation rate" was determined by dividing the number of gestational sacs identified by transvaginal ultrasound by the number of embryos transferred. If there were two or more intrauterine embryos identified by transvaginal ultrasound, it was deemed a "multiple pregnancy." The loss of the fetus before 20 weeks of gestation is commonly referred to as a miscarriage[12].

The Risk of Bias Assessment

We used the Cochrane Risk of Bias assessment tool 1 to evaluate the methodological quality of the included studies, which is suggested in the Cochrane Handbook[13]. This assessment tool considers six different factors be determined.

Statistical Analysis

We carried out statistical analysis using Cochrane Collaboration's RevMan 5.3, using a relative ratio (RR) with a 95% confidence interval (CI) as the effect estimate. We determined significance by a p-value of less than 0.05 and calculated the I^2 value to determine statistical heterogeneity. We chose to use a random effects model because of possible variations in PRP preparation and induction techniques. As our meta-analysis used a limited number of studies, we did not assess publication bias. Additionally, we conducted sensitivity analysis to account for heterogeneity in LBR outcomes between studies. We also conducted a subgroup analysis to identify the origin of heterogeneity in PIET using the time difference between PRP infusion and endometrial thickness measurements.

Results

Search Results

We found 2602 search results through a comprehensive electronic computer-based database search. In addition, six articles were identified via manual screening of reference lists of relevant review articles. After removing duplicated results, we found 877 unique publications. Of these, 865 were excluded by title and abstract screening. Then, we evaluate the full texts of the 12 potentially relevant articles for their adherence to our prespecified selection criteria. Only eight articles[14-21] were eligible to be included in both qualitative and quantitative analysis. Figure 1 shows the PRISMA flow diagram for our study.

Study Characteristics

All trials were conducted between 2017 and 2022 in Iran for Obidniak et al.[21], which was conducted in Russia. The overall included participants were 1,038 (518 patients received PRP intrauterine infusion). The average age of the included patients was 34.7±6.3 years. All studies for the Russian trial reported using the GnRH antagonist protocol for ovarian stimulation.

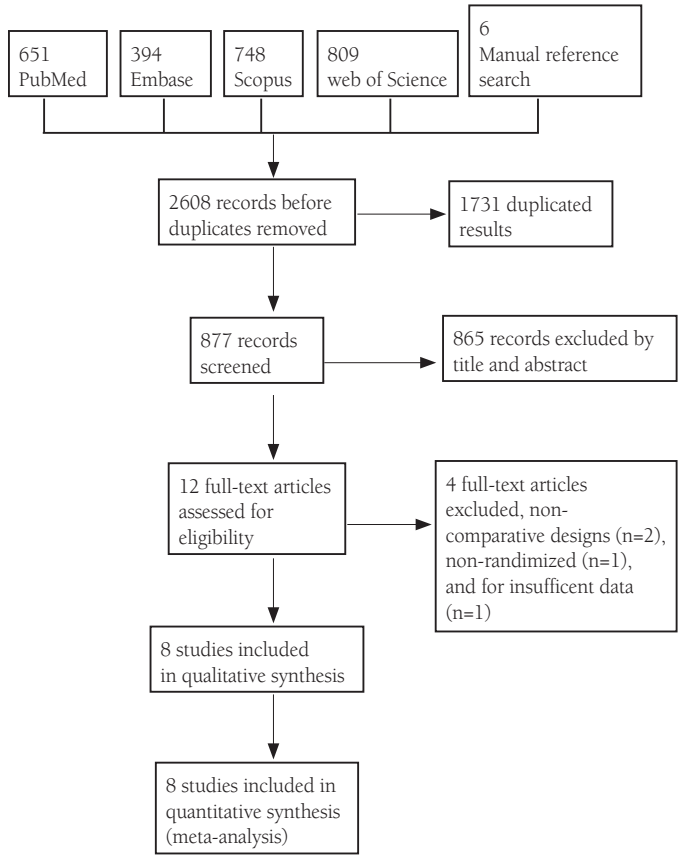

Figure 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram

Five studies compared PRP versus standard treatment, two trials used no therapy in the control group, and one compared PRP versus sham catheter. All trials used the same PRP infusion protocol by administering 0.5 mL of PRP 48 h prior to the time of ET except in two studies that used a dose of 0.5-1 mL (Eftekhar et al.[15]) and 2 mL (Obidniak et al.[21]). The transfer of the embryo in frozen condition was done in all of the included studies (Table 1).

Primary Outcomes

Our 3 primary outcomes of interest were found to be significantly increased in the PRP group compared to the control group (Figure 2). CPR was mentioned in all included studies (n=981) and it was found to be significantly higher in patients who received PRP intervention compared to women in the control group [RR: 2.35, 95% CI: (1.92, 2.88), p<0.001]. The chemical pregnancy rate was evaluated in six studies (n=851). Our meta-analysis showed a significantly higher probability of chemical pregnancy in women who received PRP intervention [RR: 1.96, 95% CI: (1.61, 2.39), p<0.001]. Three RCTs (n=553) reported LBR following the intervention compared with standard treatment. They revealed a significantly higher incidence of live birth in patients treated with PRP [RR: 4.03, 95% CI: (1.29, 12.63); p=0.02]. However, substantial heterogeneity was found between the effect estimates of 3 studies (I^2=83%).

Secondary Outcomes

Regarding the secondary outcomes (Figure 3), IR was reported in 4 studies (n=391). A significantly successful and higher IR was associated with the PRP group compared to the control group [RR: 1.98, 95% CI: (1.43, 2.75), p<0.001]. Two hundred and fifty clinically pregnant women were evaluated for miscarriage in 5 studies. PRP infusion could achieve a significant reduction in MR compared to women who received standard treatment or sham catheters [RR: 0.44, 95% CI: (0.23, 0.83), p=0.01]. MPR was mentioned in three RCTs (n=511). There was a significant increase in multiple pregnant cases in the PRP group [RR: 2.56, 95% CI: (1.02, 6.42), p=0.04]. PIET was measured in 4 trials (n=617). PRP intrauterine infusion was not associated with a significant increase in endometrial thickness [MD: 1.18, 95% CI: (-0.04, 2.40), p=0.06]. Substantial heterogeneity was found between the effect estimates of 4 studies (I^2= 98%).

Heterogeneity Assessment

Regarding LBR heterogeneity (I^2=83%), we performed a sensitivity analysis excluding Safdarian et al.[18] from the pooled

Table 1. characteristics of the included trials

Study ID	Location	Participants (PRP/ Control)	Age (Years) Mean ± SD	Ovarian Stimulation Protocol	PRP Infusion Protocol	Control	Reported Outcomes
Nazari et al.[14], 2022 (1)	Iran	418 (209/209)	34.1±3.7	(GnRH) Antagonist	0.5 mL, 48 h before ET	Standard treatment	CPR, Chemical pregnancy, LBR, IR, PIET, MPR, MR
Nazari et al.[17], 2022 (2)	Iran	50 (25/25)	35.7±5.1	(GnRH) Antagonist	0.5 mL, 48 h before ET	Standard treatment	CPR, LBR, MR
Safdarian et al.[18], 2022	Iran	120 (60/60)	33.4±4.9	(GnRH) Antagonist	0.5 mL, 48 h before ET	No therapy	CPR, Chemical pregnancy, LBR, IR, MPR, MR
Zamaniyan et al.[20], 2021	Iran	120 (60/60)	33.8±6.3	GnRH) Antagonist	0.5 mL, 48 h before ET	Standard treatment	CPR, Chemical pregnancy, IR, PIET, MPR, MR
Nazari et al.[19], 2020	Iran	97 (49/48)	35.7±3.4	(GnRH) Antagonist	0.5 mL, 48 h before ET	Standard treatment	CPR, Chemical pregnancy
Nazari et al.[16], 2019	Iran	60 (30/30)	33.9±2.7	(GnRH) Antagonist	0.5 mL, 48 h before ET	Sham catheter	CPR, Chemical pregnancy, PIET
Eftekhar et al.[15], 2018	Iran	83 (40/43)	31.9±2.2	(GnRH) Antagonist	0.5-1 mL on day 13th of cycle	Standard treatment	CPR, Chemical pregnancy, IR, PIET, MR
Obidniak et al.[21], 2017	Russia	90 (45/45)	35.2±6.4	N/R	2 mL before ET	No therapy	CPR, IR

PRP: Platelet-rich plasma, CPR: Clinical pregnancy rate, LBR: Live birth rate, MPR: Multiple pregnancy rate, MR: Miscarriage rate, PIET: Postinterventional endometrial thickness, IR: Implantation rate, GnRH: Gonadotropin releasing hormone, and N/R: Not reported

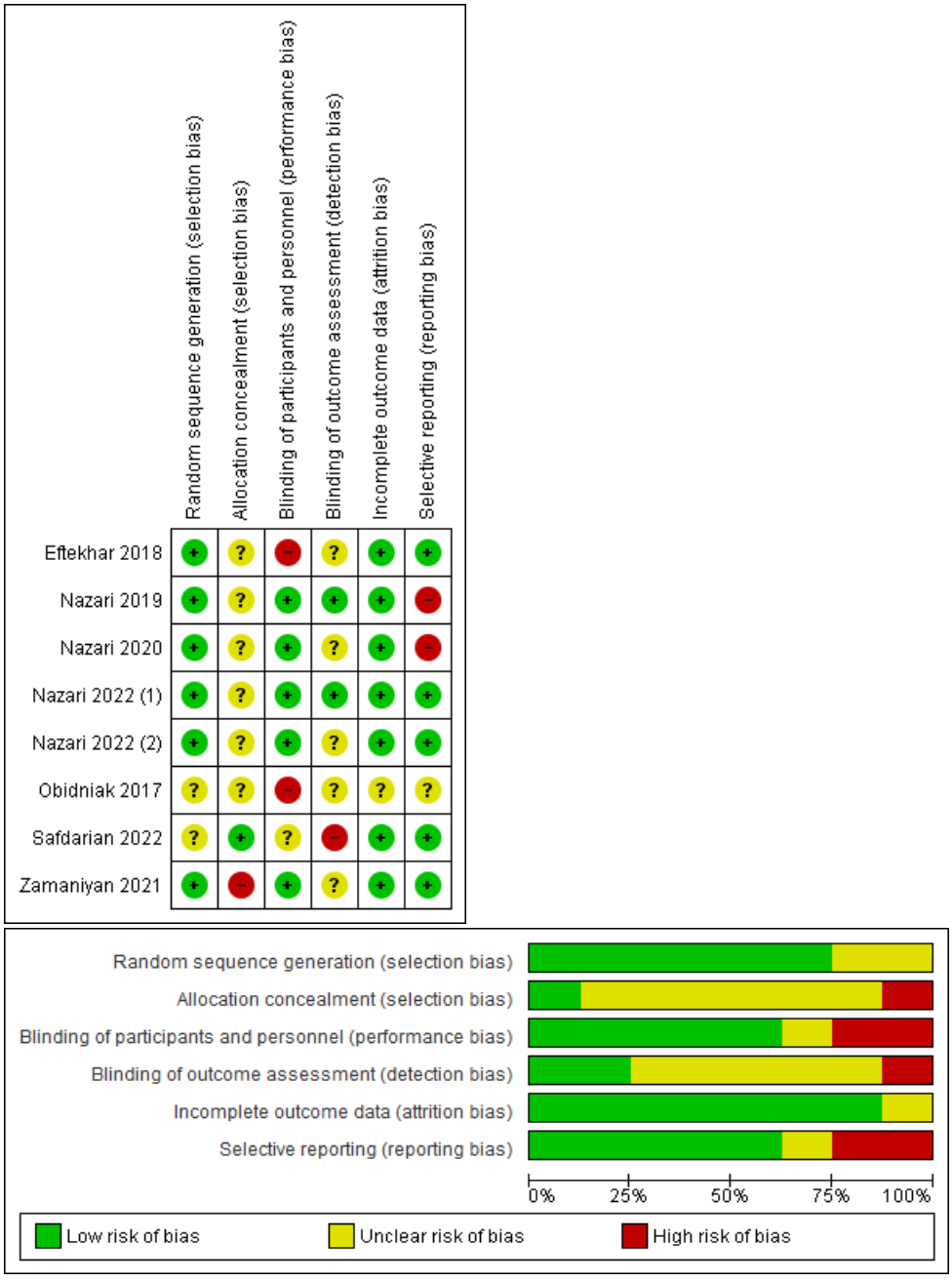

Figure 2. Risk of bias (ROB) summary and graph

analysis, which resolved heterogeneity (I^2=0%). This resulted in a significant increase in the effect estimate and CI [RR: 7.03, 95% CI: (3.91, 12.66), p<0.001]. To solve PIET heterogeneity (I^2=98%), subgrouping analysis was performed according to the period from PRP infusion to time to measure endometrial thickness and revealed a significant increase in endometrial thickness after 48 h of PRP intrauterine infusion [SMD: 0.64, 95% CI: (0.20, 1.08), p=0.005].

The Risk of Bias Assessment

The overall risk was low. However, one trial was evaluated to have some concerns due to unclear determination of the patient selection process. Another trial was judged to have a high risk of bias due to inadequate allocation concealment. Additionally, two studies had a high risk of performance bias. With regard to attrition bias, one study was deemed to have a high risk. While all trials had a low risk of reporting bias, two studies were found to have a high risk. We have provided the risk of bias summary and graph in the figures file (Figures 4-9).

Discussion

Repeated embryo implantation failure is a challenging complication in assisted reproductive techniques. Despite advancements in fertility treatment, some women continue to

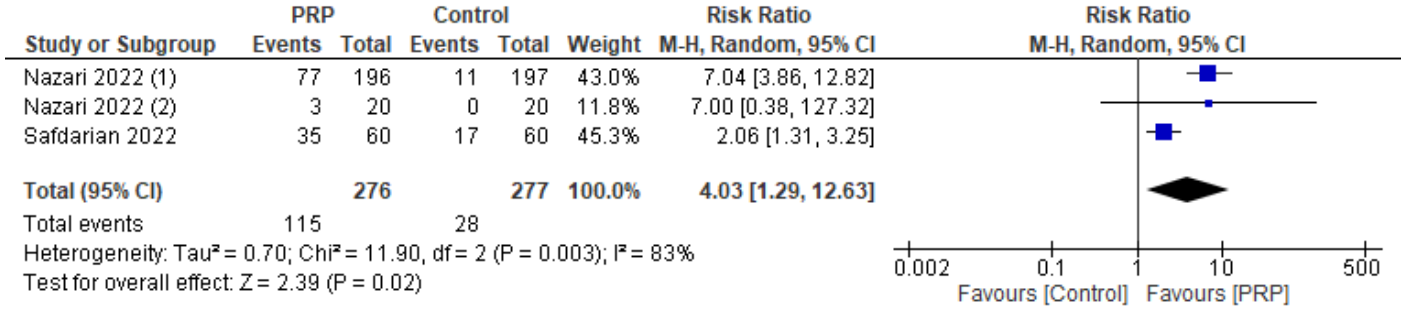

Figure 3. Meta-analysis of live birth rate (LBR) per patient

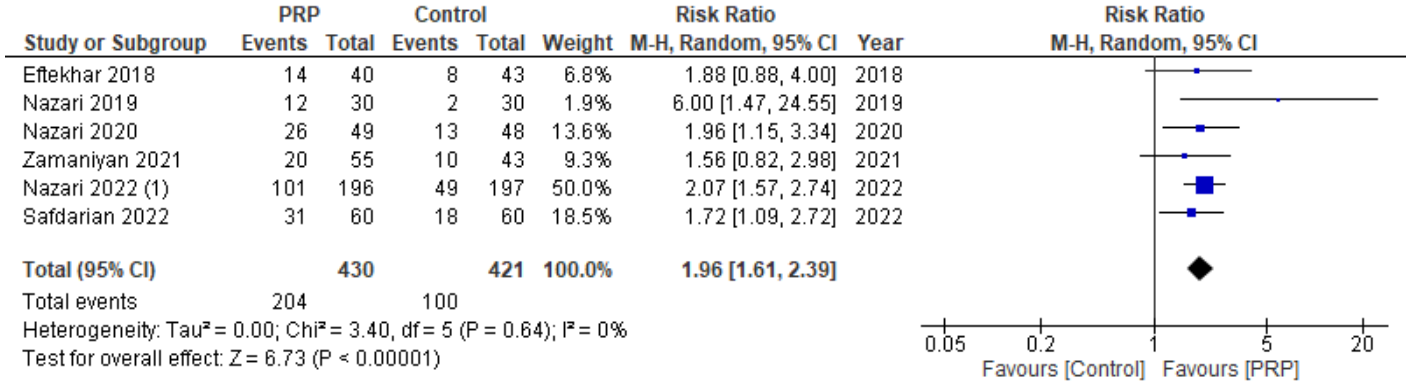

Figure 4. Meta-analysis of Chemical pregnancy rate per patient

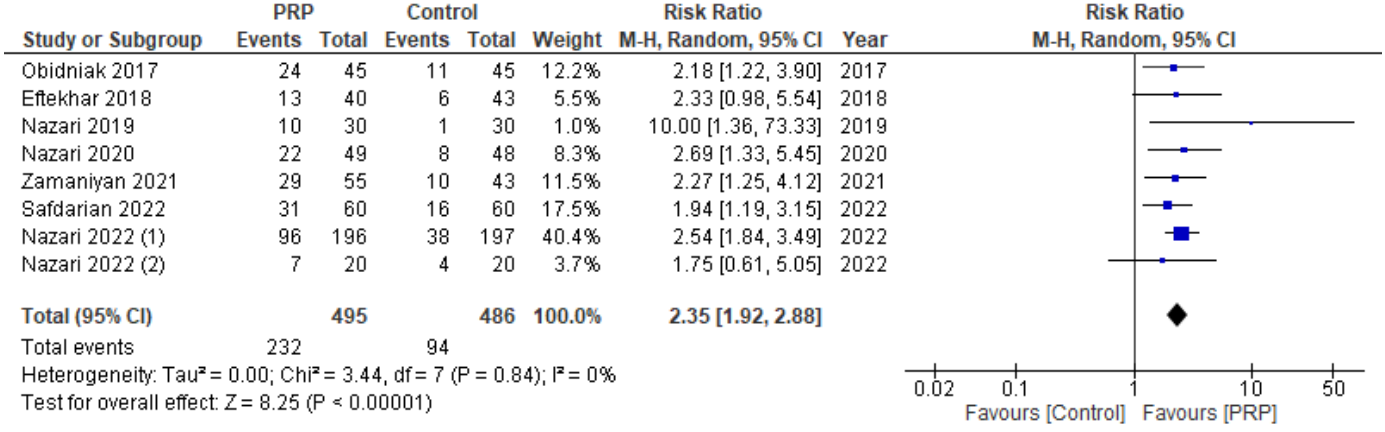

Figure 5. Meta-analysis of Clinical Pregnancy Rate (CPR) per patient

struggle with failed implantations, leading to disappointment and emotional distress. PRP has recently emerged as a promising intervention for improving pregnancy outcomes in women who have experienced RIF. PRP contains a high concentration of growth factors that have been shown to promote angiogenesis, cell proliferation, and differentiation. These mechanisms can enhance endometrial receptivity, increase IRs, and ultimately improve pregnancy rates. PRP therapy has been investigated in several studies and has shown encouraging results, particularly in patients with thin endometrium, RIF, or poor ovarian response to assisted reproductive techniques. In this way, PRP therapy can improve patient outcomes and offer hope to women struggling with RIF.

Findings Summary

Our review encompassed eight randomized clinical trials involving 1038 women who had undergone at least three unsuccessful embryo implantation attempts. We identified three main findings: first, intrauterine infusion of PRP before ET was linked to a notable increase in implantation, chemical, and CPRs and a significant rise in LBR. Second, in contrast to the control group, intrauterine PRP infusion was found to reduce the MR in patients with RIF. Third, despite a higher PIET in the PRP group,

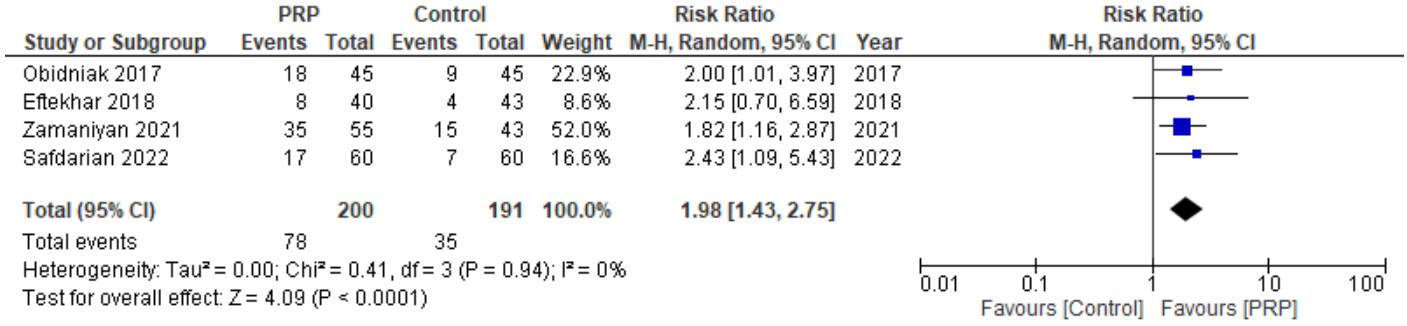

Figure 6. Meta-analysis of Implantation Rate (IR) per embryo

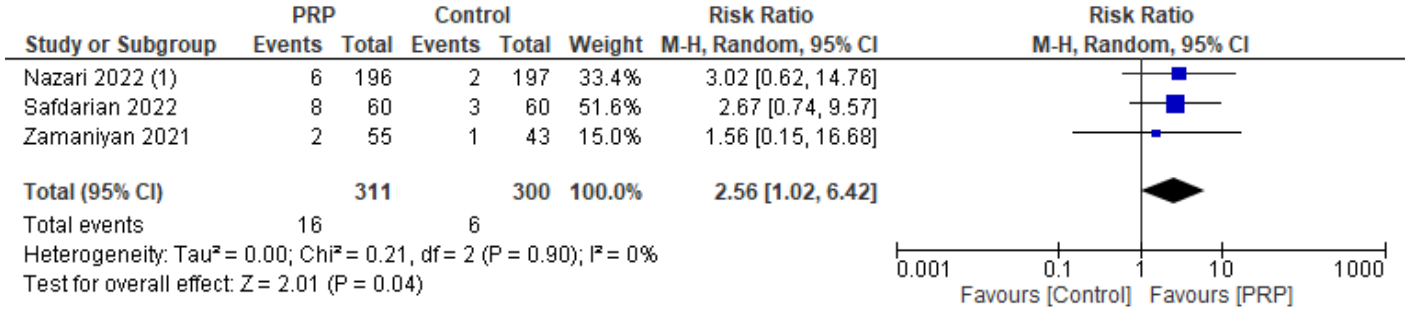

Figure 7. Meta-analysis of Multiple Pregnancy Rate (MPR) per patient

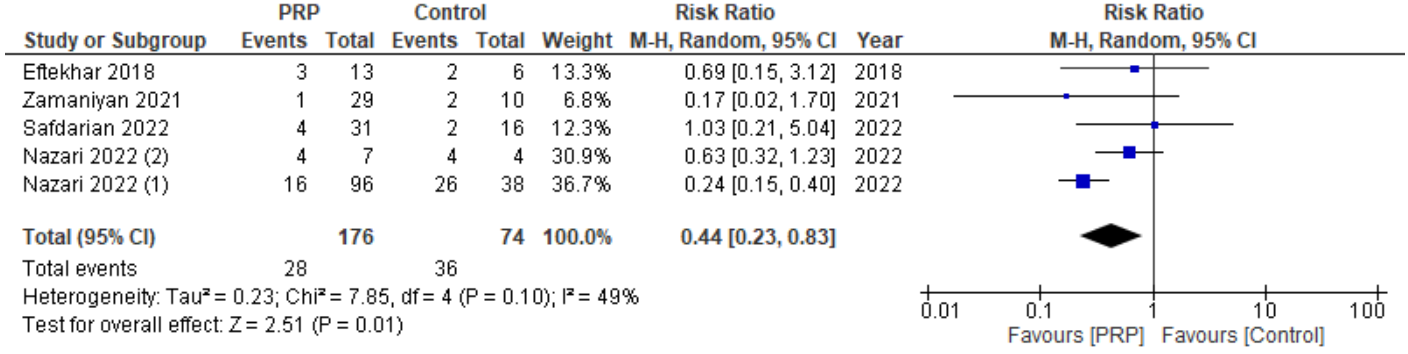

Figure 8. Meta-analysis of Miscarriage Rate (MR) per clinical pregnancy

there was no significant difference between the two groups concerning this factor. Nonetheless, based on subgroup analyses, a substantial increase in thickness was detected after 48 h.

Possible Physiological Basis

There are several physiological mechanisms by which PRP can improve pregnancy and birth outcomes in RIF women. These mechanisms include promoting angiogenesis, stimulating cell proliferation and differentiation, and modulating inflammation. One of the crucial factors for successful implantation is adequate endometrial receptivity. Studies have shown that PRP contains growth factors that can improve angiogenesis, cell proliferation, and differentiation[22]. Angiogenesis increases blood flow to the endometrium, which can improve thickness, vascularity, and receptivity, consequently facilitating embryo implantation. PRP also releases cytokines and other growth factors that have anti-inflammatory properties. Chronic inflammation plays a detrimental role in endometrial receptivity by interfering with important processes such as angiogenesis, cell proliferation, and differentiation. PRP's anti-inflammatory properties can combat this by reducing inflammation and resulting damage, therefore improving endometrial receptivity and promoting successful implantation.

Moreover, PRP has been found to contain a high concentration of growth factors such as PDGF, VEGF, and TGF-β1 that aid in the formation of a suitable endometrial microenvironment and enhance endometrial regeneration that can lead to successful embryo implantation. Overall, PRP regenerative and immunomodulatory actions prove promising for optimizing endometrial receptivity, promoting successful implantation, and improving pregnancy rates in patients with RIF.

Additionally, some recent reports attributed these to the

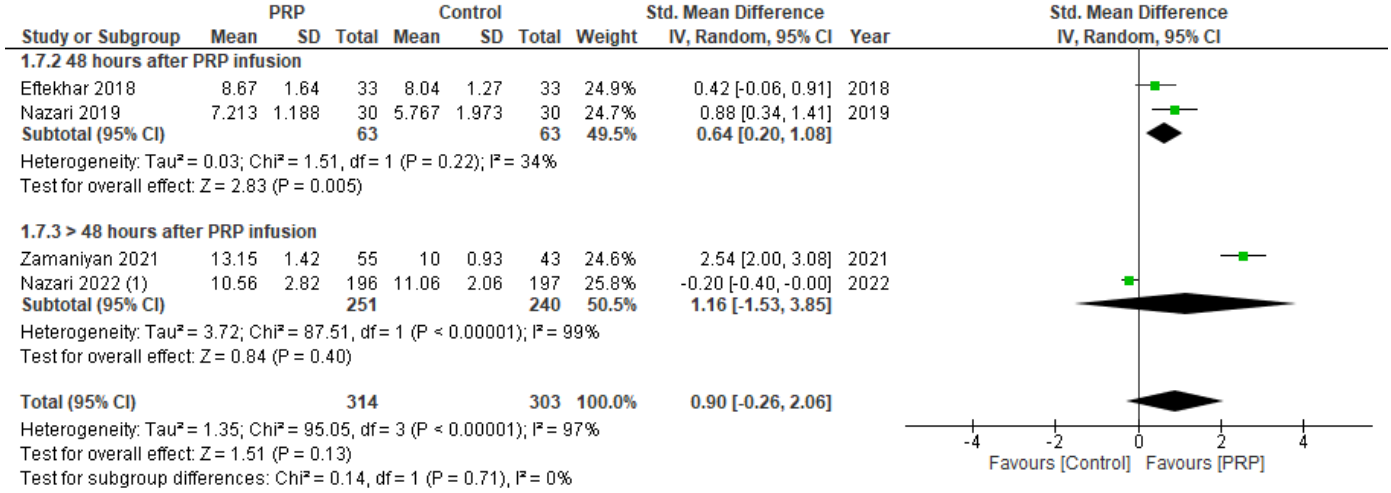

Figure 9. Meta-analysis of Post-interventional endometrial thickness (PIET), subgrouped according to the period from PRP infusion to time to measure the endometrial thickness

numerous growth factors that improve endometrial thickness and vascularity, which help tissue proliferation and differentiation, enhancing its receptivity and stabilizing pregnancy[22,23]. Additionally, platelets and extravillous trophoblast cells take the place of the endothelium and muscle layer in spiral arteries, leading to their expansion and creating sufficient blood flow to the intervillous region of the placenta[24].

Our Results in the Context of Literature

A recent meta-analysis was published by Liu et al.[25] investigating PRP efficacy on pregnancy outcomes. They included both cohort studies and clinical trials (5 RCTs and 3 Cohorts) that enrolled women with at least 2 failed attempts of ET, which may have limited generalizability when applied to RIF patients. However, our results were in the same context in pregnancy outcomes (CPR, LBR, and MR) but not in MPR. Safdarian and his colleagues recently conducted a clinical trial that evaluated the effectiveness of 0.5 mL intrauterine infusion of PRP before 48 h of ET[18]. Their results revealed that pregnancy outcomes and LBR could be improved by the effect of PRP, despite having some concerns about a significant preterm delivery detected in the PRP group compared with the control group.

Multiple studies have reported that intrauterine infusion of PRP leads to a significant increase in endometrial thickness in women with RIF[15,26]. This increase in endometrial thickness has been linked with higher pregnancy rates, suggesting that PRP impact on endometrial thickness is a crucial factor in improving pregnancy outcomes in women with RIF. PRP has been found to have a positive impact on endometrial thickness in women with RIF. Studies have shown that PRP contains a high concentration of growth factors such as PDGF and TGF-β1, which promote angiogenesis and cell proliferation. This increases blood flow and oxygenation to the endometrium, which ultimately increases endometrial thickness.

In addition to promoting endometrial angiogenesis and cell proliferation, PRP's anti-inflammatory properties can play a role in increasing endometrial thickness. Inflammation can cause fibrosis and scarring in the endometrium, which can lead to thinning of the endometrial lining.

PRP plays a key role in reducing inflammation and promoting tissue repair and regeneration. This can prevent injury and scarring, leading to thicker and more receptive endometrium. Another two recent clinical trials suggest a substantial benefit when using PRP intrauterine catheter in women with a thin endometrium (<7 mm) that interferes with successful embryo implantation[15,26]. They reported that PRP could improve endometrial growth quality in addition to increasing the chance of obtaining a clinical pregnancy in women with thin endometrium. This may be promising for patients with impaired implantation due to thin endometrium.

Study Implications

PRP is a treatment method that involves using a patients own blood, which is centrifuged to separate the plasma and platelets. In the case of RIF, PRP injections may help to improve the quality of the endometrial lining. This, in turn, may improve the chances of successful embryo implantation and reduce the risk of miscarriage. This study is the only review that pools the effect estimates of only RCTs evaluating the effectiveness of PRP on women with RIF, which generates a higher level of evidence regarding the use of PRP in resistant cases undergoing assessed reproductive techniques. Moreover, we predetermined our population to include patients who couldnot achieve a clinical pregnancy after at least 3 failed attempts of high-quality ET. This guaranties a piece of evidence that could help in decision making for RIF patients.

Study Limitations

A major limitation of this study is that 7 out of 8 are from the same research group. All trials were investigated on an Iranian population for Obidniak et al.[21], which is a strong limitation for the evidence generalizability. However, there are some

ongoing clinical trials outside Iran. Moreover, we noticed some variations regarding the PRP preparation protocols which may result in a variable composition and concentrations of its different contents. Studies have reported different centrifugation techniques for different timings and different processing[27,28].

Conclusion

We provided strong evidence on how intrauterine infusion of PRP can increase implantation and pregnancy outcomes in RIF patients, which should direct clinicians to consider this intervention as a very effective tool in assisted reproductive techniques.

Ethics

Peer-review: Internally and internally peer-reviewed.
Authorship Contributions
Concept: A.S., S.E., Y.S., D.H., H.M.F., Design: A.S., S.E., Y.S., D.H., H.M.F., Analysis or Interpretation: A.S., S.E., Y.S., D.H., H.M.F., Literature Search: A.S., S.E., Y.S., D.H., H.M.F., Writing: A.S., S.E., Y.S., D.H., H.M.F.
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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Supplementary File 1. PubMed search strategy

(((implantation failure) OR (RIF) OR (recurrent implantation failure) OR (assisted reproductive technology) OR (in vitro fertilization) OR (intracytoplasmic sperm injection) OR (embryo transfer)) AND ((platelet-rich plasma) OR (PRP)))